THE PREVENTION AND DIAGNOSIS OF CENTRAL VENOUS CATHETER-RELATED INFECTION

Anna Louise Casey

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The University of Aston in Birmingham

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SUMMARY

Central venous catheters (CVC) are widely used in clinical practice, however they continue to be the main cause of hospital-acquired bacteraemia. In this current study, the epidemiology, diagnosis, surveillance and prevention of CVC-related infection was investigated.

The potential source of CVC colonisation was assessed. Isolates of coagulase-negative staphylococci (CoNS) recovered from the skin and CVC components of 3 cardiothoracic surgery patients were characterised by pulsed-field gel electrophoresis (PFGE). The genetic heterogeneity of CoNS isolated from the skin was demonstrated and specific genotypes implicated in catheter colonisation. In addition, phenotypic and genotypic typing techniques were assessed for their ability to characterise strains of CoNS recovered from 33 patients who developed catheter-related bloodstream infection (CR-BSI) on a bone marrow transplant (BMT) unit and *Staphylococcus aureus* recovered from 6 cardiothoracic surgery patients with surgical site infection (SSI) following median sternotomy. This epidemiological investigation revealed that common strains of CoNS and *S. aureus* where not associated with infection in patients with CR-BSI or sternal SSI during the study period. Furthermore, there was no correlation between phenotypic and genotypic characterisation results. The variable expression of phenotypic traits within strains of staphylococci was evident whilst PFGE and randomly amplified polymorphic DNA (RAPD) were highly discriminatory for the molecular characterisation of *S. aureus* and CoNS. This was highlighted in 8 stem cell transplant (SCT) patients whereby it was demonstrated that routine identification and characterisation of CoNS by phenotypic techniques may not be adequate for the diagnosis of CR-BSI by current guidelines. The potential of the lipid S ELISA to facilitate the diagnosis of CR-BSI in 38 haematology/SCT patients and sternal SSI in 57 cardiothoracic surgery patients was also assessed. The ELISA proved to be a sensitive test for the rapid serodiagnosis of infection due to staphylococci in immunocompetent patients. The acridine orange leucocyte cytospin test (AOLC) was also evaluated for the rapid diagnosis of CR-BSI in 16 haematology/SCT patients with Hickman CVC *in situ*. Although the sensitivity of the test was low, it may provide a useful adjunct to conventional methods for the *in situ* sampling of catheters to predict and diagnose CR-BSI, preventing the unnecessary removal of CVC.

The Meditrend® CVC audit programme was evaluated in 108 CVC insertions in haematology/SCT patients. The programme identified CVC infection rates and highlighted potential areas whereby preventative measures may be implemented. Many strategies have been investigated for the prevention of CVC-related infection. In this study, the potential infection risk associated with the PosiFlow® and Clave® needleless connectors (NC) compared to the conventional luer and cap was assessed. In addition, the efficacy of 70% (v/v) industrial methylated spirit (IMS), 0.5% (w/v) chlorhexidine in 70% (v/v) IMS and 10% (w/v) aqueous povidone-iodine to disinfect the intravenous connections was determined. The results suggested that NC may reduce microbial contamination of CVC luers compared to the standard cap. Furthermore, disinfection of NC with chlorhexidine or povidone-iodine significantly reduced microbial contamination. Both of these strategies may reduce the risk of CR-BSI acquired via the intraluminal route.

Key words: Pulsed-field gel electrophoresis, randomly amplified polymorphic DNA, acridine orange leucocyte cytospin, lipid S ELISA, needleless connector.

Dedication

To my family

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Abbreviations

AA	aplastic anaemia
ACTC	American Collection of Type Cultures
ALL	acute lymphoblastic leukaemia
AML	acute myeloid leukaemia
AOLC	acridine orange leucocyte cytospin
API	analytical profile index
AP-PCR	arbitrarily-primed - polymerase chain reaction
ARR	aortic root replacement
ASD	atrial septal defect
ASTA	antistaphylolysin
AVR	aortic valve replacement
BC	blood culture
BHI	brain heart infusion
BMT	bone marrow transplant
bp	base-pairs
BSAC	British Society for Antimicrobial Chemotherapy
BZC	benzalkonium chloride
CABG	coronary artery bypass graft
CAPD	continuous ambulatory peritoneal dialysis
CFU	colony-forming units
CHEF	clamped homogeneous electric fields
C/L	central line
CLED	cystine lactose electrolyte deficient agar
Clf	clumping factor
CLI	clindamycin
CLL	chronic lymphobastic leukaemia
cm	centimetre(s)
CML	chronic myeloid leukaemia
CoNS	coagulase-negative staphylococci
CPA	capsular polysaccharide adhesin
CR-BSI	catheter-related bloodstream infection
CRI	catheter-related infection
CRP	C-reactive protein
CT	computerised tomography

CVC	central venous catheter
CVP	central venous pressure
DI	discriminatory index
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
Efb	extracellular fibrinogen binding protein
ELISA	enzyme linked immunosorbent assay
ERY	erythromycin
ES	EDTA-sarcosyl
ESP	EDTA-sarcosyl-proteinase K
ESS	extracellular slime substance
FUS	fusidic acid
g	gram(s)
g	specific gravity
GEN	gentamicin
HL	Hodgkins lymphoma
Hr	hour(s)
ICU	intensive care unit
IMS	industrial methylated spirit
ISA	Iso-sensitest agar
IV	intravascular
kb	kilobases
L	litre(s)
LIN	linezolid
LMT	low-melting point agarose
LTA	lipoteichoic acid
M	molar
MDS	myelodysplasia
MM	multiple myeloma
MRSA	methicillin-resistant *Staphylococcus aureus*
µg	microgram(s)
µl	microlitre
mg	milligram(s)
ml	millilitre(s)
min	minute(s)

MUP	mupirocin
MVR	mitral valve replacement
NAG	N-acetylglucosamine
NAM	N-acetylmuramic acid
NC	needleless connector
NCTC	National Collection of Type Cultures
NET-100	NaCl-EDTA-Tris
NHL	non-hodgkins lymphoma
NNIS	National Nosocomial Infection Surveillance System
nm	nanometres
NPV	negative predictive value
OD	optical density
PBSC	peripheral blood stem cell
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PIA	polysaccharide intercellular adhesin
PICC	peripherally-inserted central catheter
PG	peptidoglycan
POEMS	polyneuropathy, organomegaly, endocrinopathy, M protein and skin changes
PPV	positive predictive value
PS/A	capsular polysaccharide adhesion
PVC	polyvinyl chloride
PVR	pulmonary valve replacement
Q/D	quinupristin/dalfopristin
RAPD	randomly-amplified polymorphic DNA
REA	restriction endonuclease analysis
REAP	restriction endonuclease analysis of plasmids
Rep-PCR	repetitive-element PCR
RFLP	restriction fragment length polymorphism
RIF	rifampicin
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
RTI	respiratory tract infection
SA	staphylococcal antigen
SAA	slime-associated antigen

SCT	stem cell transplant
sec	second(s)
S_D	dice coefficient
SDS-PAGE	sodium dodecylsulphate polyacrylamide gel electrophoresis
SSI	surgical site infection
TA	teichoic acid
TAE	Tris-EDTA-glacial acetic acid buffer
TBE	Tris-boric acid-EDTA
TE	Tris-EDTA
TET	tetracycline
TMB	tetra methyl benzidine
TRI	trimethoprim
UPGMA	unweighted pair group method of arithmetic averages
UTI	urinary tract infection
UV	ultraviolet
VAN	vancomycin
VSD	ventricular septal defect
v/v	volume per volume
WBC	white blood cell count
WM	Waldenstroms Macroglobulinaemia
w/v	weight per volume

1. Introduction

Since intravascular (IV) catheters were first introduced into clinical practice they have become one of the most essential features of modern medical patient management. Indeed, each year in the U.S.A, approximately 150 million IV devices are purchased by hospital and clinics, of which more than 5 million are central venous catheters (CVC) (Maki and Mermel, 1998). IV catheters are reliable devices used to administer drugs, fluids, electrolytes, blood products, nutritional support and in some cases to monitor a patients' haemodynamic state. Specialised CVC can also be utilised for organ substitution processes such as haemodialysis (Bach, 1999). An example of a CVC is the non-tunneled multi-lumen catheter designed for short-term use (<8 days) which is frequently used in intensive care units (ICU). Figure 1-1 shows a typical triple-lumen CVC. The wide variety of different types of CVC and their functions are summarised in Table 1-1.

The use of CVC is often associated with the occurrence of iatrogenic conditions including infection (Table 1-2). Indeed, the first account of infectious complications associated with the use of IV catheters was published just 2 years following their implementation into clinical practice in 1945 (Neuhof and Seley, 1947). Since then, the problem of catheter-related infection (CRI) has steadily increased. Catheter-related bloodstream infection (CR-BSI) is now the largest cause of hospital-acquired bacteraemia with approximately 1 episode occurring per 100 hospital admissions (Correa and Pittet, 2000). In a recent surveillance report of bacteraemia acquired in English hospitals between 1997 and 2002, CVC alone were responsible for the highest proportion of hospital-acquired bacteraemias (Figure 1-2). The proportion of bloodstream infections due to CVC varies widely between clinical specialities (Figure 1-3). This thesis is based on studies of patients undergoing cardiothoracic surgery and haematology patients, which have reported hospital-acquired bacteraemia rates of 1.1 and 4.4 per 1000 patient days respectively. Of these bacteraemias, 41% percent were due to CVC in patients undergoing cardiothoracic surgery patients and 74% with haematology patients. Overall recorded rates of CRI associated with the use of CVC ranges from 5% to 20% (Bach, 1999) and it is estimated that 400 000 cases of CVC related infections occur in the U.S.A annually (Oppenheim, 2000). The cost associated with the management of infected patients is approximately $296 million - $2.3 billion per annum (Mermel, 2000). The number of cases of CR-BSI reported to the Communicable Disease Surveillance Centre is increasing, which may be due to several factors; increased use of IV catheters; enhanced awareness and ability to diagnose a CRI; increased population of immunocompromised patients and the acceptance of low-virulent coagulase-

negative staphylococci (CoNS) as pathogens which are frequently associated with the colonisation and infection of IV catheters.

Figure 1-1 A triple-lumen CVC

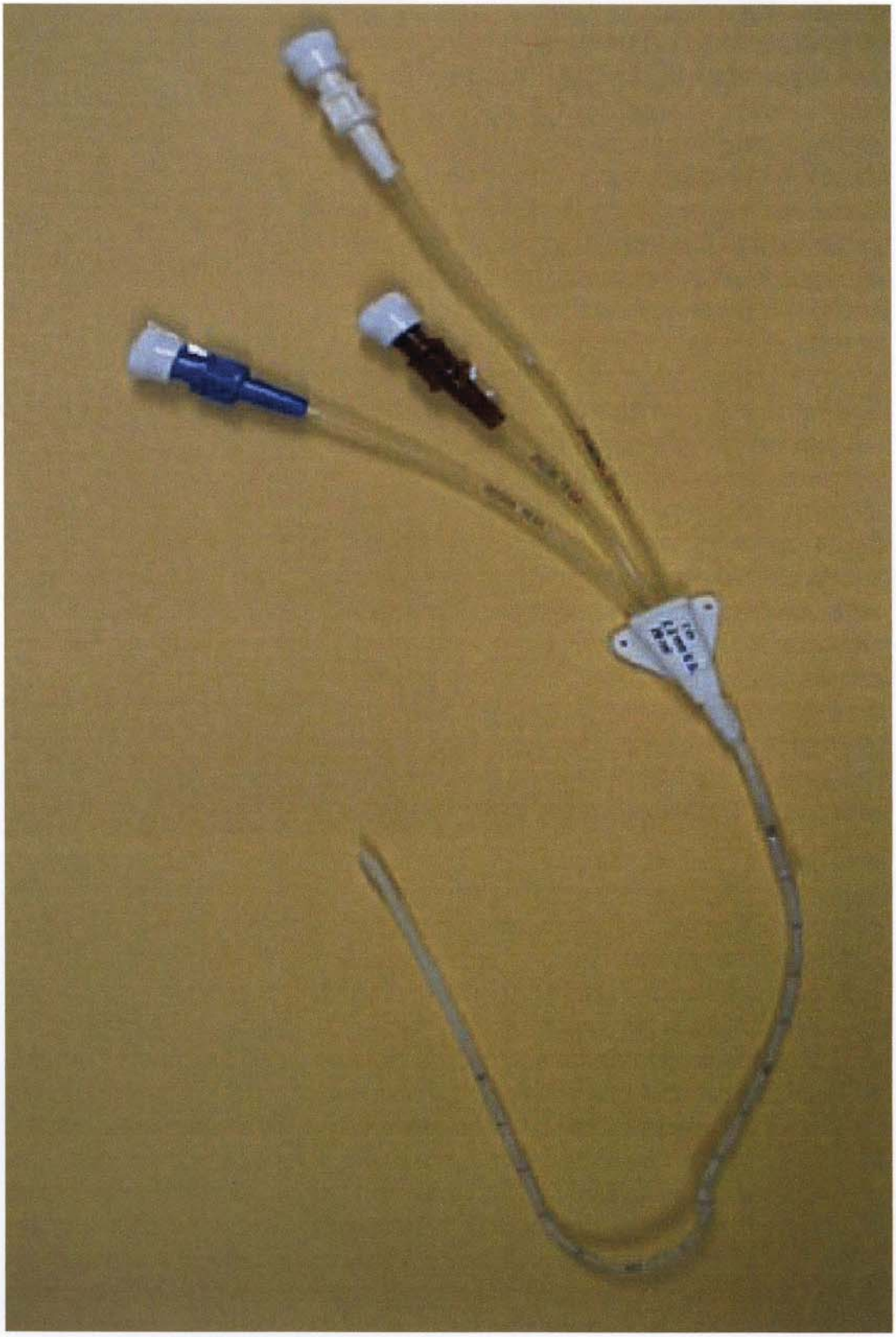


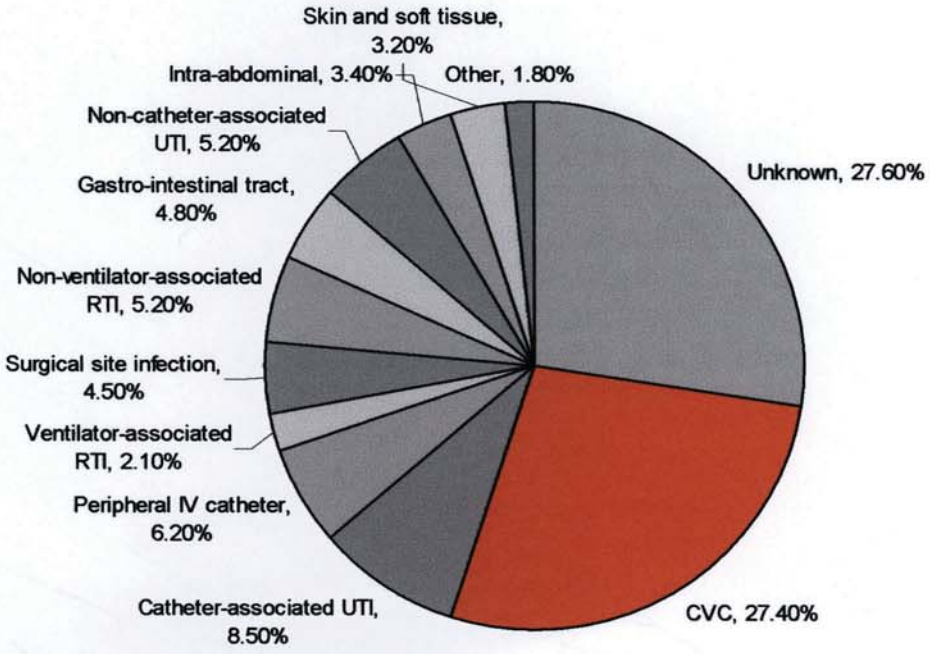
Table 1-1 Types of CVC, their functions and normal entry site

DURATION OF USE	TYPE OF CATHETER	DESCRIPTION/NORMAL FUNCTION	NORMAL ENTRY SITE/S
SHORT TERM	• Non-tunnelled multi-lumen CVC	Drug/fluid administration, haemodynamic monitoring and blood sampling	Subclavian, jugular and femoral veins
	• Haemodialysis catheters	Y – shaped CVC allowing blood to be donated and received simultaneously	Subclavian, jugular and femoral veins
	• Swan sheath	An introducer sheath used to guide a swan ganz into the pulmonary artery	Subclavian, jugular and femoral veins
	• Swan Ganz (pulmonary artery catheter)	A balloon tipped catheter. Inflated in the pulmonary artery to perform left atrial monitoring.	Via subclavian, jugular or femoral vein to the pulmonary artery
LONG TERM	• Peripherally Inserted Central Catheter (PICC)	Drug/fluid administration including chemotherapy and TPN and blood sampling	Basilic, cephalic or brachial veins then advanced to superior vena cava
	• Implantable CVC i.e. Port-a-cath	A subcutaneous port attached to a tube accessible via a huber needle. Used for drug/fluid administration and blood sampling	Implanted in subclavian or internal jugular vein
	• Non-tunnelled multi-lumen CVC	Drug/fluid administration, haemodynamic monitoring and blood sampling.	Subclavian, jugular and femoral veins

Table 1-2 Iatrogenic conditions associated with CVC use (adapted from Elliott and Faroqui, 1992)

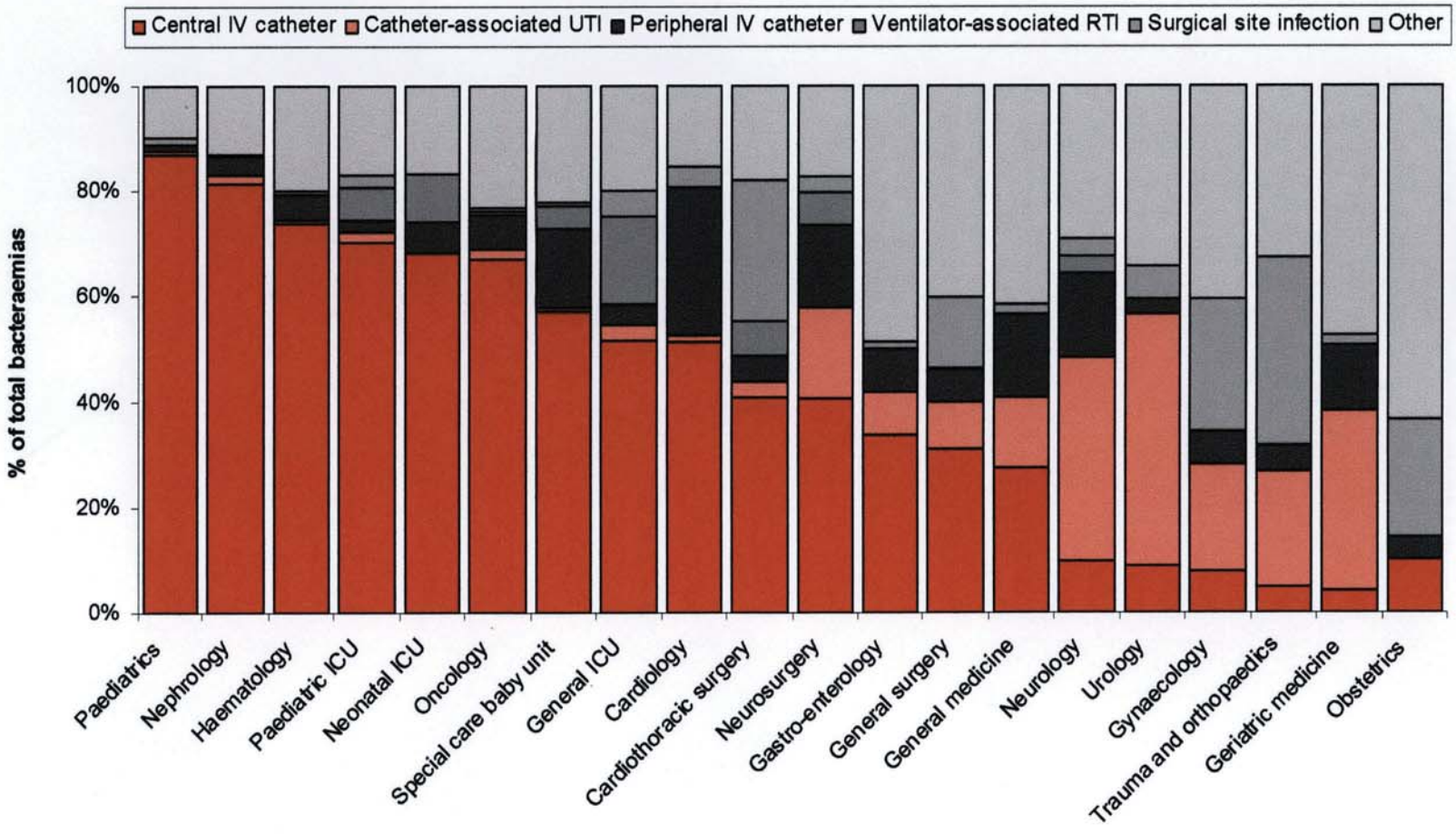
Condition
Catheter malposition
Haemothorax
Pneumothorax
Nerve damage
Haematoma
Catheter embolism
Cardiac dysrrhythmias
Air embolism
Venous/arterial damage
Subcutaneous emphysema
Pleural effusion
Cardiac perforation
Thrombosis
Thrombophlebitis
Thromboembolism
Hydrothorax
Endocardial damage
Infection

Figure 1-2 Sources of hospital-acquired bacteraemia in English hospitals between 1997 and 2002 (data supplied by the nosocomial infection national surveillance service)



IV, intravascular; UTI, urinary tract infection; RTI, respiratory tract infection

Figure 1-3 Known sources of hospital-acquired bacteraemia in English hospitals between 1997 and 2002 (data supplied by the nosocomial infection national surveillance service)



1.1 Types of infections associated with IV catheter use

CRI are classified into 3 groups; localised infection, catheter-related bloodstream infection or metastatic infection.

1.1.1 *Localised Infection*

1.1.1.1 Exit Site Infections

Exit site infections are characterised by signs of inflammation including warmth, tenderness, erythema, oedema, induration and/or purulence within 2 cm of the catheter exit site (O'Grady *et al.*, 2002). However, these symptoms may be due to clinical or mechanical irritation therefore exit site swabs positive for microbial growth aids in diagnosis of an exit site infection.

1.1.1.2 Tunnel Infection

Tunnel infections occur when a local infection extends subcutaneously along the track of a tunneled long-term catheter (figure 1-4). Clinical symptoms of tunnel infections include; erythema, tenderness, cellulitis and induration in the tissues overlying the catheter and <2 cm from the exit site (Press *et al.*, 1984). A differential diagnosis between infection and chemical or mechanical irritation such as infusion phlebitis is required (Maki and Mermel, 1998).

1.1.1.3 Pocket Infection

This infection is associated with the use of a totally implantable device. It is characterised by erythema and necrosis of the skin overlying the reservoir and/or purulent exudate in the pocket containing the device (Pearson,1996).

1.1.1.4 Suppurative Phlebitis

Suppurative phlebitis is a purulent inflammation of a vein and is a serious infection associated with intravenous therapy. It occurs frequently in burns patients (Pruitt *et al.*, 1980) and is commonly associated with catheters inserted by the cut-down method (Kristinsson, 1997). The incidence of suppurative phlebitis increases with prolonged duration of cannulation. Local inflammation is often absent and other clinical symptoms such as fever, chills and hypotension may present days after the initial onset of infection (Maki and Mermel, 1998). Suppurative phlebitis is often identified by the presence of purulent exudate when 'milking' the vein by digital pressure and/or positive blood cultures (Kristinsson, 1997).

1.1.1.5 Septic Thrombophlebitis

Septic thrombophlebitis is inflammation of a vein accompanied by the presence of a septic thrombus. The thrombus may become dislodged and embolise elsewhere, spreading the infection.

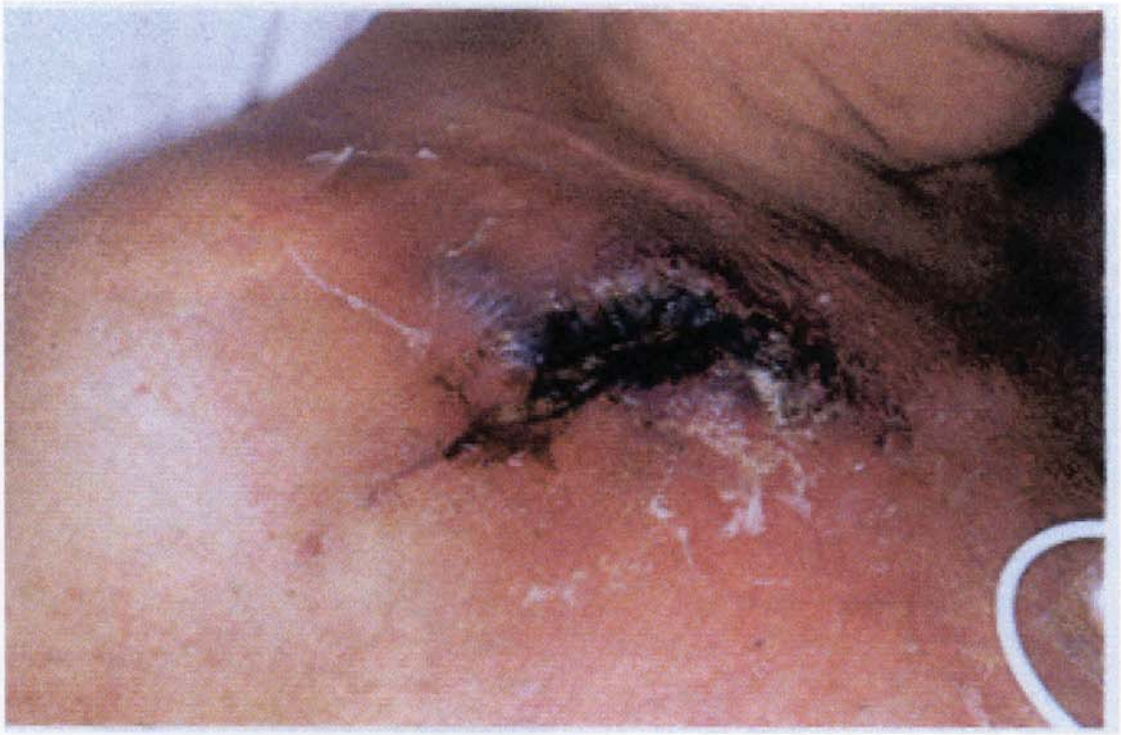
1.1.2 *Catheter-related bloodstream infection*

Systemic infections associated with IV catheters usually arise from colonisation of the distal tip of the catheter by microorganisms. Unlike localised infections, there is usually no obvious indications of infection other than pyrexia, unresponsiveness to broad-spectrum antibiotics, no other obvious source of infection, evidence of infection at the insertion site, septic rigors on flushing, hypotension and positive microbiological cultures (Elliott, 1997). Diagnosis of a systemic infection associated with an IV catheter is difficult especially in patients with numerous foci of infection for example, immunocompromised patients and patients with numerous indwelling devices. Diagnosis is essentially performed by means of exclusion and is often confirmed after catheter removal.

1.1.3 *Metastatic infections*

Endocarditis is an serious infection condition associated with CVC use. It is characterised by inflammation of the endocardium and valves which may lead to embolism and heart failure. CVC can cause endocarditis if the catheter is malpositioned and a septic thrombus develops at the distal tip of the catheter which is located near to a heart valve (Elliott, 1997). Septic thrombi may also dislodge from catheters and embolise causing infection of the lung, brain abscesses, osteomyelitis and endophthalmitis (O'Grady *et al.*, 2002).

Figure 1-4 Cellulitis in the tissues overlying the subcutaneous track of a tunneled CVC (taken from Elliott *et al.*, 1997)



1.2 Microorganisms responsible for intravascular catheter-related infection

Since the 1980s, there has been a marked shift in the microorganisms responsible for bloodstream infections reported to the National Nosocomial Infection Surveillance System (NNIS). The trend has switched from primarily Gram-negative to Gram-positive microorganisms (Banerjee *et al.*, 1991). The microorganisms responsible for bacteraemia can vary widely depending on the source. For example, in England from 1997-2001 the main microorganisms associated with bacteraemias acquired from catheter-associated or ventilator-associated infection were *Esherichia coli* and *S. aureus*, respectively (Coello *et al.*, 2003). This study was undertaken by the nosocomial infection surveillance unit using data supplied by 17 teaching and 56 non-teaching hospitals in the UK. The major microorganisms associated with CVC associated bacteraemia were CoNS, *Staphylococcus aureus* and *Enterococcus* spp (table 1-3).

The CoNS are now frequently resistant to several classes of antibiotics including beta-lactams, macrolides, quinolones and aminogylcosides. Vancomycin remains the primary treatment for CR-BSI due to CoNS as resistance is still detected in less than 1 percent of bloodstream infection isolates (Anonymous, 2003). In addition, 54% of *S. aureus* strains involved in hospital-acquired bacteraemia are now resistant to methicillin (methicillin resistant *S. aureus* – MRSA). MRSA strains are also frequently resistant to many other antimicrobial agents such as gentamicin, ciprofloxacin, erythromycin and tetracyline (Anonymous, 2003). This again leaves vancomycin the only drug of choice for treatment of CR-BSI which is of concern as there have now been several cases of infection due to *S. aureus* with intermediate resistance and total resistance to vancomycin (Bartley, 2002). *Enterococcus faecium* is now highly resistant to both ampicillin and gentamicin. Vancomycin resistance has now reached 17% in isolates from this species from bloodstream infections (Anonymous, 2003).

Fungal pathogens represent an increasing proportion of nosocomial bloodstream infections. It has been documented that 10% of *Candida albicans* strains are now resistant to fluconazole (Pfaller *et al.*, 1998). However, the number of bloodstream infections caused by *Candida* spp other than *C. albicans* is also increasing and these may exhibit higher antifungal resistance rates (Pfaller *et al.*, 1998b). It has often been assumed that candidaemia arises from endogenous flora of colonized patients but several studies have demonstrated that other external factors are associated including; contaminated infusates

(Moro *et al.*, 1990), contaminated equipment (Weems *et al.*, 1987), cross infection (Lee *et al.*, 1991) and contaminated healthcare workers' hands (Burnie, 1986). In addition, factors such as the increase in immunocompromised patients and the excessive use of antibacterial agents have contributed to the continual high rates of fungal-induced bloodstream infections.

Gram-negative microbes account for most catheter-related infections associated with pressure monitoring systems and contaminated infusates (Gahrn-Hanson *et al.*, 1988). Clusters of bloodstream infections due to Gram-negative microorganisms are likely to be due to a common source such as contaminated infusates.

Table 1-3 Microorganisms responsible for CVC-associated bacteraemia in England between 1997 and 2001 (adapted from Coello *et al.*, 2003)

Microorganism	Percent of microorganism causing CVC-associated bacteraemia.
CoNS	38.3
Staphylococcus aureus	24.8
Enterococcus spp.	7.3
Candida spp.	3.8
Klebsiella spp.	3.6
E. coli	3.3
Enterobacter spp.	3.1
Stenotrophomonas maltophilia	2.2
Pseudomonas aeruginosa	1.8
Acinetobacter spp.	1.6
Other *Pseudomonas* spp.	1.4
Serratia spp.	0.8
Proteus spp.	0.6
Other isolates	8.3

1.3 Sources of microorganisms

Microorganisms associated with CRI originate from a number of sources including the environment and the patient's and healthcare worker's skin. These microorganisms may gain access to the catheter to cause infection via a number of routes (figure 1-5);

- Haematogenous seeding
- Impaction of microorganisms during insertion
- Contaminated infusates
- Intraluminal migration
- Extraluminal migration from the exit site
- Contiguous infection

1.3.1 *Haematogenous seeding*

Microorganisms from other foci of infection within a patient may colonise the CVC surface via haematogenous seeding, however this is a rare cause of infection (Cleri *et al.*, 1980). This type of infection occurs almost exclusively in ICU patients due to a high incidence of bacteraemia whilst an IV catheter is in place. In a study by Maki and Will (1990), it was concluded that exposure to a non CVC-related bacteraemia was the strongest risk factor in the development of a systemic CVC infection in catheterised ICU patients.

1.3.2 *Impaction of microorganisms during insertion*

Microorganisms associated with CR-BSI may be impacted onto the catheter from the patients skin during insertion of the CVC (Elliott, 1995; Livesley *et al.*, 1998). Disinfection procedures are currently being investigated to determine the most effective method of reducing the number of CRI due to impaction. The catheter may also be contaminated from another source such as the healthcare workers' hands before the insertion process, emphasising the need for stringent aseptic technique prior to and during catheterisation.

1.3.3 *Contaminated infusates*

Many microorganisms have been implicated in infections associated with contaminated infusates, in particular the aerobic Gram-negative bacilli which are able to multiply at room temperature in many infusion solutions (Maki and Mermel, 1998). One widely used general anaesthetic drug, propofol has been found to support the growth of pathogenic microorganisms (Wachowski *et al.*, 1999). Contaminated infusates are responsible for only 2-3% of IV CRI due to the use of preservatives such as chlorbutol which has both

antibacterial and antifungal properties (Mermel *et al.*, 1991; Elliott and Curran, 1989). Infusates may also become contaminated during the manufacturing process (intrinsic contamination) which could potentially lead to infection outbreaks. More commonly infusates are contaminated during use (extrinsic contamination). Pre-filled flush syringes containing sterile infusions may be the way forward in preventing extrinsic contamination (Worthington *et al.*, 2001).

1.3.4 *Intraluminal migration of microorganisms*

Sitges-Serra *et al.* (1984) described microbial colonisation of the catheter hub as the initial step in the pathogenesis of CR-BSI acquired via the intraluminal route. This was concluded following a study which demonstrated that 88% of CR-BSI episodes in patients on parenteral nutrition were attributable to colonised hubs. Indeed, hub contamination followed by subsequent intraluminal migration is thought to be responsible for the highest proportion of CR-BSI in patients with long-term catheters (Linares *et al.*, 1985). A meta-analysis of CR-BSI conveyed by Sherterz (1997) concluded that 66% of patients with long-term CVC had CR-BSI attributable to hub/internal lumen contamination compared to 42% of patients with short-term CVC. Hubs may become contaminated by the patients own skin flora or by the hands of healthcare workers during repetitive handling of the hub associated with infusion therapy.

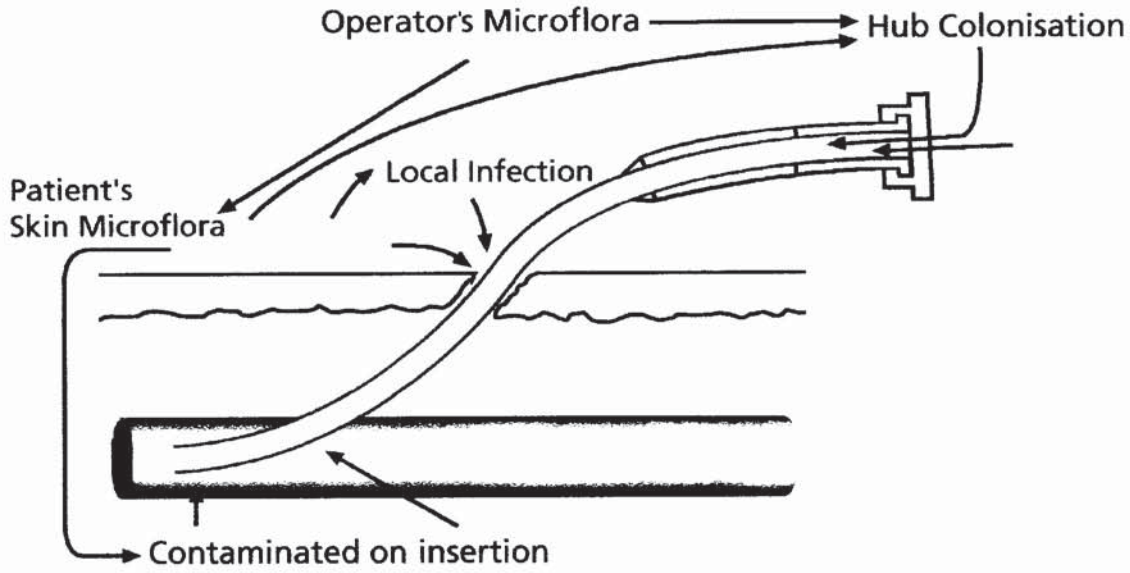
1.3.5 *Extraluminal migration from the exit site*

Microflora from the patients skin may migrate down the external surface of the catheter to the distal tip from the insertion site. The skin of the patient is thought to be the most common source of microorganisms in CR-BSI associated with IV catheters (Sherertz, 1997). The meta-analysis of CR-BSI conveyed by Sherterz (1997) concluded that 92% of patients with short-term CVC had CR-BSI attributable to contamination from their skin compared to 26% of patients with long-term CVC. Microorganisms contaminating the external surface of the catheter may also originate from the healthcare worker's skin or may be endogenous from other sites of the patient such as the respiratory or gastrointestinal tract (Elliott, 1997).

1.3.6 *Contiguous infection*

Contiguous infection may occur in burns patients whereby there is a direct spread of microorganisms from adjacent infected tissue to a catheter for example, during catheterisation of infected burned skin (Sherertz, 1997).

Figure 1-5 A schematic representation of the main routes by which microorganisms gain access to intravascular catheters



1.4 Colonisation of catheters

The adhesion of microorganisms to prosthetic devices is recognised as an important initial step in the pathogenesis of infections associated with such devices (Hogt *et al.,* 1983). Initial adhesion depends on several microbial-biomaterial surface interactions including van der Waals forces, electrostatic and hydrophobic interactions (Pascual, 2002). Hydrophobic strains of *S. epidermidis* are more likely to colonise medical devices than hydrophilic strains due to the hydrophobic nature of most modern plastic prostheses (Fleer *et al.*, 1986). When 2 hydrophobic surfaces interact in an aqueous environment, they attach non-specifically by removal of water molecules between them (Pascual, 2002). Following this non-specific association, there are specific interactions between adhesins expressed on the surface of microorganisms and extracellular host proteins. These specific interactions take place once the prosthesis is 'conditioned' by a film of human proteins. Indeed, proteins and glycoproteins from connective tissue and plasma promote the adherence of *S. aureus* and some species of CoNS. Examples of conditioning proteins include; fibronectin, fibrinogen, fibrin, collagen, lamini, vitronectin, elastin, von willebrand factor and thrombospondin (Vaudaux *et al.,* 2000; Lopes *et al.*, 1985). The adherence of staphylococci to fibrinogen is mediated by a fibrinogen binding protein expressed in all strains of *S. aureus* but rarely in strains of *S. epidermidis* (Foster and Hook, 2000). Most CoNS achieve weaker associations with host proteins and have fewer adhesins (Pascual, 2002). For example, *S. epidermidis* binds less avidly to fibronectin than does *S. aureus* (Foster and Hock, 2000). *S. aureus* binds efficiently to protein coated polymers compared to CoNS which depend more on hydrophobic interactions. Furthermore binding of CoNS may even be impaired by the presence of plasma proteins (Gotz and Peters., 2000).

However several antigens which are present in *S. epidermidis* which are thought to be responsible for adherence to plastic polymers have been identified. A capsular polysaccharide adhesin (PS/A or CPA) has been isolated and is thought to be required for the adherence of *S. epidermidis* to naked plastic (Tojo *et al.*, 1988). Several proteinacious adhesions produced by *S. epidermidis* have also been identified and implicated in adherence (Timmerman *et al.,* 1991; Heilmann *et al.*, 1996).

Many species of staphylococci, once attached to a prosthetic device, produce a fibrous exopolysaccharide material termed a glycocalyx 'slime' in which they become embedded. Indeed, many antigens have been recovered from slime producing strains of *S. epidermidis*.

Polysaccharide intercellular adhesin (PIA) has been identified and is associated with intercellular adherence of microorganisms and biofilm accumulation (Mack *et al.*, 1996). In addition, a proteinaceous antigen termed 'accumulation associated protein' (AAP) whose function is similar to PIA has been identified (Hussain *et al.*, 1997). Another antigen, slime associated antigen (SAA) which is composed of polysaccharide, has been associated with slime producing strains of *S. epidermidis* although its exact function is still unknown (Christensen *et al.*, 1990).

The exact composition of the extracellular slime substance (ESS) produced by *S. epidermidis* is a subject of much debate. It is thought to contain a mixture of sugars and proteins (Herrmann and Peters, 1994). Characterisation of ESS is complex as it is difficult to distinguish the substance from cellular components of the microorganism and its nature is also influenced by the type of media used for culture (Hussain *et al.*, 1993). However, teichoic acid appears to constitute a large component of ESS (Hussain *et al.*, 1991) and a 30kDa protein has also been shown to be present in most slime-producing strains of *S. epidermidis* (Kotilainen *et al.*, 1990). The ESS functions to modify the local environment in favour of the pathogen by accumulating nutrients and protecting the microorganism from antimicrobial agents and from opsonophagocytosis (Christenson *et al.*, 1982). Indeed, Costerton and colleagues (1995) first suggested that microorganisms within biofilms are at least 500 times more resistant to antibiotics than planktonic cells. Furthermore, a large number of genes from microorganisms within the biofilm are depressed allowing these sessile cells to react to their microniche environment and are consequently phenotypically distinct from their planktonic counterparts (Costerton *et al.*, 1995).

Slime production has been implicated as a virulence factor in the pathogenesis of CRI caused by CoNS. Christensen and co-workers (1982) demonstrated that, 63% of strains of *S. epidermidis* responsible for CRI were slime producers compared to 37% of blood culture contaminants and skin isolates. This is of clinical concern, particularly in immunocompromised patients as Passerini and colleagues (1992) demonstrated that 81% of arterial and CVC were colonised by slime-producing CoNS after 1-14 days *in situ*.

Coagulase-negative staphylococci are often an uncommon cause of infection in the absence of medical prostheses, however, they are the most frequent cause of device-related infection (Lowy and Hammer, 1983). This suggests that the pathogenicity of CoNS is

related to their ability to survive in biofilms on biomaterials such as IV catheters *in vivo* by means of the mechanisms discussed.

1.5 Diagnosis of CVC-related infection

Central venous catheter-related infection may be diagnosed both clinically and through laboratory investigation.

1.5.1 Clinical diagnosis

A catheter-related infection may be clinically diagnosed by the presence of symptoms outlined in section 1.1. Defervescence or absence of other clinical symptoms associated with this type of infection is also indicative of a CRI. Fletcher and Bodenham (1999) stated that CRI is characterised by fever and/or other symptoms in an otherwise well patient with an IV catheter *in situ*. Diagnosis is however confounded in immunocompromised patients or other patients who are susceptible to infection, for example, haematology or surgical patients.

The accurate clinical diagnosis of CR-BSI is complicated due to the non-specific clinical presentation. Indicative symptoms including inflammation, erythema, oedema is evident in only 35-50% of patients (Michalopoulos and Geroulanos, 1996). Due to the difficulty in making the clinical diagnosis of CRI, it is estimated that 75-85% of CVC are removed unnecessarily from febrile patients (Eggiman and Pittet, 2002). Therefore supporting laboratory diagnosis is required to confirm the diagnosis.

1.5.2 Laboratory diagnosis

1.5.2.1 Post catheter removal

1.5.2.1.1 Broth culture of catheter tip and segments

When CRI is clinically suspected, the catheter is often removed and sent for microbiological investigation. To confirm that a bacteraemia is catheter-related, identical microorganisms must be recovered from the catheter as in blood culture. However, in practice catheter tip cultures are very often performed in isolation thus complicating the interpretation of positive cultures (Peacock *et al.*, 1998). A simple method to determine if microorganisms are present on a catheter segment is to culture the specimen in an enrichment broth. However the disadvantage of this method is that contaminating microorganisms arising from the skin on catheter removal will yield positive cultures generating false-positive results. This method therefore has a low positive predictive value. However, a negative result using this method is highly predictive when CRI is absent, except in the case where the colonizing microorganism is a slow-growing species

in a culture (Kristinsson, 1997). This qualitative method is now rarely used in the routine Microbiology laboratory and has been replaced by semiquantitative methods of culture.

1.5.2.1.2 Semiquantitative culture of the external catheter surface

The most common method of culturing catheter segments is the roll-plate technique which was first described by Maki and co-workers (1977). This method involves culture of the intracutaneous portion of the catheter and/or the distal tip. The segment is placed on an agar plate and rolled backwards and forwards over the surface of the plate at least 4 times, after which the plate is then incubated in air at 37°C for 24 hr. Catheter segments yielding ≥15 colony forming units (cfu) is indicative of local inflammation at the catheter exit site whilst colony counts ≥1000 are suggestive of CR-BSI. The study of Maki and colleagues included a high percentage of short peripheral catheters which are more commonly associated with infection caused by extraluminal migration of microorganisms from the exit site. The positive predictive value of this test may therefore be falsely high when applied to longer catheters which are frequently associated with intraluminal contamination.

Several studies in patients with different types of catheter have been conducted to determine the optimal number of cfu yielding the highest sensitivity and positive predictive values for CR-BSI. Collignon and colleagues (1986) found that a threshold of ≥5 cfu resulted in a sensitivity of 92%, a specificity of 83% and a positive predictive value of 99.8%, however the negative predictive value was only 9%. When the thresholds were increased to ≥15cfu and ≥100cfu the diagnostic parameters were similar with a low negative predictive value which may have reflected the low incidence of CR-BSI during the study. Kristinsson and co-workers (1989) determined that the positive and negative predictive values for CR-BSI in patients with long CVC with a threshold of ≥15cfu are 46% and 99% respectively and with a threshold of ≥100cfu are 56% and 93% respectively. In a further study conducted by Rello and colleagues (1992) it was determined that a 92% sensitivity could be achieved with thresholds of both 15 and 50 cfu with specificities of 99% and 93% respectively. Positive and negative values were similar to those found in the study by Kristinsson, for a threshold of 15 colonies the positive predictive value was 34% and for a threshold of 50, this increased to 44%. Both thresholds resulted in a negative predictive value of 99%. There are several other problems with the roll plate technique including the possible contamination of catheters on removal, the fact that the internal lumen surface is not cultured to test for CRI acquired via the intraluminal route and that adherent microorganisms (i.e. slime producers) may be difficult to remove. Despite this

the roll-plate method has been widely adopted by routine Microbiology laboratories despite conflicting results on its sensitivity and threshold level of 15cfu.

1.5.2.1.3 Quantitative culture of the internal lumen

A method of sampling the internal lumen surface of the catheter was first described by Cleri and co-workers (1980). In this method, the intradermal segment of the catheter was placed in trypticase soy broth and flushed 3 times, after which the broths were diluted and cultured onto agar plates. After appropriate incubation the numbers of cfu are counted and calculated. A count of $\geq 10^3$ cfu./ml is indicative of CR-BSI however the sensitivity is further increased to 100% if the threshold is increased to 10^6 cfu/ml. The sensitivity of this culture technique correlates well with the roll plate method (Linares *et al.*, 1985).

1.5.2.1.4 Quantitative culture of the catheter after vortexing/sonication

This method was first described by Brun-Buisson and co-workers (1987). The catheter segment is vortexed in sterile water for 1 min after which it is diluted for quantitative culture. A colony count of $\geq 10^3$ cfu./ml is indicative of CR-BSI, the sensitivity and specificity values for this test was 97.5% and 88% respectively. A disadvantage of this method and other methods discussed previously is that microorganisms existing in a biofilm on the surface of the catheter may be difficult to remove therefore falsely low counts may be achieved. In an attempt to remove adherent microorganisms, Sherertz and co-workers (1990) evaluated sonication of catheter segments. In a later study which compared the roll plate method, sonication and flushing, it was concluded that sonication of catheters was the most sensitive method at detecting catheter colonization (Sherertz *et al.*, 1997).

1.5.2.1.5 Direct staining of catheter segments

Catheter segments may be directly stained with either the Gram stain or with acridine orange and examined under a microscope. The method of Gram staining catheters was first described by Cooper and Hopkins (1985) who concluded that the method is simple, inexpensive and accurate (100% sensitivity, 97% specificity). However, in a study conducted by Spencer and Kristinsson (1986), it was demonstrated that the sensitivity of this technique was considerably lower than originally stated. This technique requires 200 microscopic fields to be examined with an oil immersion objective (x1000 magnification). A positive result is designated as 1 microorganism found per 20 microscopic fields. Although simple and inexpensive, this technique has been found to be extremely time-consuming and impractical for routine microbiology laboratories (Kristinsson, 1997).

Acridine orange dye may also be used to stain catheters however a fluorescence microscope is needed to examine the segments (Zufferey *et al.*, 1988). This test utilises the technique of dry magnification of x100 for 3 min and if no fluorescence is observed the result is considered negative. If positive, results are further examined to investigate morphology of the microorganism. This test has a sensitivity and specificity of 84% and 99% respectively. However, the sensitivity of these methods has been questioned by Coutlee and co-workers (1988) who demonstrated that the Gram stain had sensitivity and specificity of 44% and 91% respectively and the acridine orange stain, 71% and 77% respectively.

Examining catheter segments under a microscope is a complex technique due to their cylindrical shape, however, Collignon and colleagues (1987) devised a method to overcome this problem. The catheter segment is smeared over a moistened glass slide, Gram stained and examined under the microscope. This technique has a high sensitivity and specificity but low positive predictive value. Again, the disadvantage with this method is that adherent microorganisms may not be completely removed from the catheter yielding falsely low numbers of microorganisms per microscope field.

1.5.2.2 *In situ* diagnostic techniques

1.5.2.2.1 Blood cultures

Guidelines published for diagnosis of CRI highlight that the recovery of identical microorganisms from both the catheter segment and from a blood culture, or isolation of identical microorganisms from blood cultures taken via the catheter and from a peripheral venepuncture is strongly indicative of a CR-BSI (Pearson, 1996). It is essential that catheter-drawn blood alone is not used for diagnosis as positive results may simply reflect contaminated or colonised catheter and/or hubs in a healthy patient (Everts *et al.*, 2001).

Several studies have been performed which suggest that quantitative blood cultures are of value in diagnosing CR-BSI (Douard *et al.*, 1991; Capdevila *et al.*, 1992; Quilici *et al.*, 1997). The finding of a 5-10 fold increase in the concentration of microorganisms in blood drawn via the CVC compared to the peripheral sample is strongly suggestive of CR-BSI. There are 4 main quantitative methods for culturing blood samples: the 'pour plate' method, whereby a known volume of anticoagulated blood is mixed with a known volume of molten agar which is allowed to solidify. Following incubation the number of cfu are estimated (Capdevila, 1992); direct inoculation of the blood sample onto agar plates followed by standard incubation and estimation of cfu; lysis centrifugation whereby blood

is inoculated into special tubes containing a lytic agent and centrifuged. The supernatant is then removed and the concentrate cultured (Dorn and Smith, 1978); automated blood culture systems, for example, Bactec® (Becton Dickinson, UK).

Rogers and Oppenheim (1998) investigated the use of continuous monitoring blood culture systems and differential time to positivity for diagnosing CR-BSI. Serial dilutions of a CoNS were inoculated into the BacT/Alert blood culture system (Biomerieux, France) and time to positivity and length of lag period monitored. The study demonstrated that the concentration of microorganisms correlated with time to positivity of blood culture bottles. Furthermore, blood cultures taken via the infected catheter became positive significantly quicker than blood taken peripherally. Several other studies have also investigated differential time to positivity as a method of diagnosing CR-BSI with favourable results (Blot *et al.*, 1998, Blot *et al.*, 1999, Malgrange *et al.*, 2001, Seifert *et al.*, 2003). In the study by Blot and colleagues (1999) a sensitivity and specificity of 94% and 92% respectively was achieved if a differential time to positivity of 2hrs or more between paired samples was applied as the threshold.

1.5.2.2.2 Skin swab cultures (from exit sites)

Several studies have evaluated the potential of swabs of the skin surrounding the exit site or the exit site itself in order to predict or diagnose CRI (Fan *et al.*, 1988, Guidet *et al.*, 1994). Fan and co-workers (1988) swabbed the exit site alone twice weekly in a study of 142 subclavian and jugular CVC insertions and demonstrated that this method had a sensitivity and specificity for predicting CRI of 37.9% and 71.7% respectively. However, when this method is combined with surveillance hub cultures, the sensitivity is increased to 79.3% and the specificity to 74.3%. Conversely, in a study by Guidet and colleagues (1994) an area of skin surrounding the exit site was swabbed. This resulted in a sensitivity of 100% for the detection of catheter colonisation.

Skin swab/exit site cultures may only be useful as a predictor of CRI in patients whereby the exit site is likely to be the source of the infection, otherwise the normal skin flora may be misinterpreted as false positive results.

1.5.2.2.3 Hub cultures

Catheter hub cultures are only useful in aiding the diagnosis of CRI in patients whereby it is likely that the hub is a potential source of infection. This is most commonly found in patients with long-term CVC *in situ* for example, Hickman lines for administering

chemotherapy in haematology patients. Since the catheter hub has been implicated as the biggest source of microorganisms for infections in long-term CVC, this suggests that routine surveillance cultures may be of value in the prediction of CRI. In the study by Fan and colleagues (1988), hub surveillance cultures had a sensitivity of 34.5% and a specificity of 87.6% for predicting CR-BSI. A combination of both hub and skin cultures yields a higher sensitivity for diagnosis of CRI, probably due to the fact that infection is not always acquired via the same route (Fan *et al.*, 1988).

1.5.2.2.4 Endoluminal brush

The endoluminal brush is used to sample microorganisms and biofilm from within the lumen of IV catheters. A brush is passed down the internal lumen to the distal tip after which it is removed and cultured for microorganisms. In a study conducted by Kite and colleagues (1997) the endoluminal brush was compared to the standard roll plate method (Maki *et al.*, 1977) and the flush technique described by Cleri and co-workers (1980). This study demonstrated that over half of the CVC removed on clinical suspicion of CRI yielded no significant growth by any of the techniques investigated and the majority of the remaining catheters were colonized with microorganisms. Line colonization was also found to be approximately twice as prevalent when using the Maki roll technique when compared to the Cleri flush and the endoluminal brush. This suggests that the roll plate method may yield more false positive results, which may be in part due to external contaminaton of the catheter with microorganisms during removal. Of the 3 methods investigated, the endoluminal brush had the highest sensitivity and specificity for the diagnosis of CRI (95% and 84% respectively. The Maki roll technique had a sensitivity of 82% and specificity of 66% compared to the Cleri flush with values of 75% and 84% respectively. Furthermore, it demonstrated that 52% of CVC removed due to clinical suspension of CRI could have been saved if diagnosis was based on endoluminal brush culture results.

Originally, the procedure to sample the internal lumen with an endoluminal brush required the operator to pass the device out of the distal tip of the catheter prior to withdrawing the brush (McLure *et al.*, 1997). This technique however has caused clinical concern as it has the potential to dislodge biofilms and/or fibrin clots within the internal lumen of the catheter, increasing the risk of subsequent bacteraemia or embolism. Indeed, McLure and co-workers (1996) reported 1 case of post-brushing bacteraemia during a study investigating the optimal frequency for catheter brushing. Brushing methodology has now been modified in order that the endoluminal brush is passed down the internal lumen of the

catheter to a known length without emerging from the distal tip. However there are still concerns in the clinical setting, of the potential hazards associated with catheter brushing, especially in immunocompromised patients (Blot *et al.*, 2000).

It is normal clinical practice in some hospitals to regularly brush IV catheters to remove fibrin clots in order to reduce the risk of blockage and potential infection in patients with long term IV access. However, if the endoluminal brush is also employed as a technique for the diagnosis of CRI false negative results may be obtained from patients where catheters are regularly brushed (McLure *et al.*, 1996).

1.5.2.2.5 Microscopy of blood aspirated through the catheter

A rapid technique for diagnosing CR-BSI is to stain and examine blood drawn through the hub of the catheter. Methods include the Gram stain and the acridine orange leucocyte cytospin test (AOLC). The AOLC test was first evaluated as a method for diagnosis of CR-BSI by Rushforth and co-workers (1993). The trial was undertaken using neonatal subjects and resulted in AOLC sensitivity and specificity of 87% and 94% respectively. The method incorporated ultraviolet microscopy to examine a centrifuged and acridine orange stained blood sample aspirated through the hub of the CVC. The test is rapid, with results available within 1 hr and employs standard cytospin techniques and fluorescence microscopy, which is available in many routine laboratories.

The success of the AOLC as a method for the diagnosis of CR-BSI has been demonstrated in neonates but has generated conflicting results in adult patients (von Baum *et al.*, 1998). Neonatal CVC have a narrower internal lumen diameter which aids increased velocity and turbulence of blood flow through the catheter during aspiration. This narrow lumen also has an increased surface area in contact with the blood aspirated, which potentially increases bacterial yield and therefore positive results (Kite, 1999). Tighe and co-workers (1995) also evaluated the AOLC test in adult patients and demonstrated negative results in those patients without a narrow lumen CVC. Furthermore, clinically significant bacteraemias in adults are characterised by a relatively small number of microbes per ml of blood when compared to neonates (Tighe *et al.*, 1995). The sensitivity of the AOLC test is however increased if the assay is used in combination with the endoluminal brush (Tighe *et al.*, 1996). Moonens and co-workers (1994) investigated the potential of Gram staining blood drawn directly through the CVC in patients undergoing total parenteral nutrition. A positive predictive value of 100% and negative predictive value of 42% for diagnosing

CR-BSI was demonstrated as all patients who had fever unrelated to catheter infection yielded negative results.

1.5.2.2.6 Serological diagnosis of staphylococcal infection in patients with CR-BSI

There are several serological tests for facilitating the diagnosis of infection due to *S. aureus* including the detection of antibodies to alpha and beta-toxin, teichoic acid (TA), staphylolysin (ASTA), cell-bound clumping factor (Clf), extracellular fibrinogen binding protein (Efb), peptidoglycan (PG), crude staphylococcal antigen (SA), whole cells, and lipases. The ASTA test is widely used for aiding diagnosis of infection due to *S. aureus*, however the assay lacks sensitivity and specificity (Larinkari, 1982). Indeed, a study which evaluated the ASTA in patients with osteomyelitis due to *S. aureus* demonstrated a sensitivity of 47% and specificity of 96%. Antibodies to alpha and beta toxin and teichoic acid have also been widely studied to facilitate diagnosis. A study by Granstrom and co-workers (1983) concluded that measurement of serum IgG titres to alpha toxin was more sensitive than IgM and IgA titres. A significant correlation between IgG titres to alpha and beta toxins was demonstrated in infected patients however measurement of anti-beta toxin was shown to be less sensitive. Furthermore the anti-alpha IgG test was found to be more sensitive than the widely used ASTA. The study also demonstrated that a combined use of anti-alpha toxin and anti-teichoic acid assays was the most favourable method for diagnosing infection. Indeed, sensitivities of 91% for endocarditis, 86% for complicated septicaemia and 68% uncomplicated septicaemia were demonstrated. The overall sensitivity of the assay for diagnosis of septicaemia was increased to 97% when paired samples of serum were tested. A study by Julander and colleagues (1983) which evaluated the methods described by Granstrom also found that measurement of IgM titres was of limited value. The value of the anti-alpha toxin and teichoic acid assays was further confirmed in a study by Larinkari and Valtonen (1984) who demonstrated that 2 patients with culture-negative infective endocarditis yielded elevated serum titres of antibodies and subsequently received successful antibiotic therapy. Colque-Navarro and colleagues (2000) investigated serum antibody responses to 2 *S. aureus* fibrinogen proteins (Clf and Efb) in a wide range of infections. The tests were shown to have low sensitivities, especially in the acute phase of the infection. However the assay had potential for diagnosing chronic infection including osteitis, septic arthritis and endocarditis. The use of antibodies to lipases and whole cells has also been demonstrated to be of some diagnostic value (Ryding *et al.*, 1992).

The number of serological tests currently available for the detection of infection due to *S. aureus* is large, however, there are limited serological assays for the diagnosis of infection due to other species of staphylococci. Christensson and co-workers (1985) observed a significant rise in serum anti-PG and anti-SA titres in some streptococcal and *S. epidermidis* infections. Another test with potential for serodiagnosis of infection due to *S. epidermidis* was highlighted by Karamanos and colleagues (1997). A sulphated 20kDa acidic polysaccharide was recovered from the slime of *S. epidermidis* and evaluated for diagnostic potential. It was demonstrated that it may facilitate serodiagnosis of infection due to *S. epidermidis* and also differentiate between slime-positive and negative strains.

1.5.2.2.7 Lipid S ELISA

A more recently described assay for the serodiagnosis of staphylococcal infection is the lipid S enzyme-linked immunosorbant assay (ELISA) (Elliott *et al.*, 2000). ELISA is a frequently utilised, inexpensive, simple serological technique used for the serodiagnosis of several types of infections. A rapid, indirect ELISA incorporating lipid S, a previously unrecognised exocellular short-chain length form of cellular lipoteichoic acid (LTA) (figure 1-6) produced by strains of *S. aureus* and *S. epidermidis* has been developed, for the diagnosis of deep-seated staphylococcal infection (Worthington *et al.*, 2002; Elliott *et al.*, 2000; Lambert *et al.*, 2000). Lipid S shares common antigenic determinants with LTA but contains only 6 glycerophosphate units compared to 40-42 in whole cell LTA (figure 1-7) (Worthington *et al.*, 2002). The lipid S antigen used to coat the ELISA plates was prepared from a total of 7 strains of CoNS which had been recovered from the blood cultures of patients with CR-BSI.

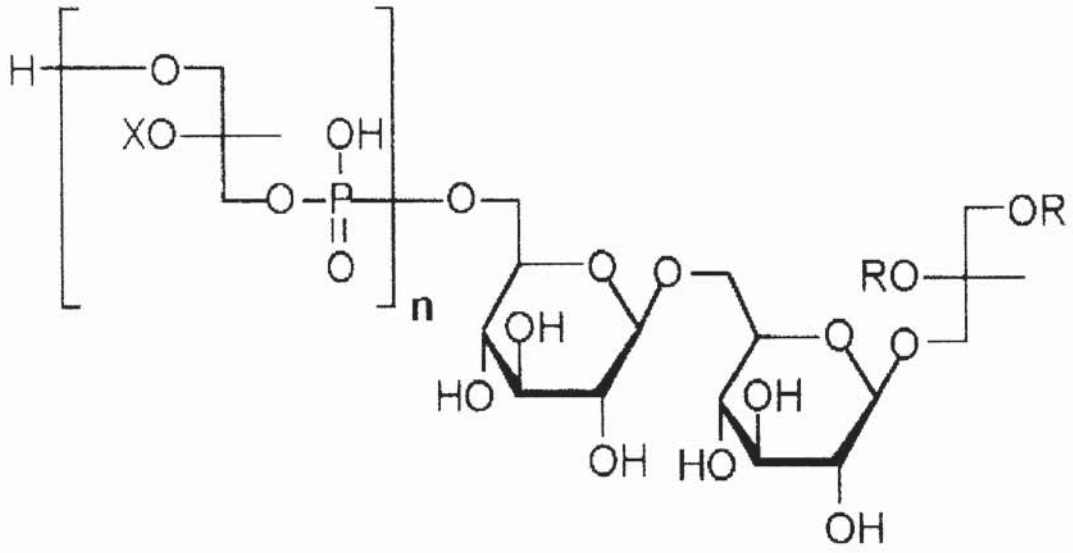
The ELISA has previously been used to facilitate the diagnosis of deep-seated staphylococcal infection including CR-BSI. In a study by Elliott and colleagues (2000) anti-lipid S IgG and IgM titres were determined in patients with CR-BSI due to *S. epidermidis.* The ELISA had a sensitivity of 75% and a specificity of 90% for detecting IgG antibodies to lipid S, however the IgM assay had lower values of 52% and 85% respectively. This IgG assay has also been used to facilitate the diagnosis of pyogenic spondylodiscitis caused by Gram-positive microorganisms which resulted in sensitivity and specificity rates of 70% and 97% respectively (Worthington *et al.*, 2000).

The anti-lipid S IgG ELISA was further developed by Worthington and co-workers (2002) to reduce the assay time from 24hrs to 4hrs. This rapid ELISA was further evaluated on patients with CR-BSI due to CoNS and demonstrated a sensitivity and specificity of 70%

and 100% respectively. The rapid anti-lipid S IgG ELISA has also been evaluated in patients with Gram-positive bacterial endocarditis (Connaughton *et al.*, 2001). In this study the ELISA demonstrated a sensitivity and specificity of 88% when used for the diagnosis of infection due to *S. aureus*, CoNS, *streptococcus* spp and enterococci. The 24hr lipid S ELISA has also been used to facilitate the diagnosis of prosthetic joint infection due to Gram-positive microorganisms and demonstrated a sensitivity and specificity of 93% and 97% respectively when estimating levels of anti lipid S IgG (Rafiq *et al.*, 2000). Further work by Worthington and colleagues (2000) to evaluate the anti lipid S IgG ELISA in the diagnosis of patients with confirmed prosthetic joint infection due to staphylococci demonstrated on assay a sensitivity and specificity of 70% and 97% respectively.

Figure 1-6 A cross-section of the structure of a Gram-positive bacterial cell wall (from Dr Kaiser's microbiology website - www.cat.cc.md.us/~gkaiser/welcome.html)

Figure 1-7 Lipid S molecule



R = ester-linked fatty acids, X = ester-linked D-alanine or *N*-acetylglucosamine, *n*=6.

1.5.2.3 Identification and characterisation of staphylococcal species

There are several clinical scenarios whereby microbial isolates require identification and typing, for example in an epidemiological investigation of an outbreak of infectious disease. Clinical suspicion of an outbreak may be triggered by the increased isolation of a particular pathogen, a cluster of similar infections in a clinical area or the detection of an increased number of isolates with unusual antibiograms or biotypes in the microbiology laboratory (Tenover *et al.*, 1997). When investigating potential persistent strains it is important to include epidemiologically unrelated isolates as controls to differentiate between new outbreaks and endemic problems (Tenover *et al.*, 1997). Identification of cross transmission of infection may allow additional infection control procedures to be employed in order to control such transmission of the offending microorganism.

Speciation and typing of microorganisms is also essential when confirming a diagnosis of CR-BSI. One of the diagnostic criteria for this infection is the isolation of the same microorganism from the catheter tip as the blood culture (Pearson *et al.*, 1996). Since there are over 30 species of CoNS the major cause of CR-BSI, it is important that identification is made to species level. However, as *S. epidermidis* is the most frequently implicated species of CoNS in infection and is a member of the resident skin flora (Kloos and Bannerman, 1994), both, phenotypic and genotypic typing methods need to be employed to distinguish between contaminating and infecting strains.

An ideal typing system must fulfil 4 main criteria, it should: a) be able to type the vast majority of clinical strains encountered including natural isolates; b) have good discrimination with the ability to recognise a reasonable number of types; c) show good reproducibility over a long period of time and in different centres and d) should not be too complicated or expensive to perform or interpret (Tenover *et al.*, 1997).

1.5.2.3.1 Phenotypic methods of characterisation

All microorganisms within in a particular species must be typeable by the chosen method of phenotypic characterisation used. Some phenotypic methods are incapable of this, for example, those based on antibody reactions (Olive and Bean, 1999). Phenotypic methods characterise the products of gene expression therefore results may vary due to changes in a number of factors, for example, growth conditions or spontaneous mutation (Tenover *et al.*, 1997).

1.5.2.3.1.1 Biotyping

The ease of obtaining a biotype makes this method of phenotypic characterization attractive to routine Microbiology laboratories, which now frequently uses commercial kits for the rapid identification of bacterial species. Systems available for the identification of staphylococcal species include: the STAPH-IDENT, API ID 32 STAPH, API STAPH and the ATB 32 STAPH (Biomerieux, France). Which have an identification accuracy of 70% to >90% (Kloos and Bannerman, 1994). However variations in biochemical reactions even within single strains have been observed when using these commercial systems (Tenover *et al.*, 1997). Variations in biochemical reaction may be due to several factors: occasional loss of plasmids encoding metabolic functions; variation in incubation conditions; inoculum size and the age of the cultures (Towner and Cockayne, 1993). Biotyping also has poor reproducibility as microorganisms may alter the expression of genes (Tenover *et al.*, 1997).

Biotyping is also of little value for microorganisms which lack biochemical diversity, for example, the enterococci. Furthermore, several studies have shown biotyping to be indiscriminative when typing CoNS, despite the high genetic variation within these species. Indeed, in a study by Geary and colleagues (1997) which investigated phenotypic and genotypic typing methods, *S. epidermidis* recovered from nosocomial infections were restricted to a limited number of biotypes following characterisation by ATB 32 STAPH. This did not correlate well with results from other typing methods including antibiograms, bacteriophage typing, plasmid profiling and SDS-PAGE protein profiling. It was therefore concluded that biotyping by ATB 32 STAPH was of value only when identifying unusual biotypes of CoNS. These results were also reflected in a study using API ID 32 STAPH to type *S. epidermidis* implicated in septicaemia (Sloos *et al.*, 2000). However, the discriminative power of biotyping CoNS by API was enhanced by Hebert and co-workers (1988) with the addition of adherence and haemolysis tests as an adjunct to the STAPH-IDENT biochemical panel. Conversely, when this system was used by Tenover and colleagues (1994) to type isolates of *S. aureus*, it was shown that although the tests were easy to interpret, too many subtypes were identified and this did not enable outbreak-related strains to be grouped together.

Biotyping of microorganisms may be useful in short-term epidemiological investigations where local strains are investigated. However long-term or larger investigations may need additional methods of typing to discriminate fully between similar isolates (Towner and Cockayne, 1993).

1.5.2.3.1.2 Antibiograms

Similar microorganisms can be compared on the basis of their susceptibility to a panel of antibiotics. This method is rapid, cheap, easy to perform and interpret, and used widely in the routine Microbiology laboratory. However, this technique has many disadvantages including lack of reproducibility and discriminatory power. Antimicrobial resistance is often associated with mobile genetic elements including plasmids and transposons and is often under extreme selective pressure in healthcare institutions (Tenover *et al.*, 1997; Geary *et al.*, 1997). Furthermore microorganisms that are genetically indistinguishable and epidemiologically related may have differing antimicrobial sensitivity patterns (Worthington *et al.*, 2000). Spontaneous point mutations or the gain or loss of extrachromosomal genetic material may account for this (Tenover *et al.*, 1997). Conversely, genetically unrelated strains may have identical antibiograms, which may be due to basic lack of variation within a species or acquisition of a plasmid (Tenover *et al.*, 1997). It has been demonstrated that categorization of antibiotic sensitivity patterns based on quantitative measurement of inhibition zones is a more discriminative method of typing *S. aureus* isolates when compared to simple classification by qualitative measurement (Tenover *et al.*, 1994; Blanc *et al.*, 1996). However, inhibition zones may be affected by environmental factors such as changes in culture conditions (Weller, 2000). Another approach to enhancing the discriminatory power of antibiograms is to increase the number of antimicrobials tested, however, this may be expensive and time consuming (Weller, 2000). The efficacy of increasing the antimicrobial test panel must be considered to determine if there is likely to be any increase in discriminative power. A carefully selected extended panel of antimicrobials may be useful when typing *S. epidermidis* as multi-antimicrobial resistance is common within this species (Geary *et al.*, 1997). Whilst antibiograms are useful to identify recurring resistance patterns locally or identifying outbreaks caused by microorganisms with distinct sensitivity patterns, the technique has had little value when used in isolation in multicentre comparative studies (Towner and Cockayne, 1993). The epidemiologist therefore should consider the use of additional typing techniques in combination with antibiograms.

1.5.2.3.1.3 Phage typing

Phage typing is based on bacterial sensitivity to specific bacteriophages. However, the technique has several disadvantages. Many variables have to be controlled as environmental conditions influence the assay. Phage types may be variable by genetic mechanisms such as lysogenic conversion, acquisition or loss of R plasmids or loss of prophages (Towner and Cockayne, 1993). Also, there is the need to maintain

bacteriophage stocks by serial passage. Phage typing is a sensitive technique but is time consuming and complex therefore is only undertaken in reference laboratories.

Phage typing has been one of the major methods for typing *S. aureus*. However, a high proportion of isolates are non-typeable by this method (Bannerman *et al.*, 1995; Andrasevic *et al.*, 1999; Tambic *et al.*, 1997; Schlichting *et al.*, 1993; Tenover *et al.*, 1994). Phage typing lacks reproducibility and discriminatory power, however this technique is superior to many other phenotypic methods currently used to type *S. aureus* (Schlichting *et al.*, 1993; Richardson *et al.*, 1993).

Attempts have also been made to phage type CoNS. However, phage collections for *S. epidermidis* have not been internationally standardised, have low reproducibility and low discriminatory power (Geary *et al.*, 1997; Kloos and Bannerman, 1994). Phage typing of *S. saprophyticus, S. haemolyticus, S. hominis* and multi-resistant CoNS has also been investigated but with little success (Pereira and Melo Cristino, 1991; Rosdahl *et al.*, 1990).

1.5.2.3.2 Genotypic methods of characterisation

Genetic variation in the DNA of bacterial species is normally high therefore differentiation of strains is relatively simple (Goering, 1993). However, the strains of microorganisms which are associated with infection normally constitute a small subset of a species and consequently may illicit limited genetic variation making them difficult to type. Genotypic typing methods are less subject to natural variation than phenotypic methods although they can be affected by: DNA insertions or deletions in the chromosome; the gain or loss of extrachromosomal DNA or by random mutations (Tenover, 1997).

1.5.2.3.2.1 Plasmid analysis and restriction endonuclease analysis of plasmids (REAP)

Plasmids are the easiest nucleic acid molecules to examine. Indeed, plasmid isolation and analysis can be performed in any laboratory which contains a high-speed centrifuge, agarose gel electrophoresis equipment and a ultraviolet (UV) transilluminator (Towner and Cockayne, 1993). This simple technique differentiates isolates based on the number and size of plasmids (Weller, 2000). One pre-requisite of this technique is the presence of plasmids in the bacteria under investigation. The long-term stability of plasmids within microorganisms may also be a limiting factor as plasmids are readily lost or gained *in vivo*

and *in vitro* (Hartstein *et al.*, 1995; Locksley *et al.*, 1982). Plasmid analysis may be useful for detecting transmission of plasmids encoding resistance to a particular antibiotic, however false impressions of clonal outbreaks may be observed if DNA is transferred between unrelated isolates (McGowan *et al.*, 1979; Tenover *et al.*, 1994). A further problem associated with plasmid typing is the fact that the DNA may exist in more than 1 form (i.e. supercoiled, nicked or linear) all of which have different electrophoretic properties (McGowen *et al.*, 1979). In order for this method to be effectively discriminative, large numbers of plasmids need to be present within the microorganism (Melo Cristino and Pereira, 1989). However, a limited discriminatory capacity was demonstrated in a study which typed *S. epidermidis* from the blood cultures of patients with septicaemia, despite the fact that *S. epidermidis* normally contains multiple plasmids (Sloos *et al.*, 2000).

Plasmids can be analysed in more detail with the use of restriction endonuclease digestion of the purified genetic material – restriction endonuclease analysis of plasmids (REAP). A restriction endonuclease is an enzyme which recognizes specific base pair sequences and cleaves them to produce a 'fingerprint' once the material is electrophoresed onto an agarose gel. This method is particularly useful when comparing plasmids of the same size. However, if this technique is performed with large plasmids, many restriction fragments may be produced making the band patterns difficult to interpret. For the staphylococci whereby plasmids are typically <50 kb, the discriminatory power is increased. DNA fragments or whole plasmids can be compared by DNA-DNA hybridization, following the transfer of DNA to a membrane support.

1.5.2.3.2.2 Restriction enzyme analysis (REA) of chromosomal DNA

Restriction endonucleases can also be used to cleave chromosomal DNA afterwhich conventional electrophoresis may be used to resolve fragments that are 25kb to 0.5kb into a banding pattern on an agarose gel (Tenover *et al.*, 1997). The gel is usually stained with ethidium bromide and visualized on a UV transilluminator. All bacterial isolates are typeable by this method, however profiles with numerous bands are difficult to interpret. Also, as conventional electrophoresis will not separate fragments larger than 20 kb, the banding pattern may contain a large number of overlapping fragments, making interpretation difficult (Mulligan and Arbeit, 1991).

1.5.2.3.2.3 DNA hybridization and restriction fragment length polymorphisms (RFLP)

The combined use of REA and southern hybridization can decrease the number of bands for analysis to a more manageable level (Weller, 2000). Following REA, the DNA fragments are transferred onto a nitrocellulose or nylon membrane by Southern blotting (Sambrook *et al.*, 1989). The DNA is then hybridised with a radioactive or chemically labelled DNA or RNA probe which binds to a limited number of fragments with complementary nucleic acid sequences. As only the genomic DNA fragments that hybridise to the probes are visible, the analysis is simplified. Differences in the number and sites of the bands can then be used to discriminate between strains. These variations are referred to as restriction fragment length polymorphisms (RFLP), which refers to the polymorphic nature of the locations of the restriction enzyme sites within specific genetic regions (Tenover *et al.*, 1997; Olive and Bean, 1999). This simple, inexpensive and universally applicable technique may have enhanced discriminatory power with the use of multiple probes. However, the blotting process is laborious and is frequently replaced by PCR-based locus-specific RFLP.

1.5.2.3.2.3.1 *Ribotyping*

The most commonly used probe for hybridization is ribosomal RNA. Within DNA there are multiple copies of rRNA transcriptional sequences which are relatively conserved between species (Weller, 2000). Labelled rRNA or corresponding genes will hybridise with these sequences wherever they appear. Hybridisation should occur in different positions on the electrophoresed DNA based on the fact that restriction site heterogeneity is present between unrelated strains. The number of bands may also vary if a restriction site is present within the rRNA transcriptional sequence (Stull *et al.*, 1988). Probes originating from the same species for which it's targeted against have been shown to hybridise more rapidly than those from other species (Richardson *et al.*, 1994). All isolates of *S. aureus* are typeable by this method which has been shown to be more discriminative and reproducible than many phenotypic typing methods however less valuable than genotypic methods (Blumberg *et al.*, 1992; Prevost *et al.*, 1992). This deficiency in discriminatory power may be due to the reduced number of bands which result from ribotyping. Furthermore, ribotyping may be technically complex, time consuming and its results quite difficult to interpret (Weller, 2000).

1.5.2.3.2.4 Pulsed-field gel electrophoresis (PFGE)

One complication with the use of conventional electrophoresis is that resolution of DNA fragments >50kb can not be undertaken and fragments <50kb cannot be easily separated and visualised on a gel. Pulsed-field gel electrophoresis (PFGE) has been designed to overcome these problems. Rare-cutting restriction endonucleases are utilised to cleave the chromosomal DNA into a small number of fragments which can be up to 10mb long. These large fragments are subjected to electrophoresis whereby the electric field is alternated between spatially distinct pairs of electrodes which force the migrating fragments through an agarose gel. The DNA fragments continuously change their direction of migration and separation is based on the fact that larger sections of DNA change direction more slowly than smaller ones, resulting in separation by retardation of DNA fragments by size (Towner and Cockayne, 1993). There are several types of pulsed-field systems currently available. The most popular is based on the clamped homogenous electric field technique (CHEF). The gel is placed in a horizontal mode submerged in a buffer and an electric field is generated on all points of the gel, with an optimal 120° angle of alternating pulses by a hexagonal structure of 24 electrodes. The resulting gel is stained with ethidium bromide and directly visualized using a UV transilluminator to observe the DNA 'fingerprints'. Trial experiments to determine the optimum conditions for high resolution is required for each microorganism under investigation. In order to efficiently separate a wide range of fragment sizes it is standard practice to increase the pulse time over the duration of the experiment. This is referred to as 'ramping'. Since chromosome-sized DNA is prone to random shearing during the preparation process, the DNA is extracted from intact cells embedded in low-melting point agarose (LMT) plugs. A culture of the microorganism under investigation is mixed with the LMT and is decanted into a mould after which the plugs are exposed to detergents, enzymes and EDTA to lyse the cells, remove membranes, proteins and RNA and inactivate cellular nucleases *in situ*. A portion of the plug is then subjected to restriction with a specific restriction endonuclease prior to electrophoresis

.

All species of bacteria are typeable by PFGE however, for some there are complications in the DNA isolation process. PFGE is one of the most discriminatory and reproducible typing methods available but is technically and financially demanding requiring specialist equipment (Schmitz *et al.*, 1998; Bannerman *et al.*, 1995; Sloos *et al.*, 2000). Macrorestriction profiles generated by PFGE are easy to interpret since the publication of consensus guidelines for correlating variations in restriction profiles with epidemiological relatedness (Tenover *et al.*, 1995). These guidelines take into consideration that single

band differences may be due to genetic switching mechanisms and may not actually represent different strains. For cross-laboratory comparisons of bacterial strains, the protocol for PFGE should be standardised (Van Belkum *et al.*, 1998). An example of this is the Harmonisation guidelines produced for typing MRSA in a trial undertaken by ten European laboratories (Murchan *et al.*, 2003). Pulsed-field gel electrophoresis is a time consuming process, however modifications in methodology have been developed to reduce the total time (Chang and Chui, 1998; Matushek *et al.*, 1996; Leonard and Carroll, 1997; Frumin *et al.*, 2001; Flanagan *et al.*, 1989; Birren and Lai, 1994; Goering and Winters, 1992). Despite the disadvantages associated with PFGE it is considered to be the gold standard of molecular typing methods.

1.5.2.3.2.5 Polymerase chain reaction (PCR) typing

The polymerase chain reaction (PCR) has the ability to produce millions of copies of a particular DNA segment within 3-4 hr (Tenover *et al.*, 1997). Template DNA is required (or RNA if a reverse transcriptase is initially used), 2 oligonucleotide primers which flank the template DNA sequences to be amplified and a thermostable DNA polymerase. Each cycle in a typical PCR assay consists of a heat denaturation phase whereby double stranded DNA is melted into single strands; an annealing phase where the primers bind to the target sequences in the single strands; and an extension phase in which the DNA synthesis begins from the primers along each stand of template DNA, generating 2 new double-stranded copies of the original template. After 30 of these cycles, a single piece of template DNA can be amplified into 1 billion copies (Tenover *et al.*, 1997). The PCR is a less demanding and more rapid technique than most molecular typing methods and has been used extensively for diagnostic purposes. This technique is easy to master and numerous isolates can be typed in a relatively short period of time. However, this method has the disadvantage of reduced reproducibility and discriminatory power.

1.5.2.3.2.5.1 *Random amplification of polymorphic DNA (RAPD)*

Random amplification of polymorphic DNA (RAPD), also referred to as arbitrarily primed-PCR (AP-PCR) is a variation of the PCR technique. This method uses a single short primer (normally 10 base pairs) that is not targeted towards any specific DNA sequence. Instead, at low annealing temperatures the primer will hybridise at several random chromosomal locations and initiate DNA synthesis, it is assumed that there will always be some areas of DNA sufficiently similar to the primers for them to anneal. If a copy of the primer binds on one strand in close proximity to one that has annealed on the opposite strand of DNA, a DNA fragment will be synthesized and amplified. The PCR

products will contain a variety of different sized DNA fragments which are then visualized by agarose gel electrophoresis. This technique is the subject of much debate and it has been suggested that it is much more susceptible to technical variation than standard PCR which uses primers specifically directed at known sequences. For example, the pH of the buffer used and the reaction temperatures may influence the efficiency with which the primers initiate DNA synthesis at a particular site i.e. many priming events might be the result of imperfect hybridization between the primer and the target site (van Belkum, 1994; van Belkum *et al.*, 1995; Tyler *et al.*, 1997; Olive and Bean, 1999). Therefore the same strain on different occasions may be amplified into differing numbers of copies, changing the intensity of the banding patterns on the agarose gel and making it difficult to interpret. Thus isolates that are to be directly compared should be subjected to the same PCR reaction and run on the same agarose gel, this makes it difficult for inter-laboratory comparisons to be made (van Belkum *et al.*, 1995). As amplification of minute amounts of DNA can take place with this method it is important that steps are taken to prevent contamination of the reaction mixture. The interpretation of these results are further disabled by the fact that there are no standard guidelines for the interpretation of results acquired using this technique (Tenover *et al.*, 1997). The discriminatory power of this technique is also being questioned (Saulnier *et al.*, 1993; Schmitz *et al.*, 1998; Raimundo *et al.*, 2002). However the level of discriminatory power depends heavily on the number and sequences of the primer employed (Weller, 2000). Despite these disadvantages associated with the technique many investigators have found RAPD to have promising results when typing CoNS (Nouwen *et al.*, 1998) and MRSA (Tambic *et al.*, 1997; Andrasevic *et al.*, 1999; Fung *et al.*, 2001).

1.5.2.3.3 Interpretation of typing results and relatedness of isolates

One method of determining the level of similarity between microbial isolates is by conducting pairwise comparisons of banding profiles by using the Dice coefficient (S_D). The Dice coefficient is expressed as:

$$S_D = \frac{2ni}{(n_A + n_B)}$$

n_A and n_B are the numbers of fragments in patterns $_A$ and $_B$ and ni is the number of common bands. Interpretation of profiles can be performed using computer software, for example

GelCompar™ (Applied Mathematics, Belgium). Gel analysis data can be transferred to this database programme to allow comparison of isolates by calculation of the Dice coefficient. Isolates are then clustered together based on their profiles by the unweighted pair group method of arithmetic averages (UPGMA) to construct a dendrogram.

1.6 Auditing catheter-related infection

Audit plays a crucial role in the prevention of CR-BSI as it provides invaluable information on infection rates, current clinical practice, determines if current guidelines are effective and highlights areas where weaknesses can be improved upon (Elliott *et al.*, 1995). Indeed, several CVC audit programmes have been documented. Gowardman and colleagues (1998) used a 1-year prospective audit to a) determine the incidence of central CR-BSI, b) identify patient and/or CVC characteristics associated with CR-BSI and c) assess the efficacy of the AOLC method for detecting CR-BSI. Their findings, unfortunately did not identify any risk factors for CR-BSI, however it was demonstrated that the AOLC test, used alone, was an unhelpful tool to diagnose CR-BSI.

Other investigators, however demonstrated that retrospective audit of CVC care was able to identify risk factors associated with CR-BSI. Indeed, the results of a study by Herbst (1978) demonstrated that inexperience of the physician inserting the CVC was an important cause of CVC complications. In addition, Lawrance (1994) described how hand washing was identified by audit as the single most effective means of improving CVC care on a BMT unit.

1.7 Prevention of CRI

There are many strategies which may be employed to prevent CRI. Good clinical practice during catheter insertion and care of the CVC post insertion may facilitate to prevent CRI. Furthermore, many elements of the CVC itself will also influence CRI including its design and the polymer it's made with.

1.7.1 *During Catheter Insertion*

1.7.1.1 Aseptic technique

During CVC insertion, full barrier precautions be implemented. Prior to insertion, the healthcare worker should disinfect their hands for 3-4 min with chlorhexidine or povidone-iodine (Elliott *et al.*, 1997) and wear sterile gloves, a gown, a cap and a mask. Large sterile drapes should also be used as CVC insertion is a surgical procedure (O'Grady *et al.*, 2002).

1.7.1.2 Prophylactic antibiotics

The use of prophylactic antibiotics at the time of catheter insertion has been widely investigated. McKee and colleagues (1985) discovered that the prophylactic use of vancomycin in patients referred for intravenous nutrition had no significant effect on catheter infection rates. Conversely, Lim and co-workers (1991) found that the use of prophylactic teicoplanin reduced early Hickman catheter-related sepsis in patients receiving chemotherapy for haematological malignancies. In a study by Barriga and colleagues (1997), a vancomycin solution was found to prevent bacteraemia caused by vancomycin sensitive microorganisms when used prophylactically for tunneled CVC insertion in non-neutropaenic patients. The use of oxacillin prophylactically for CVC insertion was also found to be of benefit in a study of patients undergoing immunotherapy with interleukin-2 (Bock *et al.*, 1990). However, some antibiotic treatments have side effects such as nausea and it is believed that the widespread use of antibiotics may add to the problem of emerging antibiotic resistance.

1.7.1.3 Skin preparation prior to CVC insertion

Microorganisms may contaminate the CVC by impaction during insertion. Therefore it is fundamental to thoroughly disinfect the skin of the potential insertion site. Maki and colleagues (1991) investigated the efficacy of 3 antiseptics for insertion site disinfection; 2% aqueous chlorhexidine, 10% povidone iodine and 70% alcohol. The results indicated

that chlorhexidine was associated with 2.3 cases of CRI per 100 patients compared to 7.1 and 9.3 for alcohol and povidone iodine respectively.

1.7.1.4 Method of CVC Insertion

The Seldinger technique which is a method used for inserting CVC may reduce the risk of CRI due to limited handling of the IV device and a reduction in blood leakage at the insertion site. Indeed, a study by Ahmed and Mohyuddin (1998) investigated the effects of percutaneous and surgical cut-down insertion techniques of Hickman catheters in patients with haematological malignancies. Excessive bleeding and haematoma formation occurred in a significantly higher proportion of patients in the cut-down group (61% and 41% respectively) compared to patients in the percutaneous group (8% and 0% respectively) which may increase the incidence of CRI. Indeed, exit site infection and septicaemia were observed in 26% and 41% respectively of patients in the cut-down group compared to 7% and 19% in the subcutaneous insertion group.

1.7.1.5 Site and position of CVC insertion

Numerous studies indicate that CVC inserted in the internal jugular vein have a higher incidence of CRI than those inserted in the subclavian vein. This is due to increased skin colonisation rate associated with the insertion site of the internal jugular vein (Jansen, 1997). Furthermore, proximity of the insertion site to oropharyngeal secretions may promote CRI (Pittet, 1994). Insertion into the subclavian veins is also associated with a reduced rate of CRI compared to CVC inserted into the femoral veins (Merrer *et al.*, 2001).

1.7.1.6 Type of CVC insertion

Long-term catheters, for example, Broviacs and Hickmans carry an associated risk of CRI due to the duration of the catheterisation and also ineffective outpatient catheter care. Attempts have been made to reduce the level of infections associated with these devices such as tunneling and the addition of a collagen cuff. The tunneling and cuff combined are designed to reduce the microbial migration down the catheter from the skin. Tunneling has been found to reduce the risk of infectious complications in CVC placed in the femoral (Timsit *et al.*, 1999; Nahum *et al.*, 2002) and internal jugular veins (Timsit *et al.*, 1996). However a study by Andrivet and colleagues (1994) demonstrated that there was no clinical benefit of subcutaneous tunneling in immunocompromised patients.

1.7.2 *Catheter care*

1.7.2.1 Specialised care teams

Teams responsible for catheter insertion and care may be appropriate in a hospital setting with patients at high risk of infectious or other complications. The use of teams whose job role is focused solely on this area has been suggested to have significant cost benefits when compared to the costs associated with CRI (Elliott and Tebbs, 1998). In addition, educational programmes aimed at reducing CRI have proved beneficial. In a trial by Coopersmith and colleagues (2002), registered nurses were required to take a self-study module on risk factors and practice modifications involved in CRI. This resulted in a reduction of CR-BSI by 66%. This educational approach has also been assessed with medical students and physicians and has also resulted in a decrease of CRI by 28% (Sherertz and *et al.*, 2000).

1.7.2.2 Dressings

Dressings must be water permeable otherwise the accumulation of moisture leads to overgrowth of microflora. Conventional gauze and tape dressings have traditionally been associated with reduced levels of CRI (Hoffmann *et al.*, 1992). However rates of colonisation of the CVC and risk of CRI are similar for gauze and transparent dressings (Maki *et al.*, 1994). One advantage of using transparent dressings is that it allows visual inspection of the exit site without the need to remove the dressing and further increase the risk of infection. In a study by Wille and colleagues (1993) a moderately permeable dressing, 'Opsite' was compared to a highly permeable 'Opsite IV3000'. The investigation demonstrated that there was no benefit to using the 'IV3000' as far as infection rates were concerned, however, the latter was found to be easier to handle and apply. The 'IV3000' was also compared to the less-permeable 'Tegaderm' dressing in a study by Reynolds and co-workers (1997). Similarly, there was no added advantage to using the more permeable dressing.

Antiseptic incorporated dressings have also been produced. However, Maki and Ringer (1987) concluded that they do not reduce microbial colonisation. In a prospective, randomised study, later it was shown that the use of a chlorhexidine-impregnated sponge dressing (Biopatch) at the site of CVC and arterial catheters insertion led to a 3-fold reduction in CR-BSI (Maki *et al.*, 2000). There is also debate as to how often CVC dressings should be changed. Engervall and co-workers (1995) found during a clinical trial using patients with haematological malignancies that Tegaderm dressings should be changed twice rather than once a week to reduce the risk of CRI.

1.7.2.3 Insertion site care (antibiotics and topical antiseptics)

Care of the exit site of the CVC is essential to prevent CRI. Antiseptics including 10% povidone iodine, 70% isopropanol and 2% aqueous chlorhexidine have all been associated with reducing the number of cases of CRI (Maki *et al.*, 1991). In addition, a topical antiseptic containing a combination of 0.25% chlorhexidine gluconate, 0.025% benzalkonium chloride and 4% benzyl alcohol has been evaluated clinically and compared to 10% povidone-iodine in reducing CRI (Mimoz *et al.*, 1996). This antiseptic was associated with a significantly lower level of CRI and was effective in reducing the number of infections due to Gram-positive microorganisms.

Antimicrobial ointments have also been shown to reduce bacterial colonisation and potential cases of CRI, however their use has been associated with a higher incidence of CRI due to *Candida* spp. (Flowers *et al.*, 1989). Mupirocin has been illustrated to reduce catheter colonisation and subsequent CRS, however increased bacterial resistance to this antibiotic ointment is an associated disadvantage (Zakrzewska-Bode *et al.*, 1995). Furthermore, the use of topical mupirocin ointment at CVC exit sites may be associated with a deleterious effect on the polyurethane catheter integrity (Riu *et al.*, 1998). Bacitracin, neomycin and polymyxin have been shown to delay microbial colonisation of CVC, but do not reduce the risk of CRS, which could be due to resistant bacteria causing the CRI (Maki and Band., 1981).

1.7.2.4 CVC patency

In order to maintain catheter patency and reduce CRI, it is recommended that the CVC be flushed with a solution containing heparin or low dose warfarin in patients with long term CVC (O'Grady *et al.*, 2002). Preservatives including chlorbutol are often present in flush solutions and are also thought to reduce the risk of associated infection in addition to maintaining patency (Elliott and Curran, 1989). Antibiotic solutions have been suggested to reduce the risk of infection (Henrickson, 2000), however excessive use of antibiotics is not encouraged due to the emergence of resistance.

1.7.2.5 Duration of catheterisation

It is well documented that there is an increased risk of CRI with increasing duration of central venous catheterisation. Indeed, a recent hospital infection surveillance programme undertaken by McLaws and Taylor (2003) illustrated that a significantly higher proportion of patients whose CVC was *in situ* for $\geq$6 days developed a CR-BSI compared with the proportion of patients whose CVC was *in situ* for $\leq$5 days. In order to reduce the risk of

CRI it is therefore recommended that CVC are removed immediately at the end of their clinical use.

1.7.2.6 Routine replacement of catheters

Routine replacement of CVC does not reduce the risk of CRI (Cobb *et al.*, 1992) Furthermore, replacement CVC may also become contaminated during insertion. The method by which CVC are exchanged needs to be considered. Indeed, animal studies have demonstrated that guide wire exchange of deliberately infected CVC may result in rapid colonisation of the new catheter, with subsequent endocarditis and metastatic pneumonia (Olson *et al.*, 1992).

1.7.2.7 Catheter hub decontamination

Several methods have been developed in order to reduce microbial contamination of CVC hubs including; frequent heating of metallic hubs with an electrical soldering iron (Sandstedt *et al.*, 1985), covering the hub with an iodine gauze (Sitges-Serra *et al.*, 1985) and the use of disinfectants to clean hubs (Salzman *et al.*, 1992). However, with the exception of using disinfectants to clean hubs, none of these methods have been widely implemented in the clinical setting. There are limited clinical trials which have evaluated the most appropriate disinfectant for cleaning intravenous hubs. In a study by Salzman *et al* (1992) the efficacy of 1% (w/v) aqueous chlorhexidine, 1% (w/v) chlorhexidine in 70% (v/v) ethanol, 97% (v/v) ethanol and 0.9% (w/v) saline was assessed. It was demonstrated that all methods of cleaning were successful in reducing microbial counts, particularly the alcohol-based disinfectants. 1% (w/v) aqueous chlorhexidine demonstrated less efficacy in the study however, a longer dwell time may have improved the activity. This, however, may be impractical in the clinical setting. Lucet *et al* (2000) described the use of hub 'protection' boxes for preventing microbial contamination of hubs. The study involved a clinical comparison of microbial hub contamination rates in patients who either had protection boxes or closed connectors. There was no significant difference in the level of microbial contamination of hubs associated with either device however the study was confounded by the lack of a control group with standard hubs alone.

1.7.3 *Intravascular Device*

1.7.3.1 Polymers

The type of material from which CVC are manufactured is an important factor in preventing CRI. The material should be non-thrombogenic and non-antigenic (Elliott, 1997). The most common materials used for catheter production are silicone, polyurethane, polyvinyl chloride (PVC), Teflon and Vialon. In a study by Lopez-Lopez and colleagues (1991) polyurethane was associated with the lowest levels of *S. epidermidis* adherence followed by vialon, Teflon, silicone and PVC.

The use of a hydrophilic coating on CVC has also been associated with lower levels of CRI (Tebbs and Elliott, 1994). Many microbial cells are hydrophobic and bind to hydrophobic surfaces including hydrophobic silicone catheters, thus encouraging colonisation and infection. If a hydrophilic coating is incorporated the microorganisms are less likely to adhere. Indeed, Tebbs and colleagues (1994) showed that adherence of *S. epidermidis* to Hydrocath® CVC; a hydrophilic catheter, was significantly reduced in comparison to standard polyurethane catheters.

1.7.3.2 Catheter surface topography

Topography of CVC will also influence microbial colonisation and infection. A smoother surface topography results in decreased microbial attachment compared to CVC with surface irregularities (Cheesebrough *et al.*, 1985). Indeed, Tebbs and co-workers (1994) used scanning electron microscopy and laser profilometry to demonstrate a reduction in adherence of *S. epidermidis* to catheters with a smooth topography. Hydromer® CVC; have since been developed which have a smooth hydrophilic coating and have been shown to reduce microbial colonization. Furthermore, a Teflon® biliary stent has been investigated and was also found to be associated with a reduction in polymer colonisation (Rees *et al.*, 1998).

1.7.3.3 Heparin bound catheters

The addition of heparin to CVC reduces the risk of thrombosis after catheter insertion. Heparin is bound to CVC by the quaternary ammonium compound benzalkonium chloride (BZC). Although heparin-bound pulmonary artery catheters have been associated with a reduced rate of CRI, it is thought that BZC used to bind the heparin is the active compound (Mermel, 1993).

1.7.3.4 Silver incorporated catheters

Silver has antimicrobial activity with little toxicological effect. It has been impregnated into numerous medical devices since it was first investigated for the prevention of CRI by Maki and colleagues (1988). It was incorporated into a subcutaneous cuff composed of a biodegradable collagen matrix and was positioned below the CVC insertion site. The silver cuff was found to reduce CRI but is not widely used in the clinical setting. Silver has since been incorporated into the polymer of the CVC itself and has shown to reduce the incidence of CRI by approximately 50% (Goldschmidt *et al.*, 1995). Bach and colleagues (1999) demonstrated that the silver coating of catheters did not reduce catheter colonization in the ICU setting. A silver iontophoretic catheter has also been shown to reduce microbial colonisation in both *in vivo* and *in vitro* investigations (Raad *et al.*, 1996). Another silver containing CVC has been developed which also contains carbon and platinum. In a recent study by Ranucci and co-workers (2003), this catheter reduced catheter colonisation due to microorganisms when compared to a BZC CVC, however did not reach statistical significance when comparing the rates of CR-BSI.

1.7.3.5 Antibiotic incorporated catheters

Intravascular catheters with antimicrobial substances incorporated into the polymer have existed in clinical practice for many years and several studies have been undertaken to determine their efficacy. In 1985, Trooskin and colleagues investigated the effects of bonding penicillin to polyethylene catheters in a CRI rat model and demonstrated a reduced rate of infection in models with antibiotic catheters compared to controls. Furthermore, the efficacy of catheters containing vancomycin, clindamycin, novobiocin and minocycline alone and in combination with rifampin was compared in an *in vitro* model of CRI (Raad *et al.*, 1995). The results illustrated that the combination of minocycline and rifampin had a synergenistic effect against yeasts, Gram-negative microorganisms and slime-producing *S. epidermidis*. In further studies this antimicrobial combination was found to be more effective at preventing infection than standard and antiseptic coated catheters (Darouiche *et al.*, 1999; Raad *et al.*, 1997; Veenstra *et al.*, 2000).

The coating of a Hydromer® catheter with the glycopeptide, teicoplanin has also been investigated (Bach *et al.*, 1996; Jansen *et al.*, 1992). However the teicoplanin antibiotic coating was retained only in the short term and therefore may only be suitable for preventing early-onset CRI. Cefazolin-bonded CVC have been evaluated in the ICU setting and were shown to be associated with a significant reduction in infectious

complications (Kamal *et al.*, 1998). However, the main problem associated with the use of antimicrobial incorporated catheters in clinical practice is the risk of emerging resistance and the selection of resistant microorganisms. The use of antibiotic incorporated catheters should therefore be restricted.

1.7.3.6 Antiseptic incorporated catheters

Antiseptics including BZC and chlorhexidine have been incorporated into CVC and offer an alternative approach to antibiotics. Benzalkonium chloride is widely used as a preservative in contact lens solutions but is also used as an antiseptic for pre-operative skin disinfection.

The antiseptic properties of BZC when incorporated into the internal and external surfaces of the Hydrocath CVC have been investigated *in vitro* (Tebbs and Elliott, 1993; Tebbs and Elliott, 1994). Bacterial adherence to the BZC catheters was significantly lower than the control catheters. Furthermore, Gram-negative microorganisms were shown to be less adherent than Gram-positive microorganisms and *Candida* spp. A significant reduction in microbial colonization rates on BZC catheters has also been demonstrated in clinical practice (Moss *et al.*, 2000; Jaeger *et al.*, 2001).

A further antiseptic bonded CVC which has been fully investigated in clinical practice is a polyurethane catheter with chlorhexidine gluconate and silver sulfadiazine bonded to the external surface. Maki *et al* (1997) demonstrated in a randomised clinical trial that these CVC reduce the incidence of CRI, extends the duration for which CVC can be left in place and are less likely to become colonised as a result of contamination during catheter removal. These results were also reflected in a study by Collin (1999). However, a clinical trial conveyed by Heard and colleagues (1998) demonstrated that the use of this CVC reduced the incidence of bacterial growth on catheter segments but did not reduce the rate of CR-BSI. In comparison to heparin-BZC bonded catheters, the silver sulfadiazine-chlorhexidine catheter has been shown to retain its antimicrobial properties for longer durations (Mermel *et al.*, 1993).

Iodine (Jansen *et al.*, 1992; Kristinsson *et al.*, 1991) and irgasan (Kingston *et al.*, 1992) have also been assessed for their antiseptic properties when incorporated into CVC. Both antiseptics were shown to have initial activity however, the efficacy is not sustained over time.

1.7.3.7 Electrical catheters

Application of an electrical current to a carbon impregnated catheter has been shown to reduce microbial colonisation *in vitro* (Crocker *et al.*, 1992). As most microorganisms carry a negative surface charge associated with their cell wall, application of an additional negative charge, for example, from an electrical CVC, will prevent microorganisms from attaching and thus colonising the CVC. The antimicrobial activity associated with an electrical catheter is also thought to arise as a result of hydrogen peroxide production and free chlorine formed by electrolysis (Lui *et al.*, 1993). A major advantage of using electricity to prevent CRI is that the problem of antimicrobial resistance associated with the use of antibiotics is avoided. Furthermore, application of electricity may have clinical applications in other areas, for example, in preventing infection associated with prosthetic devices including hips and knees (Elliott and Tebbs, 1998).

1.7.3.8 Multi-lumen vs. single lumen catheters

Contaminated hubs are a major source of CR-BSI via the internal lumen, particularly in long-term CVC. The number of lumens associated with the CVC may also be linked with increased rates of CR-BSI (Raad *et al.*, 1993). However, several studies have been undertaken that have demonstrated that there is no additional risk of CR-BSI associated with multi-lumen CVC (Lee *et al.*, 1988; Gupta *et al.*,1995; Reed *et al.*, 1995). The use of a single lumen CVC could result in the increased numbers of peripheral catheters being employed, which may indirectly indicate that the use of single lumen catheters pose as much of a risk of CRI as the use of multi lumen CVC (Raad *et al.*, 1993). Despite this it is recommended that single lumen CVC should be used unless multiple access routes are clearly required.

1.7.3.9 Hub modifications

An example of a hub modification developed to reduce CR-BSI acquired by the intraluminal route is the antiseptic-filled hub. Leon and co-workers (2003) and Segura and colleagues (1996) found that hubs containing a chamber filled with 3% iodinated alcohol reduced endoluminal colonization and the rate of CR-BSI was also reduced 4-fold.

1.7.3.10 Needleless Connectors

Needleless connectors (NC) allow direct access to IV catheters. The design of NC is based on a male luer which attaches to a female luer such as a syringe or administration set. These devices were originally introduced to reduce the incidence of needlestick injuries which impose a serious financial burden on the health service due to laboratory

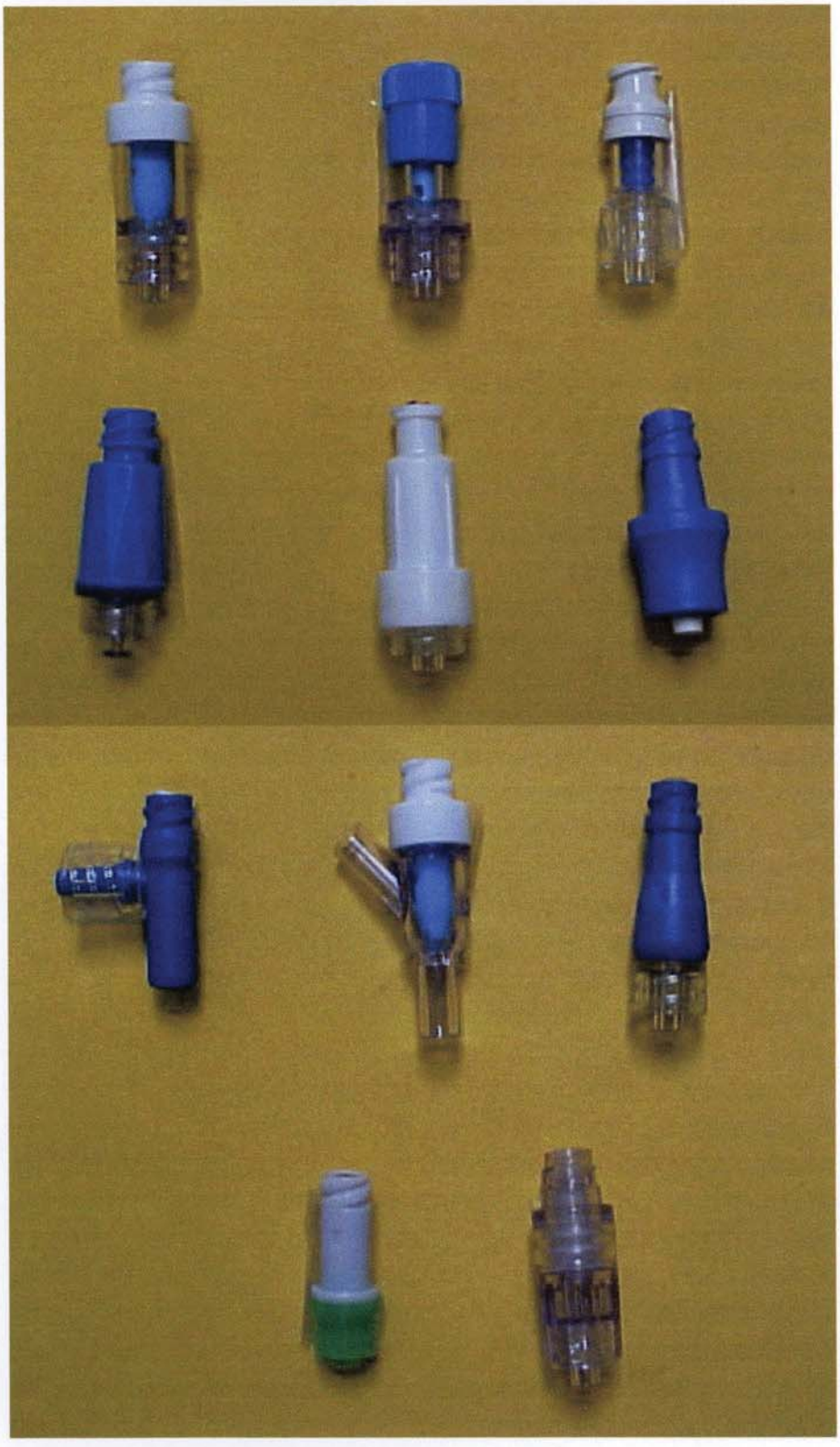
investigations, prophylactic treatment and potential legal compensatory costs (Yassi *et al.*, 1995). Indeed, the benefits of these devices to healthcare workers is now well documented (Orenstein, 1995).

The potential risk of microbial contamination and infection associated with NC is at present, unclear. Needleless connectors automatically seal upon removal of the female luer and it is thought that this closed-system design may reduce manipulation time and create a barrier against the entry of microorganisms into the catheter. A wide range of NC are available for use in clinical practice (figure 1-6).

The SafSite® NC (Braun Medical Inc, USA) was introduced onto a paediatric haematology/oncology home health care programme and a retrospective cohort study of the rates of CR-BSI associated with the introduction of the device was conveyed by Kellerman and colleagues (1996). The study demonstrated that the number of cases of CR-BSI increased by 80% following the introduction of this device. An increase in CR-BSI was also observed in another retrospective cohort study following implementation of the SafSite® NC. The increase in the number of cases of CR-BSI occurred shortly after the implementation of the NC and was associated with deviations from the manufacturer's recommendations with regard to care of the device (Cookson *et al.*, 1998). Conversely, 2 other clinical trials which evaluated the infection risk associated with the use of the SafSite® for IV therapy, demonstrated that its use was not associated with an increase in the number of cases of CR-BSI (Mendelson *et al.*, 1998; Zafar *et al.*, 1999).

Danzig *et al.* (1995) evaluated the infection risk associated with the Interlink® NC (Baxter Healthcare, USA). Use of the Interlink® NC in clinical practice was shown to be an additional risk factor associated with CR-BSI and was further increased if used in combination with total parenteral nutrition. *In vitro* assessment of the Interlink® NC however, has demonstrated that there is no difference in the rate of microbial contamination of the fluid pathway when compared to conventional intravenous access systems (Arduino *et al.*, 1997; Luebke *et al.*, 1998). These results have been confirmed in an *in vivo* investigation undertaken by Adams and colleagues (1993).

Figure 1-8 Needleless connectors currently available for use in clinical practice



From left to right, top to bottom; NAC™ PRN (Kippmed®, Porex), Safsite® (B. Braun Medical Inc), SmartSite® (Alaris medical systems), PosiFlow® (Becton Dickinson Infusion Systems Inc), Ultrasite® (B. Braun Medical Inc), Clave® (ICU Medical Inc), CLC2000™ (ICU Medical Inc), NAC™ y-site (Kippmed®, Porex), microClave® (ICU Medical Inc), Bionector® (Vygon UK Ltd) and Clearlink® (Baxter Healthcare corp.).

The Clave® NC (ICU Medical Inc, USA) has been investigated in several studies. Seymour *et al.* (2000) assessed the infection risk associated with this device in a prospective clinical trial by recruiting patients who required a CVC prior to cardiac surgery. Microbial contamination rates of 3-way tap entry ports with either standard luer caps or the Clave® attached were compared after 72 hr *in vivo*. The microbial contamination rates of the entry ports in both groups were similar and it was concluded that the Clave®, if properly disinfected posed no additional risk in the development of a CR-BSI. Lucet and colleagues (2000) also reported no increased risk of infection associated with the use of the Clave® in another clinical trial undertaken in the ICU. *In vitro* investigations undertaken by Brown *et al.* (1996) demonstrated that the Clave® may reduce the microbial contamination of catheters via the intraluminal route if disinfected inadequately. In a clinical trial undertaken by Bouza and colleagues (2003) it was demonstrated that the Clave® offered significant protection from catheter-tip and hub colonisation. However, Donlan and co workers (2000) examined the internal surface of the Clave® to sample for the presence of biofilms. Sixty-three percent of NC collected from the Hickman lines of patients on a bone marrow transplant unit were found to have detectable levels of attached bacteria indicating that the Clave® may indeed act as a reservoir of pathogens responsible for CR-BSI.

The rates of CR-BSI associated with the SafSite®, Interlink® and Clave® were compared in a clinical trial conveyed by Do *et al* (1999). The Interlink® was shown to be associated with the lowest incidence of CR-BSI. The investigation also demonstrated that the incidence of CR-BSI decreased as the frequency of changing the NC increased suggesting that the frequent replacement of NC should be encouraged.

1.8 Treatment of CRI

1.8.1 *Catheter removal*

There are no specific guidelines relating to the removal of CVC once they become infected as patient factors and clinical circumstance will influence the decision. In a study undertaken by Raad and colleagues (1992) it was demonstrated that catheter retention was associated with a higher risk of recurrence of the CR-BSI. However, several issues need to be taken into account by the clinician when deciding if the CVC is to be salvaged, these include; if the catheter would be difficult to replace, the blood cultures are negative after 48-72 hrs of antimicrobial therapy, there are no signs of tunnel/port infection, no signs of metastatic complications, the infection relapses after discontinuation of antibiotics or the infection is caused by *Candida* spp., *Aspergillus*, other fungi, *S. aureus*, *C. jeikeium*, *Bacillus* spp., *Pseudomonas* spp., and rapidly growing mycobacteria (Rodriguez-Bano, 2002; Bouza *et al.*, 2002). Other considerations are the cost to the national health service and the fact that 75% of CVC are removed unnecessarily (Eggiman and Pittet, 2002).

1.8.2 *Systemic antimicrobial therapy*

Antimicrobial therapy for CR-BSI can be administered immediately on an empirical basis or more specifically after obtaining microbiology culture results. Empirical therapy should be considered when the catheter is to be salvaged or after removal when; the catheter is a CVC, the patient is septic, the patient has suppurative thrombophlebitis or metastatic complications, the patient is immunosuppressed or if the patient has an endovascular prosthesis (Bouza *et al.*, 2002). Furthermore, empirical therapy should be given intravenously through the infected catheter if it is to be left *in situ*. Empirical therapy should include antimicrobial cover against Gram-positive microorganisms, in particular the staphylococci (Rodriguez-Bano, 2002). Vancomycin is the drug of choice for Gram-positive cover as it is active against *S. aureus* and CoNS which are often resistant to many antibiotics. For immunosuppressed patients, teicoplanin has been found to be a well-tolerated, effective alternative to vancomycin (Webster *et al.*, 1986; Smith *et al.*, 1989). However intermediate resistance is more frequently reported in CoNS for teicoplanin than vancomycin (Cercenado *et al.*, 1996). The streptogramin, quinupristin/dalfopristin (Synercid®) may be used as an alternative to a glycopeptide in the treatment of CR-BSI due to staphylococci or enterococci.

Gram-negative cover is also essential in empirical therapy for immunosuppressed patients, burns patients, individuals with severe sepsis and when a contaminated infusate is the

suspected source of the CR-BSI. Examples of antimicrobial agents frequently used for Gram-negative empirical treatment are; aztreonam, third-generation cephalosporins, forth generation cephalosporins, aminoglycosides, piperacillin/tazobactam and the quinolones (Rodriguez-Bano, 2002). In high risk patients including the immunosuppressed or patients on broad-spectrum antibiotics, empirical treatment against *Candida* spp should also be considered. Common antifungals for treatment of CR-BSI include fluconazole and amphotericin B. Once results of microbiological tests are available, antimicrobial therapy may be modified and directed to treat the infection more efficiently and reduce the risk of inappropriate antibiotic use which may lead to development of resistance.

1.8.3 *Antibiotic locks*

Catheters which need to be salvaged may be treated with the antibiotic lock technique. This technique involves filling the catheter lumen with a high concentration of an antibiotic to which the infecting microorganism is sensitive, and leaving it to dwell whilst the catheter is not in use. Several studies have demonstrated that CVC can be salvaged in this manner (Rubin *et al.*, 1999; Berrington and Gould, 2001; Bouza *et al.*, 2002), however further work is required to investigate appropriate antibiotic concentrations and dwell times (Carratala, 2002).

1.9 Aims

Aims of the current study:

1) To investigate the epidemiology of microorganisms associated with CR-BSI in cardiothoracic surgery patients by PFGE.

2) To characterise strains of CoNS recovered from SCT patients with CR-BSI by genotypic and phenotypic methods.

3) To characterise strains of *S. aureus* recovered from cardiothoracic surgery patients with sternal SSI by genotypic and phenotypic methods.

4) To investigate if multiple strains of CoNS are implicated in CR-BSI and whether current phenotypic characterisation methods facilitate the diagnosis of this infection.

5) To determine if the lipid S ELISA facilitates the diagnosis of CR-BSI in haematology patients.

6) To determine if the lipid S ELISA facilitates the diagnosis of sternal SSI in cardiothoracic surgery patients.

7) To assess the potential of the lipid S ELISA and AOLC in the diagnosis of CR-BSI.

8) To evaluate the ability of 'Meditrend', a novel computer database to identify rates of and risk factors associated with CRI.

9) To undertake a randomised, prospective clinical evaluation of the infection risk associated with the PosiFlow® and Clave® NC and to evaluate the efficacy of 0.5% (w/v) chlorhexidine in 70% (v/v) industrial methylated spirit (IMS), 10% (w/v) aqueous povidone-iodine and 70% (v/v) IMS in the disinfection of the devices.

2. Assessment of genetic similarity amongst multiple CoNS recovered from the skin and explanted CVC of cardiothoracic surgery patients

2.1 Introduction

Microorganisms responsible for CR-BSI may gain access to the catheter via a number of routes (chapter 1, section 1.3). There has much debate over which of the sources is responsible for the highest proportion of CR-BSI. Mermel and co-workers (1991), using plasmid profile analysis found that 80% of infected Swan-Ganz catheters showed concordance with microorganisms cultured from the skin of the insertion site compared to just 17% with a contaminated hub. However, in a study which used multilocus enzyme electrophoresis to determine the source of infection in neonates with persistent CR-BSI it was found that the catheter hub is a more likely source of colonisation that the exit site. Indeed, the catheter hub is becoming more widely recognised as a major source of microorganisms which cause CR-BSI (Sitges-Serra *et al.*, 1984; Linares *et al.*, 1985; Sherertz, 1997). It is also widely accepted that most episodes of CR-BSI which occur shortly after catheter insertion are caused by microorganisms which migrate down the extraluminal surface from the skin at the insertion site whereas CR-BSI in catheters which are in place for extended periods i.e. >30 days, are due microorganisms migrating down the intraluminal surface of the catheter from a contaminated hub (Salzman and Rubin, 1995).

The molecular typing of microorganisms in order to track the source of infection is crucial in the prevention of nosocomial infection. Indeed, de Cicco and co-workers (1989) discovered that the source of CoNS colonising CVC in patients receiving parenteral nutrition was by contamination of the hub with microbial flora found on nurses' skin. Also, Van Belkum and colleagues (1995), by using PFGE and RAPD investigated the source of a clone of CoNS which was repeatedly recovered from blood taken at the end of cardiopulmonary bypass.

Aims of the study;

a) To characterise CoNS recovered from the skin and explanted CVC of cardiothoracic surgery patients by pulsed-field gel electrophoresis.

b) To investigate the genetic population of CoNS recovered from the skin and explanted CVC of cardiothoracic surgery patients.

c) To determine the genetic relatedness of CoNS recovered from patients' skin and components of the explanted CVC.

d) To investigate the skin insertion site as a source of CVC colonisation/contamination.

2.2 Materials and methods

2.2.1 Patients

Patients who underwent coronary artery bypass grafting with post-operative admission to the cardiac surgery ICU, University Hospital Birmingham NHS Trust were recruited into the study. Ethical committee approval was sought for patients requiring a CVC as part of their clinical management to be recruited into the study (see chapter 8, section 8.2.1). All patients recruited had a CVC as part of their clinical management. The CVC insertion site of each patient was swabbed prior to disinfection with a transport swab (Charcoal amies, Bibby Sterilin, Stone, U.K.). A quad-lumen CVC was then inserted into an internal jugular vein by the standard Seldinger technique and stopcocks attached to each hub. Patients had either the PosiFlow® NC or standard luer caps as designated by randomisation attached to all ports of the stopcocks. Each port/PosiFlow® was numbered 1-8. After 72 hr *in situ* the stopcocks complete with PosiFlow® or standard luer caps were aseptically removed, placed in sterile specimen bags and sent for microbiological analysis. Upon catheter removal, the distal tip of the CVC was aseptically removed and sent for microbiological examination. Patients yielding positive distal tip, intravenous connection and pre-insertion skin cultures for CoNS were investigated further.

2.2.2 Culture of specimens

2.2.2.1 Culture media and incubation conditions

Needleless connectors, stopcocks, skin swabs and CVC tips received in the laboratory were inoculated onto agar plates containing 7% defibrinated horse blood (Oxoid, Basingstoke, U.K.) and incubated for at 37°C for 48 hr in air. Following incubation, plates were examined for microorganisms.

2.2.2.2 Sampling the stopcock entry ports for microbial contamination

The entry ports of each stopcock to which PosiFlow® or standard luer caps were attached to were sampled by 3 techniques.

1. A nasopharyngeal swab moistened in sterile 0.9% (w/v) saline (Bibby Sterilin, Stone, U.K.) was inserted into the port and rotated ten times through 360°.
2. A fresh nasopharyngeal swab moistened in 0.9% (w/v) saline was used to sample the depression in the base of the entry port.

3. Fifty micro litres of brain heart infusion broth (BHI) (Oxoid, Basingstoke, U.K.) was inoculated into the port after which a sterile luer cap was attached. The port was then vortexed for 30 sec.

All swabs and fluids were cultured as described in 2.2.2.1.

2.2.2.3 Sampling the external surface of the PosiFlow® device

The silicone compression seal of each PosiFlow® was imprinted onto the surface of the 7% blood agar plate 10 times and incubated as described in 2.2.2.1.

2.2.2.4 Sampling the internal surface of the PosiFlow® device

After sampling the compression seal, the external surface was disinfected with a 70% (v/v) isopropyl alcohol swab (Sterets®, Seton Prebble Ltd, Merseyside, U.K.), and allowed to dry for 2 min. One-hundred microlitres of BHI was then flushed through the device 3 times and 80µl cultured as described in 2.2.2.1.

2.2.2.5 Sampling the external surface of the distal tip of the catheter

The distal 5cm of catheter was removed from the CVC and sampled by rolling the tip over the surface of the 7% blood agar plate (Maki *et al.*, 1977). Plates were incubated as described in 2.2.2.1.

2.2.2.6 Sampling the internal lumen of the distal tip of the catheter

Following sampling by the roll plate method, the distal tip was disinfected with a 70% isopropyl alcohol swab and allowed to dry for 2 min. Each of the 4 internal lumens of the distal tip was sampled by flushing 5 times with 1 ml of BHI. One hundred microlitres of BHI was then cultured as described in 2.2.2.1.

2.2.2.7 Skin swab cultures

Charcoal amies swabs from patients' skin were cultured as described in 2.2.2.1.

2.2.2.8 Identification of microorganisms recovered from the skin and CVC

2.2.2.8.1 Gram stain

One colony of each culture was mixed with 1 drop of sterile 0.9% (w/v) saline (Baxter, UK) on the surface of a glass slide (Ultima, UK). The suspension was allowed to dry at room temperature and then heat fixed. The smear was stained with crystal violet (Pro-Lab Diagnostics, UK) for 30 sec and washed with water. Lugol's iodine (Pro-Lab Diagnostics, UK) was then added for 30 sec and rinsed with water. The smear was decolourised by

addition of acetone (Hillcroft, UK) for 2 sec followed by rinsing with water. The smear was then counter-stained with carbol fuchsin (Pro-Lab Diagnostics, UK) for 1 min. Finally, the slide was washed with water, blotted dry and examined using the x100 objective (x1000 magnification).

2.2.2.8.2 Catalase test

A capillary tube (Bilbate Ltd, UK) was used to withdraw hydrogen peroxide 3% (v/v) (Thornton and Ross, UK) from the stock bottle. The capillary tube was then used to touch the test colony, after which it was inverted to ensure contact between hydrogen peroxide and test culture. A positive result was indicated by liberation of oxygen bubbles. The following control strains were included with each test:

Positive catalase test	Oxford strain *S. aureus* NCTC 6571
Negative catalase test	*Enterococcus faecalis* NCTC 12697

2.2.2.8.3 Staphylococcal latex test for detection of clumping factor

The staphylococcal latex test kit (Staphytect Plus®, Oxoid Ltd, UK) was allowed to reach room temperature prior to use. One drop of well-mixed test latex and control latex were dispensed separately onto a reaction card and 5 colonies of the test culture were mixed into each to achieve a smooth suspension. The reaction card was rotated for 20 sec after which the suspensions were examined for agglutination. The following control strains were included with each test:

Positive latex test	Oxford strain *S. aureus* NCTC 6571
Negative Staph latex test	*S. epidermidis*. (wild strain)

2.2.3 Pulsed Field Gel Electrophoresis (PFGE)

2.2.3.1 Standard stock solutions

2.2.3.1. 1M Tris-HCl (Tris-Hydrochloric acid)

Two solutions of Tris-HCl were prepared (pH 8.0 and pH 7.6). 12.11g of Tris base (Sigma, UK) was dissolved in 80ml of distilled water after which the pH was adjusted accordingly with concentrated hydrochloric acid (Fisher Scientific, UK). The volume was then adjusted to 100ml using distilled water. Both solutions were autoclaved at 120°C for 15 min and stored at 4°C until use.

2.2.3.1.2 0.5M EDTA (Ethylenediamine tetra-acetic acid)

Two solutions of EDTA (pH 8 and pH 9) were also prepared. 18.61g of EDTA (Sigma, UK) was added to 80ml of distilled water after which the pH was adjusted accordingly with sodium hydroxide (NaOH) pellets (Sigma, UK) and continuous stirring. The volume of each solution was then adjusted to 100ml using distilled water. Both solutions were autoclaved 120°C for 15 min and stored at room temperature until use.

2.2.3.2 In-use solutions for PFGE

2.2.3.2.1 TE (Tris-HCl and EDTA)

Five millilitres of 1M Tris-HCl pH 8.0 and 1M of 0.5M EDTA pH 8.0 were mixed together and the volume adjusted to 500ml with distilled water. The solution was autoclaved at 120°C for 15 min and stored at room temperature until use.

2.2.3.2.2 10 X TBE solution (Tris, Boric acid and EDTA electrophoresis buffer)

Tris base (60.55g), boric acid (27.5g) (Sigma, UK) 0.5M EDTA pH 8.0 (20ml) were mixed together and adjusted to 500ml with distilled water. The solution was autoclaved at 120°C for 15 min and stored at room temperature until use.

2.2.3.2.3 NET-100 solution (Sodium chloride, EDTA and Tris-HCl-100)

Twenty millilitres of 0.5M EDTA pH 8.0, 1ml of 1M Tris-HCl pH 8.0 and 0.58g of sodium chloride (NaCl) (Sigma, UK) were mixed together and the volume adjusted to 100ml with distilled water. The solution was autoclaved at 120°C for 15 min and stored at room temperature until use.

2.2.3.2.4 0.9% (w/v) chromosomal grade agarose

Chromosomal grade agarose (0.45g) (BioRad, UK) was added to 50ml of NET-100 and gently heated in a microwave oven until the agarose was completely dissolved. The agarose was allowed to solidify and stored at room temperature until required.

2.2.3.2.5 0.5%(w/v) molecular biology grade agarose

Molecular biology grade agarose (0.25g) (BioRad, UK) was added to 50ml TBE and dissolved and stored as described in 2.2.3.2.4.

2.2.3.2.6 Lysis solution

Six-hundred microlitres of 1M Tris-HCl pH 7.6 was added to 20ml 0.5M EDTA pH 8.0 and 5.8g of NaCl. The volume of the solution was then adjusted to 100ml with distilled water, autoclaved at 120°C for 15 min and stored at room temperature until use. When required, 0.5% sarcosyl (w/v) (Sigma,UK), 1mg/ml lysozyme (Sigma, UK) and 6.6 units/ml of lysostaphin (Sigma, UK) were added. For example, for 30ml of lysis solution, 0.15g sarcosyl, 0.03g lysozyme and 400µl of lysostaphin was required.

2.2.3.2.7 ES solution (EDTA and sarcosyl)

This solution consisted of 0.5M EDTA pH 9.0 with 1% (w/v) sarcosyl added. For example, for 30ml of ES solution, 0.3g of sarcosyl was required.

2.2.3.2.8 ESP solution (EDTA, sarcosyl and proteinase K)

This solution consisted of ES solution (section 2.2.3.2.7) with 1.5mg/ml proteinase K (Sigma, UK) added. For example, for 30 ml of ESP solution, 0.3g of sarcosyl and 0.045g of proteinase K were added to 30ml of 0.5M EDTA pH 9.0.

2.2.3.3 Preparation of chromosomal DNA in agarose plugs

Colonies of CoNS recovered from the study patients were subcultured onto 7% horse blood agar and incubated overnight at 37°C in air. Multiple colonies were selected for genotypic characterisation by PFGE in order to represent the different colonial types present in the patients' positive cultures. Indeed, 8 colonies were selected from each positive culture of skin swab, external catheter tip and the external surface of NC. Four colonies from positive cultures of each internal lumen of the catheter tip, internal surface of NC and the stopcock entry ports were also selected. A reference strain of *S. epidermidis* (NCTC 11047) was also subcultured for inclusion into the study. One colony from each subculture of CoNS was inoculated into separate flasks containing 20ml of sterile brain heart infusion (BHI) broth (Oxoid, UK). The flasks were then incubated on a rotary shaker at 37°C in air for 18 hr.

One millilitre of each of these broths was dispensed into a pre-weighed sterile eppendorf tube and centrifuged in a microfuge at 13,000 X *g* for 5 min. The supernatant was then removed and the tube reweighed to establish the wet weight of the microbial pellet. The weight of each pellet was adjusted with NET-100 to give a final wet weight of 20mg/ml. Five-hundred microlitres of each cell suspension was transferred to new, separate sterile

eppendorfs and were heated in a water bath to 50°C. The 0.9% (w/v) chromosomal grade agarose was heated in a microwave until molten after which it was cooled to 50°C by placing in a water bath. Five-hundred microlitres of this molten agarose was mixed with each cell suspension and dispensed into a Perspex mould (BioRad, UK) on ice. For each isolate, 3 blocks were prepared, removed from the mould and placed into a sterile bijou containing 3ml of lysis solution. Each bijou containing lysis solution and blocks was incubated in a waterbath at 37°C for 24 hr. The lysis solution was then replaced with 3ml ESP and incubated in a waterbath set at 50°C for 48 hr. The ESP was replaced with 3ml TE and the tubes were rolled on a slow roller for 2 two hr sessions followed by 2 one hr sessions with a change of TE for each. This was carried out in order to wash the blocks and remove any products that may either inhibit the action of the restriction enzyme. The blocks were then stored in 3ml of fresh TE at 4°C until required.

2.2.3.4 Digestion of the chromosomal DNA embedded in agarose blocks

A 1mm x 5mm section was cut from each agarose block with a sterile coverslip and placed into a sterile eppendorf tube containing 180µl of sterile water and 20µl of restriction enzyme buffer A (Roche, UK). These eppendorfs were then placed on ice for 15 min. This solution was then replaced with 175µl of sterile water, 20µl of restriction enzyme buffer A and 5µl of *Sma*I restriction enzyme 10units/µl (Roche, UK) after which the eppendorfs were placed on ice for 15 min then transferred to a waterbath for incubation at 25°C for 18 hrs. The enzyme solution was then replaced with 200µl of ES solution and incubated at 50°C for 15 min. The ES solution was next replaced with 1ml of TE solution and the tubes were left at room temperature for 15 min. Finally, the TE solution was removed and the digested agarose sections were stored at 4°C until use (within 48 hrs).

2.2.3.5 Electrophoresis of the SmaI digested sections

2.2.3.5.1 Casting the gel

1% (w/v) agarose was prepared by the addition of 1g of molecular biology grade agarose to 5ml 10 X TBE, adjusted to 100ml with distilled water and heated to dissolve the agarose. A casting stand (BioRad, UK) was assembled in accordance with the manufacturer's instructions. The casting tray (BioRad, UK) was adjusted with a spirit level to ensure an even surface and the comb was placed into position. Once the agarose

had cooled to approximately 50°C, it was poured into the casting tray, bubbles were removed and the gel was allowed to set at room temperature for 30 min. The comb was then removed to reveal the wells.

2.2.3.5.2 Loading the gel

The digested agarose sections were loaded into each pre-formed well. A bacteriophage lambda concatemer ladder DNA size standard (monomer to approximately a 21-mer, pulse marker 50-1000kb, Sigma, UK) was placed into at least 1 well of each gel in order to determine the band sizes of the DNA tested during analysis. Each well was then sealed with molten 0.5% (w/v) agarose (section 2.2.3.2.5).

2.2.3.5.3 Electrophoresis of digested fragments using the CHEF-DR III system

The PFGE tank (BioRad, UK) was washed twice with 2L of distilled water after which a spirit level was used to ensure that the tank was level. Distilled water (1900ml) was added to 100ml of 10 X TBE and the solution poured into the tank and cooled to 10°C by a chiller unit and recirculation pump (BioRad, UK) (1L/min). The gel was removed from the casting tray and placed in the centre of the tank after which it was electrophoresed using the following parameters:

Initial pulse time: 1 sec
Final pulse time: 50 sec
Run time: 24 hr
Voltage: 6V/cm
Angle: 120°C

2.2.3.6 Staining of the gel

The gel was removed from the tank and immersed in 500ml of distilled water containing 5µl of 10mg/ml ethidium bromide (Sigma, UK). This was left to shake gently for 25 min after which the fluid was drained off and replaced with 800ml of distilled water for 1 hr on the shaker to destain the gel.

2.2.3.7 Visualisation of the PFGE gels

The gel was visualised and photographed using a UV light scanner (UVP products, UK) with UV transillumination.

2.2.3.8 Analysis of the PFGE gel images

The Gelcompar® computer programme (Applied Mathematics, Belgium) was used to interpret the macrorestriction fragment profiles. The size of the fragment bands were determined by comparison with the DNA lambda phage standard (range 48.5 – approximately 1,000kb). Only fragments with a molecular weight of 48.5kb and larger were analysed. The gel analysis was transferred to the Gelcompar® ID database programme to enable strain comparison by calculation of the Dice correlation coefficient. Dendrograms were constructed to show clustering of the isolates by the unweighted pair group method of arithmetic averages (UPGMA).

2.3 Results

2.3.1 Patients

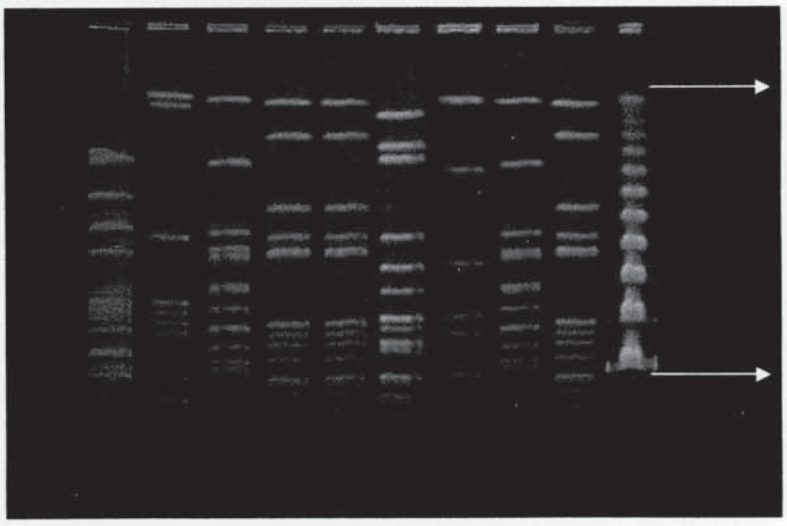
All 3 patients recruited underwent coronary artery bypass grafting (2 female and 1 male, mean age: 63, age range: 45-74). Two of the 3 patients had PosiFlow® NC attached to entry ports of the stopcocks and the remaining patient had standard luer caps attached. Three patients yielded complete sets of positive cultures with CoNS including; skin swabs, external tips, intravenous connections and internal tips.

2.3.2 Pulsed-field gel electrophoresis

2.3.2.1 Skin isolates

Eight different colonial types of CoNS were selected for genotyping from the skin swabs of each of the patients. Macrorestriction profiles of the multiple colonies of CoNS recovered from the pre-CVC insertion skin swabs of the 3 patients studied are shown in figures 2-1 to 2-3. Six genotypes of CoNS were recovered from the skin swabs of patient 1 (figure 2-1); 2 genotypes of CoNS were isolated from patient 2 (figure 2-2), whilst patient 3 yielded 5 distinct genotypes (figure 2-3).

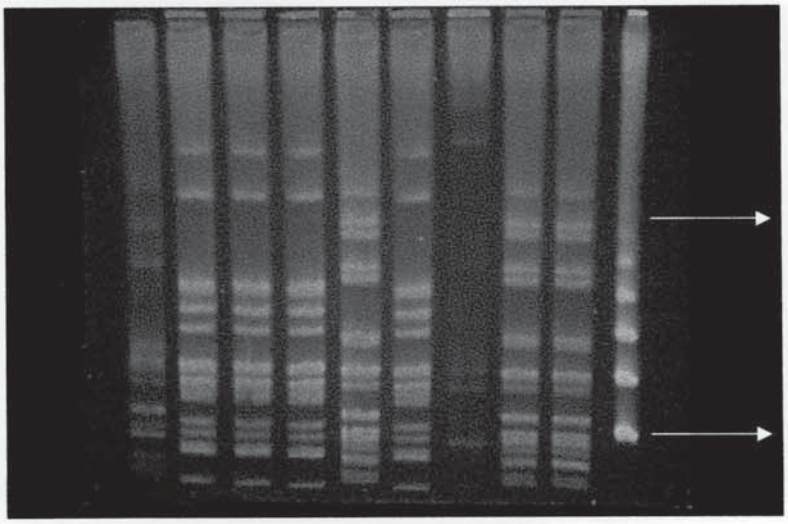
Figure 2-1 Macrorestriction profiles of isolates of CoNS recovered from the pre-CVC insertion skin swab from patient 1



Lane number	1	2	3	4	5	6	7	8	9	10
Skin isolate number		1	2	3	4	5	6	7	8	
Relatedness			*	†	†			*	†	

Lane 1 = control strain NCTC 11047, lane 10 = molecular weight size standard.
Identical 'relatedness' symbols represent identical genotypes.

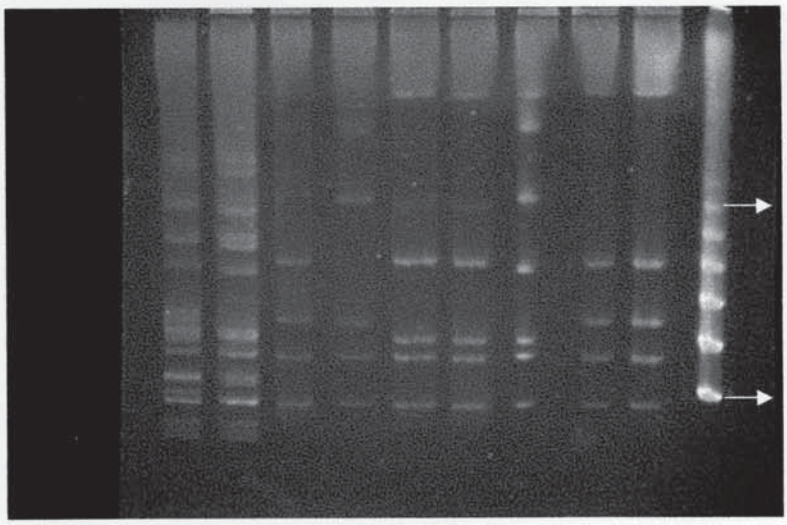
Figure 2-2 Macrorestriction profiles of isolates of CoNS recovered from the pre-CVC insertion skin swab from patient 2



Lane number	1	2	3	4	5	6	7	8	9	10
Skin isolate number		1	2	3	4	5	6	7	8	
Relatedness		*	*	*	†	*		†	†	

Lane 1 = control strain NCTC 11047, lane 10 = molecular weight size standard.
Identical 'relatedness' symbols represent identical genotypes.
Skin isolate 6, lane 7 was un-typeable.

Figure 2-3 Macrorestriction profile of isolates of CoNS recovered from the pre-CVC insertion skin swab from patient 3



Lane number	1	2	3	4	5	6	7	8	9	10
Skin isolate number		1	2	3	4	5	6	7	8	
Relatedness			†		*	*		†	†	

Lane 1 = control strain NCTC 11047, lane 10 = molecular weight size standard.
Identical 'relatedness' symbols represent identical genotypes.

2.3.2.2 Macrorestriction profiles of CoNS from patients 1 to 3

The isolates from all patients yielded 5 to 12 macrorestriction fragments within the range of 48.5 to 582kb.

2.3.2.2.1 Patient 1

A total of 24 isolates of CoNS were genotyped from patient 1 (skin n=8, ports n=4, external tip n=8, internal tips n=4). Analysis of the macrorestriction profiles of the 24 strains of CoNS by Gelcompar® software is shown in the dendrogram in figure 3-4. The overall genetic similarity of all isolates of CoNS from this patient was 56%. Two major clusters of genotypes were generated, cluster A at 62% similarity, containing 5 distinct genotypes and cluster B at 86% similarity containing 2 distinct genotypes. Six distinct genotypes were identified from 8 isolates of the pre-CVC insertion skin swab. Two distinct genotypes were also identified within the 4 isolates recovered from the internal medial-distal CVC tip lumen. However, all isolates from port 3 and the external surface of the CVC tip belonged to the same genotype, respectively. Skin isolate number 1 shared the same macrorestriction profile as all isolates from port 3 (see *, figure 2-4). Also, internal medial-distal isolates 1 and 3 shared the same macrorestriction profile as all external CVC tip isolates (see †, figure 2-4).

2.3.2.2.2 Patient 2

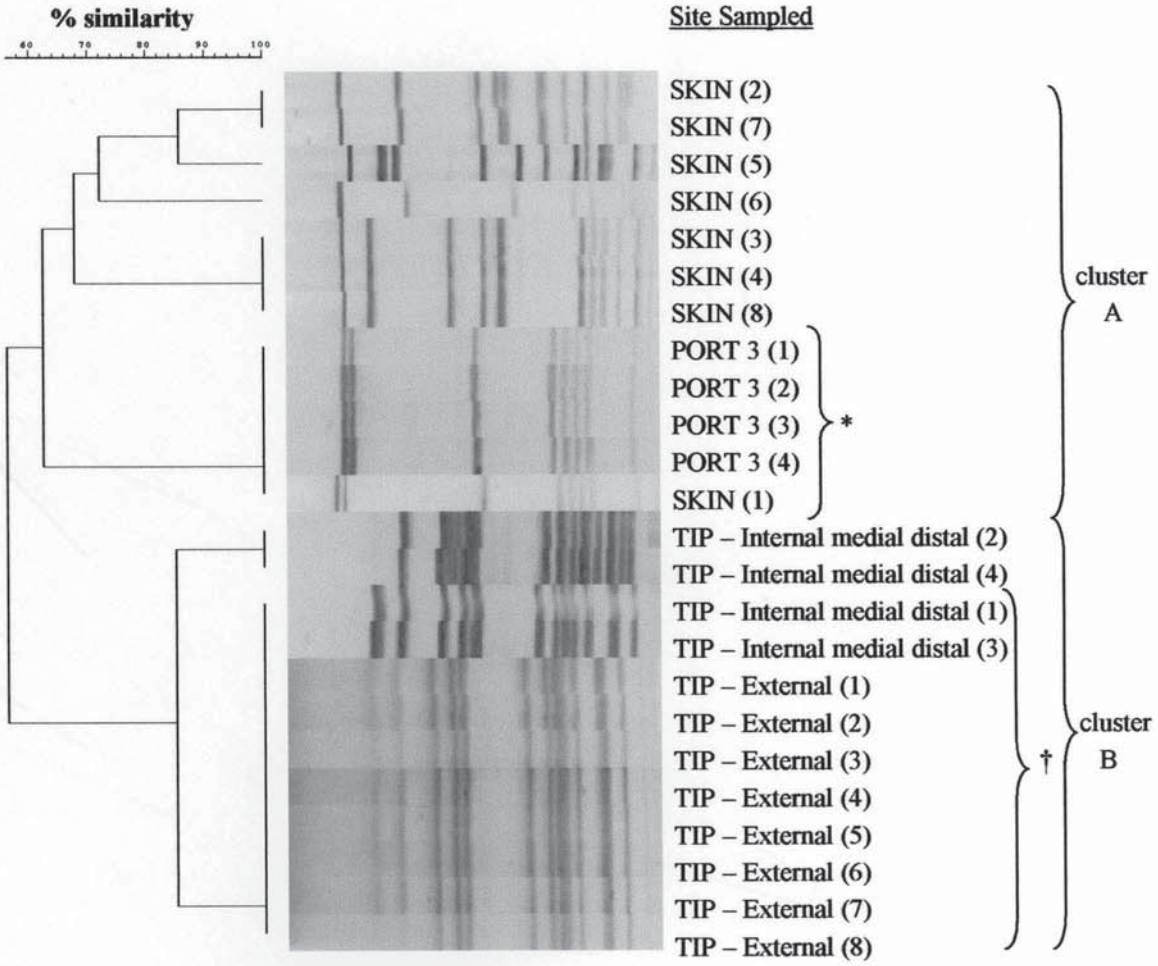
A total of 49 isolates of CoNS were genotyped from patient 2 (skin n=8, ports n=2, external tip n=8, internal tips n=8, external NC n=16, internal NC n=7). Analysis of the macrorestriction profiles of the 49 strains of CoNS by Gelcompar® software is shown in the dendrogram in figure 3-5. The overall genetic similarity of all isolates of CoNS from this patient was 44%. One major cluster (A) was generated at 66% similarity, containing 11 distinct genotypes. Another minor cluster (B), containing 2 distinct genotypes was generated at a similarity of 82%. Two distinct genotypes of CoNS were identified from the skin isolates. Other specimens which yielded multiple genotypes included the external surface of NC 3, port 1 and the external surface of the CVC tip. For all remaining specimens including; the internal and external surfaces of NC 2, the internal surface of NC 3 and the internal medial-distal and distal CVC tip lumen only 1 distinct macrorestriction fragment profile was identified, respectively. All isolates of CoNS recovered from the internal medial-distal and distal CVC tip lumen shared a common macrorestriction profile (see *, figure 2-5). A common macrorestriction profile was also shared by isolates from the skin, the external and internal surface of NC 3 and the external surface of the CVC tip

(see †, figure 2-5). One isolate from the external surface of NC 2 shared a common profile with all isolates from the external surface of NC 3 (see □, figure 2-5).

2.3.2.2.3 Patient 3

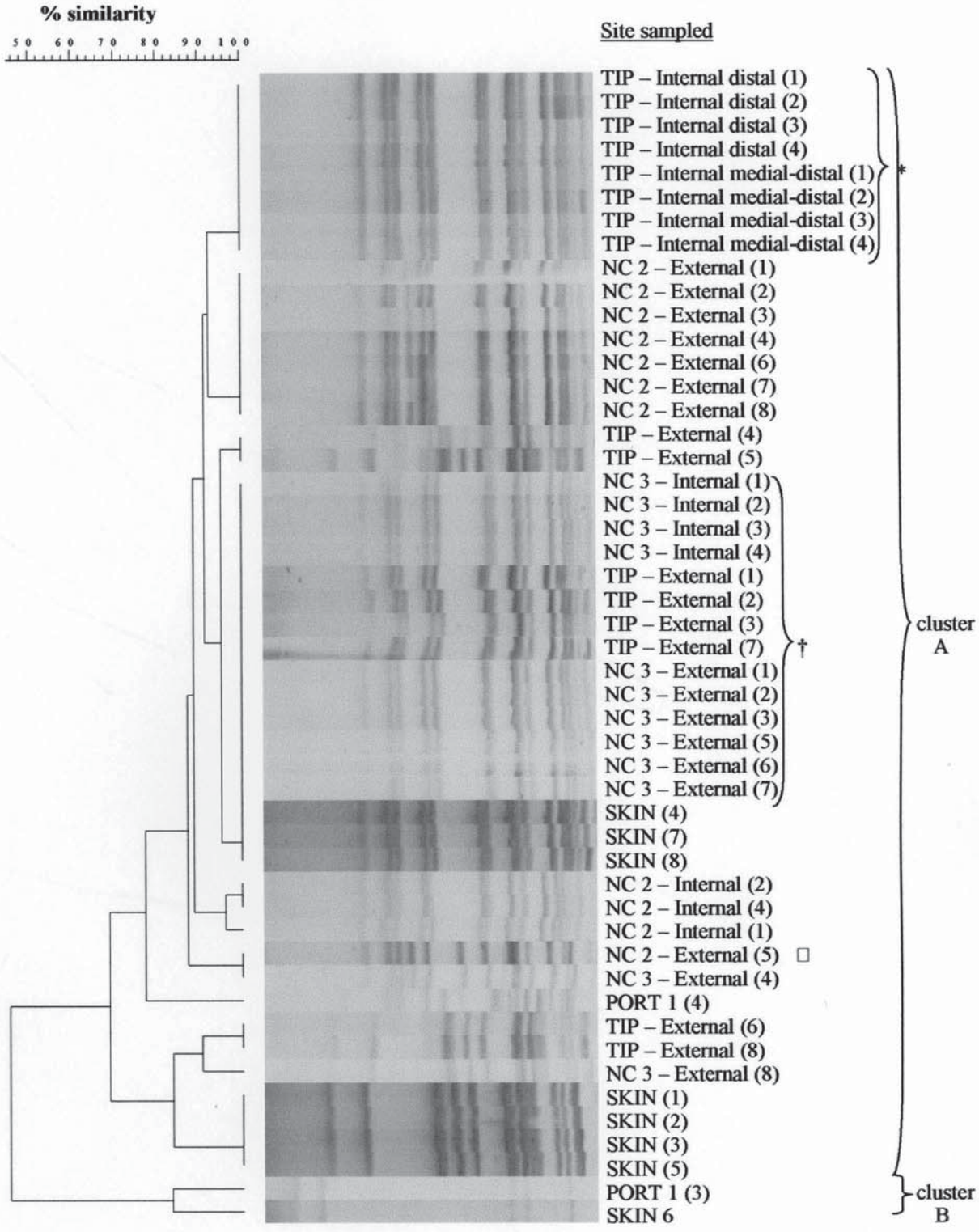
A total of 43 isolates of CoNS were genotyped from patient 3 (skin n=8, ports n=4, external tip n=7, internal tips n=4, external NC n=16, internal NC n=4). Analysis of the macrorestriction profiles of the 43 strains of CoNS by Gelcompar® software is shown in the dendrogram in figure 3-6. The overall genetic similarity of all isolates from this patient was 38%. Two major clusters were generated, cluster A at 46% similarity, containing eight distinct genotypes and cluster B at 52% similarity containing 13 distinct genotypes. Five distinct macrorestriction profiles were identified from the 8 isolates of the pre-CVC insertion skin swab alone. All isolates of CoNS from port 7 had the same macrorestriction profile. All other specimens including; the internal surface of NC 8, the external surface of the CVC tip, the internal proximal CVC tip lumen and the external surfaces of NC 7 and 8 generated multiple macrorestriction profiles, respectively. Skin isolate 6 shared the same macrorestriction profile as 2 isolates from the external surface of NC 7 (see *, figure 2-6). Also, 1 isolate from the external surface of NC 8, 1 from the internal proximal CVC tip lumen and 3 external CVC tip isolates shared a common macrorestriction profile (see †, figure 2-6).

Figure 2-4 UPGMA dendrogram of PFGE macrorestriction profiles of isolates of CoNS from the skin and explanted CVC of patient 1



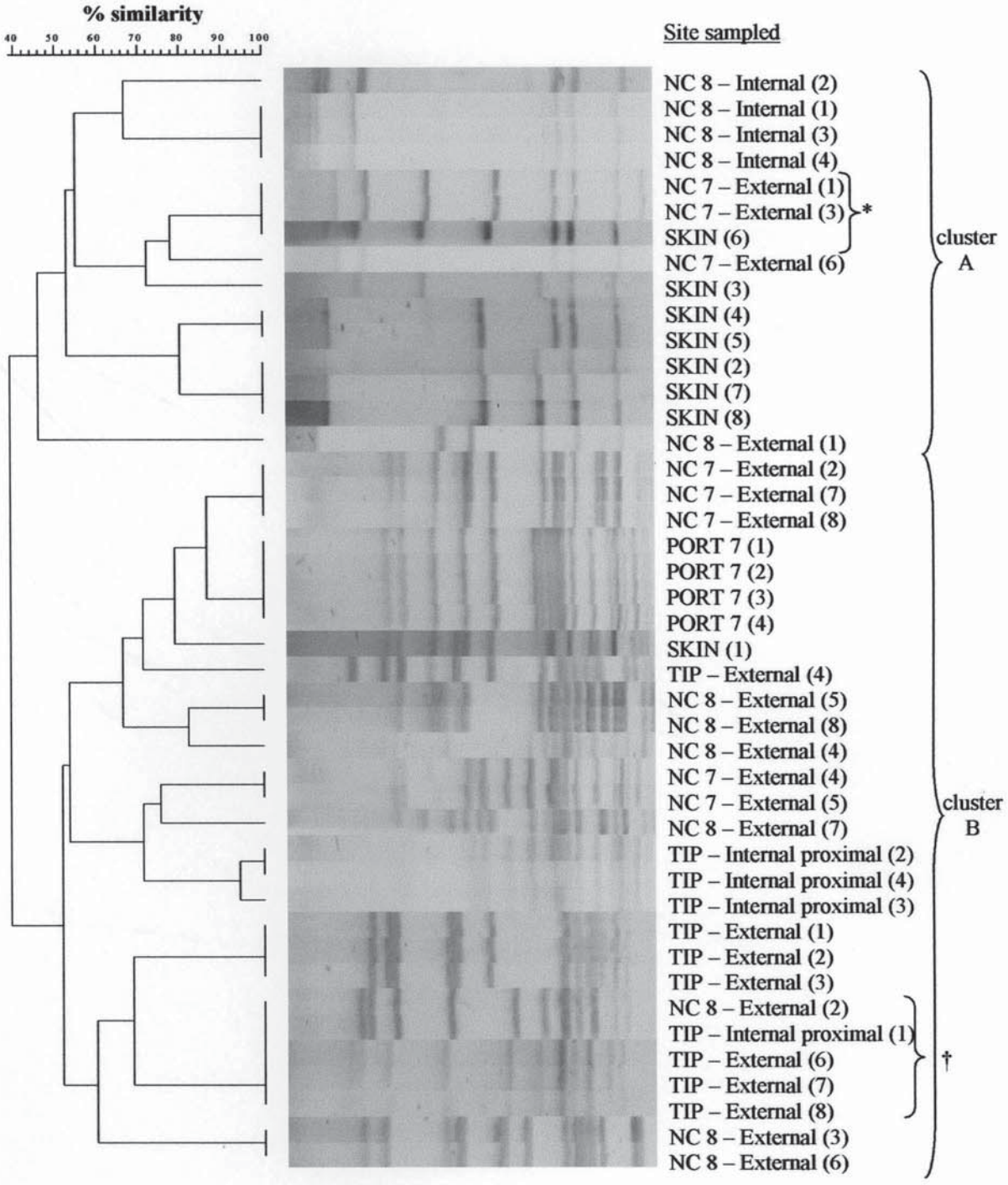
SKIN – (CVC insertion site), PORT – (on stopcock), TIP (of CVC).
***, † - denotes common macrorestriction profiles from differing specimen types.**

Figure 2-5 UPGMA dendrogram of PFGE macrorestriction profiles of isolates of CoNS from the skin and explanted CVC of patient 2



NC – Needleless connector, SKIN – (CVC insertion site), PORT – (on stopcock), TIP (of CVC). *, †, □ - denotes common macrorestriction profiles from differing specimen types.

Figure 2-6 UPGMA dendrogram of PFGE macrorestriction profiles of isolates of CoNS from the skin and explanted CVC of patient 3



NC – Needleless connector, SKIN – (CVC insertion site), PORT – (on stopcock), TIP (of CVC).
***, † - denotes common macrorestriction profiles from differing specimen types.**

2.4 Discussion

The main aim of this study was to investigate the genetic similarity of isolates of CoNS recovered from the skin of patients with CVC *in situ* and subsequently the explanted colonised CVC to determine the potential sources of CVC colonisation.

Multiple genotypes of CoNS were isolated from pre-CVC insertion site skin swabs of the 3 patients investigated (6, 2 and 5 respectively), demonstrating the heterogeneous nature of the CoNS within the normal skin flora. Indeed, it is well-known that the normal skin flora is primarily composed of many species and strains of CoNS. Overall, the isolates of CoNS recovered from patients 1 and 3 demonstrated genetic heterogeneity. In patient 2, however, the relative genetic homogeneity of CoNS isolates overall reflected the small number of genetically distinct strains recovered from the skin surface of this patient. This may indicate that the host's skin flora may play a focal role in catheter colonisation and subsequently in the pathogenesis of CR-BSI. In support of this theory, many studies investigating the source of microorganisms involved in CR-BSI have concluded that the skin is an important reservoir of microorganisms involved in such infections (Sadoyama and Filho, 2003; Almirall *et al.*, 1989; Bach *et al.*, 1999).

Indeed, in this current study, identical genotypes of CoNS recovered from the skin of the patients were also isolated from numerous distinct sites of the CVC including the stopcock entry ports, the internal and external surfaces of the NC and the external surface of the CVC tip. For example, in patient 2, an isolate of CoNS recovered from the skin surface was found to be genetically identical to isolates recovered from the external surface of the CVC tip. This observation could simply be due to contamination of the CVC tip during removal or that the microorganisms were impacted from the skin onto the CVC tip during insertion. Indeed, this theory has been investigated in 2 previous studies which both concluded that viable microorganisms are introduced onto the catheter tip during insertion (Elliott *et al.*, 1997; Livesley *et al.*, 1998). A further hypothesis for identical genotypes of CoNS being recovered from the skin and CVC is extraluminal migration whereby microorganisms from the skin surface enter at the CVC exit site and migrate down the external surface of the CVC. This could possibly be a more likely route than hub contamination and subsequent intraluminal migration for patients in this current study due to the fact that all patients studied had short-term intravenous access (Salzman and Rubin, 1995).

Our findings also indicate that microorganisms contaminating catheter hubs and subsequently causing infection may possibly originate from the patients' own skin flora. Although the hub is a well-documented route of entry for microorganisms implicated in CVC colonisation and subsequently CR-BSI, none of the patients during this study were found to have genetically identical strains of CoNS from the stopcock entry port or internal surface of the NC and the internal lumen of the CVC tip. This may be due to; short-term CVC are infrequently associated with colonisation/infection via hub contamination, the number of patients studied was too small or, the number of colonies from each culture investigated was too low. Patient 3, however, had the same clone of CoNS isolated from the external surface of the NC and the internal lumen of the CVC tip, suggesting that this strain entered the NC and stopcock entry port but was absent from these areas upon sampling. It is possible that the microorganism was flushed through the CVC to the distal tip when a fluid was administered. Also, in patients 2 and 3, the same genotype of CoNS was isolated from the external and internal surface of a NC, suggesting that the use of closed needleless access systems may not provide a protective barrier against microorganisms and thus highlighting the need for effective disinfection of connections prior to administrations (chapter 8). There were several genotypes of CoNS isolated from the CVC which were not found on the skin. This could indicate that the number of colonies characterised was insufficient or the skin is not the only source of CoNS responsible for catheter colonisation and subsequent infection. Indeed contaminating CoNS may originate from an environmental source or from the healthcare workers' hands.

All observations made during this study were based on the assumption that identical isolates were genetically identical, i.e. had the same number of macrorestriction bands and the corresponding bands were the same size. However, variations of 2 to 3 bands have been observed in strains of some species when; repeatedly subcultured over time or isolated several times from the same patient (Tenover *et al.*, 1995). This observation is consistent with the occurrence of a single genetic event such as point mutation or the insertion or deletion of DNA. Tenover and colleagues (1995) therefore stated that strains with 2 to 3 band differences are closely related. If this is the case, the interpretation of our findings may differ slightly. For example, in patient 1, we could assume that the 2 genotypes in cluster B, which share 86% similarity would be closely related. This would also be the case for most of the isolates within cluster A of patient 2 (at 88% similarity) which would indicate that closely related strains were recovered from the skin, external and internal surfaces of NC and the internal and external surfaces of the CVC tip, thus potentially providing evidence for CVC colonisation via a number of routes including

intraluminal migration. The criteria of Tenover and colleagues' (1995) for interpreting chromosomal DNA restriction patterns is relatively simple to use when interpreting small numbers of isolates but can prove difficult when the number of comparisons to be undertaken is high. During this study it proved difficult to find a strict correlation between percentage similarity of isolates (as determined by the GelCompar™ package) and the criteria outlined by Tenover and co-workers (1995). Indeed, if we were to consider that in patient 1, the 2 genotypes (at a similarity of 86%) in cluster B have 3 bands difference we could assume this percentage similarity value to be a reference point to distinguish between isolates that are closely and possibly related. However, in patient 1, skin isolates 2 and 7 shared 86% similarity with skin isolate 5 but it was clear from visual observation of these profiles that there are >3 band differences between the isolates.

It is essential that efforts are made through epidemiological studies to identify sources of microorganisms involved in CR-BSI in order to implement appropriate preventative strategies. Traditional preventative approaches including CVC site care, tunneling, aseptic barriers at the time of CVC insertion and the coating of the external surface of the CVC with antimicrobial agents only focus on preventing the microorganisms from the skin located at the insertion site from instigating infection (Sitges-Serra *et al.*, 1997). Whereas attempts to reduce CR-BSI due to hub contamination can be approached by aseptic hub manipulation, the use of protective intravenous connection devices and the reduction of the number of lumen and ports utilised (Sitges-Serra *et al.*, 1997). However, as microorganisms on the skin surface are frequently implicated in episodes of CVC colonisation and CR-BSI, this suggests that either the current methods of skin disinfection are ineffective, or that microorganisms implicated in such infections reside in the dermal layers of skin rather than the epidermis to which the antimicrobial agents activity is restricted.

In conclusion, the results from this current study highlighted that; multiple genotypes of CoNS are present on the skin, sensitive molecular methods are required to investigate genetic relatedness, strains with <100% genetic similarity may be closely related, the CoNS exist in a ubiquitous nature with wide genetic heterogeneity and the skin may serve as a source for CVC colonisation and subsequent CR-BSI. However, as all genotypes from the CVC were not recovered from the patients' skin, other sources of contamination may exist, for example, the hands of the healthcare worker. Further work in this area is

warranted and should include assessment of the genetic similarity of increased numbers of multiple isolates from a larger group of patients.

3. Epidemiological typing of strains of staphylococci implicated in nosocomial infection

3.1 Introduction

As it was demonstrated in chapter 2, accurate identification and typing of microorganisms is crucial when undertaking epidemiological investigations (Weller, 2000). Strain typing methods are becoming widely applied in the clinical setting in order to; identify outbreaks, detect cross transmission of microorganisms, determine the source of a microorganism, recognise virulent strains and monitor vaccination programmes (Olive and Bean, 1999).

In order for a typing technique to be effective it must be; highly discriminatory, reproducible, standardised, based on a stable feature, widely available, inexpensive and easy to perform and interpret (Weller, 2000; Olive and Bean, 1999). With the exception of discriminatory power, all of the above criteria are relatively simple to assess. Discriminatory power is defined as the number of types identified by a particular typing technique which doesn't allow comparison of techniques with any ease (Hunter and Gaston, 1988). However, an index of discrimination developed for the purpose of allowing comparison of techniques has been described. The Simpson's index of diversity is based on the probability that 2 unrelated strains sampled from the test population will be placed into different typing groups (Hunter and Gaston, 1988).

A number of typing techniques have been employed to investigate isolates of staphylococci. Until fairly recently, most of these techniques have been based on characterisation according to the expression of phenotypic traits. Such techniques include; biotyping, antibiograms and bacteriophage typing. Biotyping is relatively easy to perform but has been reported to have poor discriminatory power when used for characterising CoNS (Geary *et al.*, 1997). The discriminatory power of antibiogram typing of nosocomial strains of staphylococci has also been relatively inadequate which may be due to the fact that such an environment acts as a reservoir for antibiotic resistance genes (Lang *et al.*, 1999). Although the discriminatory power of bacteriophage typing has been reported to be superior to other phenotypic techniques, this technique has an unacceptable typeability record (Weller, 2000).

The introduction of genotypic typing methods such as plasmid analysis, restriction enzyme analysis (REA), DNA hybridisation, PCR typing and PFGE have provided enhanced tools for the characterisation of staphylococci (Sloos *et al.*, 2000; Kloos, 1990; Mulligan and Arbeit, 1991; Prevost *et al.*, 1992; Yoshida *et al.*, 1997; Morvan *et al.*, 1997; Symms *et al.*, 1998; Kreiswirth *et al.*, 1993; Leeuwan *et al.*, 1999; Frenay *et al.*, 1994; Kobayashi *et al.*, 1995; Nada *et al.*, 1996; van der Zee *et al.*, 1999; Deplano *et al.*, 2000; Welsh and McClelland, 1992; Kumari *et al.*, 1997; de Mattos *et al.*, 2003; Young *et al.*, 1994; Vermont *et al.*, 1998; Schlegel *et al.*, 2001; Landman *et al.*, 2003).

Pulsed-field gel electrophoresis (PFGE) has been widely used to successfully type staphylococcal isolates. The technique has been used to investigate a cluster of paediatric cases of deep-seated *S. aureus* infections contracted following cardiac surgery (Ruef *et al.*, 1996). It was found that these cases of infection were not due to cross-transmission between patients, however nasal colonisation with *S. aureus* was found to be a major risk factor for infection. This typing technique has also been used successfully to type CoNS. During 2 case studies of BMT patients suffering from chronic bacteraemia due to *S. epidermidis*, it was found that their CVC was the source of the microorganisms causing the infection (Lina *et al.*, 1995; Kennedy *et al.*, 2000). Indeed, it is well recognised that IV catheters are the most common source of bacteraemia in neutropaenic haematologic patients (D'antonio *et al.*, 1992). However, it is crucial for patient management that the source of infection is correctly identified. Macrorestriction enzyme analysis by PFGE was also successfully used to demonstrate ineffective infection control procedures on a haemato-oncology unit (van Pelt *et al.*, 2003).

Random amplification of polymorphic DNA (RAPD) otherwise known as arbitrarily primed PCR (AP-PCR) has also been used to type staphylococci with success. Kitao (2003), studied the potential transmission of methicillin-resistant CoNS on the fingers of nursing students. This study concluded that preventative measures for cross-infection weren't required since there was no evidence of cross-transmission occurring. This typing technique is also frequently used in industry, for example, for typing strains of *S. xylosus* used in food production (Di Maria *et al.*, 2002; Rossi *et al.*, 2001).

It is well-documented that genotypic techniques such as PFGE and RAPD have higher discriminatory capabilities for typing staphylococci than phenotypic methods (Andrasevic *et al.*, 1999; Tambic *et al.*, 1997; Worthington *et al.*, 2000). However, there is debate over which of these 2 techniques is superior. In a study by Schmitz and colleagues (1998) 6

genotypic techniques were evaluated. It was concluded that although PCR-based techniques (including RAPD) have the advantage of rapid performance and ease of use, their discriminatory capacity is inferior to PFGE. This could be due to the fact that with RAPD only randomly selected areas of DNA are amplified and analysed whereas with the 'gold standard' PFGE, the whole genome is sampled and analysed (Weller, 2000).

The aims of this study were to;

a) Characterise strains of *S. aureus* associated with sternal surgical site infection (SSI) (chapter 5) by biotyping, antibiograms, PFGE and RAPD.

b) Determine the phenotypic and genotypic similarities of strains of *S. aureus* associated with sternal SSI.

c) Determine if common strains of *S. aureus* are associated with a suspected outbreak of sternal SSI.

d) Characterise strains of CoNS associated with CR-BSI on a BMT unit by biotyping, antibiograms, PFGE and RAPD.

e) Determine the phenotypic and genotypic similarities of strains of CoNS associated with CR-BSI on a BMT unit.

f) Determine if common strains of CoNS are associated with CR-BSI on a BMT unit.

g) Compare phenotypic and genotypic typing methods for the characterisation of staphylococci associated with nosocomial infection.

3.2 Materials and Methods

3.2.1 Patients

3.2.1.1 Sternal surgical site infection due to S. aureus in cardiothoracic surgery patients

Cardiothoracic surgery patients at the cardiac surgery critical care unit, University Hospital Birmingham NHS Trust with a clinical and microbiological diagnosis of sternal SSI due to *S. aureus* were recruited into the study. Microbiological diagnosis was made by isolation of *S. aureus* from wound swabs, pus swabs, pus and tissue acquired from the sternum and mediastinum. The clinical criteria for defining a SSI is outlined in table 3-1.

3.2.1.2 CR-BSI due to CoNS in stem cell transplant patients

Stem cell transplant (SCT) patients in the bone marrow transplant (BMT) unit, University Hospital Birmingham NHS Trust who underwent transplantation during 2002 and during the same year had a clinical diagnosis of CR-BSI due to CoNS acquired during their stay on the BMT unit were recruited onto the study.

3.2.2 Microbiological culture of clinical specimens

3.2.2.1 Culture of wound swabs and pus from sternal SSI

Wound swabs and pus from sternal SSI were inoculated onto blood agar, Cystine Lactose-Electrolyte-Deficient Agar (CLED) (Oxoid, Basingstoke, UK) and neomycin agar (Oxoid, Basingstoke, UK). Plates were incubated in CO_2 at 37°C for 48 hr. In addition, the samples were inoculated onto blood agar and neomycin agar and incubated anaerobically at 37°C for 5 days for the detection of slow-growing anaerobic microorganisms.

3.2.2.2 Culture of tissue (from sternum and mediastinum)

Media for culture of sternal tissue was prepared by dispensing approximately 10-15 glass beads into a thick-walled glass universal. To this 4ml of BHI broth was added after which the universal autoclaved at 121°C for 15 min. 0.1g of tissue was added to the BHI broth/beads and vortexed for 10 sec. The specimen was then left to stand for at least 30 sec to allow aerosols to settle. The tissue suspension was cultured as described in 3.2.2.1.

Table 3-1 – Criteria for defining surgical site infection (SSI) (adapted from Horan *et al.*, 1992)

3.2.2.3 Blood cultures (stem cell transplant patients)

Blood cultures were performed in accordance with routine laboratory standard operating procedures. For each patient a set of Bactec bottles (BD, UK) comprising 1 aerobic and 1 anaerobic bottle, inoculated with 8-10 ml of blood was sent to the laboratory for analysis using the Bactec 9240 automated system (BD, UK). Bactec bottles were then loaded into the Bactec 9240 machine. Positive bottles were removed and examined for microorganisms. Firstly, the septum of the Bactec culture bottle was disinfected with a Steret® 70% isopropyl alcohol swab (Seton Prebble Ltd, UK) and was left to dry for 2 min. The Bactec bottle was then vented by insertion of a Bactec vent (BD, UK). A smear of the blood sample was prepared and Gram-stained (chapter 2, 2.2.2.8.1). The blood was also cultured as described in section 3.2.2.1.

3.2.3 Identification of CoNS and *S. aureus*

Colonies resembling Staphylococci were identified by the Gram-stain, catalase and Staphylococcal latex tests as outlined in section 2.2.2.8.

3.2.4 Biotyping

All isolates were biotyped by the API ID 32 STAPH (Biomerieux, France). This is a standardised system for the identification of the genera *Staphylococcus, Micrococcus, Stomatococcus* and *Aerococcus*. Microorganisms were biotyped in accordance with the kit instructions described below.

3.2.4.1 Inoculum and API strip preparation

Fresh subcultures of each CoNS were harvested on blood agar plates. A single colony was then inoculated into a tube containing 3ml of distilled sterile distilled water. A suspension with a turbidity equivalent to 0.5 McFarland was prepared for each sample. In order to prepare the strip, it was removed from its packaging, allowed to reach room temperature and the strain reference was recorded on the elongated flap.

3.2.4.2 Inoculation of the API strip

Fifty-five microlitres of each bacterial suspension was then added to each well of the test strip (reactions included on the test strip are summarised in table 3-2). Cupules 1.0, 1.1 and 1.2 were covered with 2 drops of mineral oil (sterile fluids manufacturing department, QEMC, UK) after which the lid was replaced and the strip incubated at 37°C for 24 hr in air.

3.2.4.3 Reading and Interpretation of the API strips

The API strips were read and interpreted in accordance with the manufacturers recommendations. The following reagents were added to the strip; NIT 1 and NIT 2 to the NIT test cupule (0.0), VP A and VP B to the VP test cupule (0.1) and FB reagent to tests βGAL to PyrA cupules (0.2-0.5). After 5 min the test strip was analysed in accordance with table 3-2. A 9-digit numerical profile was generated dependant upon positive/negative reactions (figure 3-1). The identity of the microorganisms was determined by entering the profile number into a computer database (APiLAB Plus, Biomerieux, France).

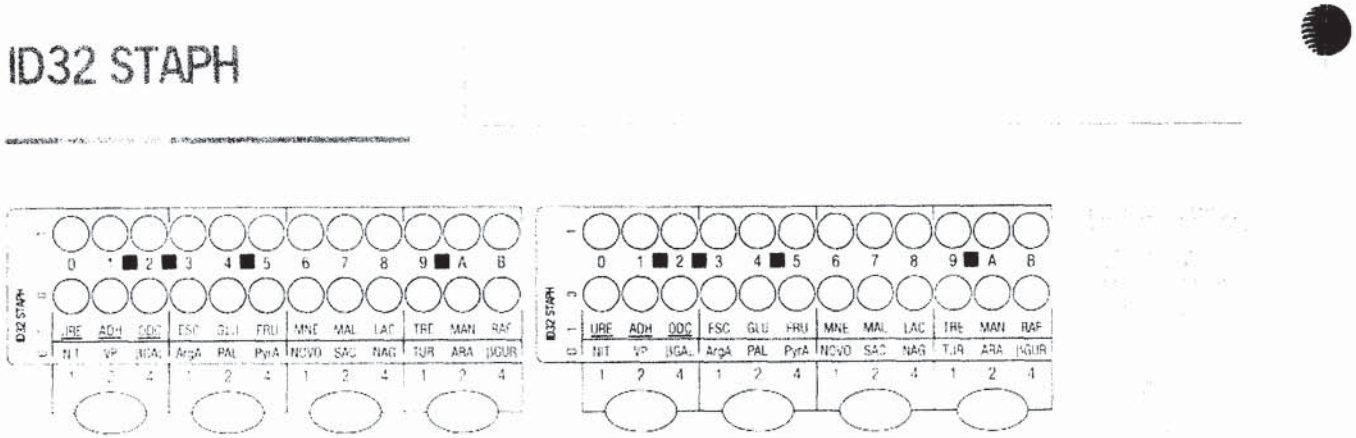
Table 3-2 – API ID 32 STAPH substrates and interpretation

Cupule	Test	Active ingredients	Quantity (mg/cup)	Reactions/enzymes	Result	
					Negative	Positive
1.0	URE	urea	1.12	Urease	Yellow	Orange red-violet
1.1 1.2	ADH ODC	L-arginine L-ornithine	0.76	Arginine Dihydrogenase Ornithine Decarboxylase	Yellow	Orange-red
1.3	ESC	Esculin Ferric citrate	0.224 0.032	Hydrolysis	Colourless-pale grey	Brown-black
1.4	GLU	D-glucose	0.56	Fermentation		
1.5	FRU	D-fructose	0.56	Fermentation		
1.6	MNE	D-mannose	0.56	Fermentation		
1.7	MAL	D-maltose	0.56	Fermentation		
1.8	LAC	D-lactose (bovine)	0.56	Fermentation	Red	Yellow
1.9	TRE	D-trehalose	0.56	Fermentation	red-orange	yellow-orange
1.A	MAN	D-mannitol	0.56	Fermentation		
1.B	RAF	D-raffinose	0.56	Fermentation		
1.C	RIB	D-ribose	0.56	Fermentation		
1.D	CEL	D-cellobiose	0.56	Fermentation		
1.E 1.F				Empty cupules		
0.0	NIT	Potassium nitrate	0.054	Reduction	Colourless	Pink-purple
0.1	VP	Sodium pyruvate	0.475	Acetoin production	Colourless	Pink-red

Table 3-2 continued

0.2	βGAL	2-naphthyl-βD-galactopyranoside	0.0364	B Galactosidase	Colourless Pale purple Pale orange	Purple
0.3	ArgA	L-arginine β-naphthylamide	0.0172	Arginine Arylamidase	Colourless Pale orange	Orange
0.4	PAL	2-naphthyl phosphate	0.0123	Alkaline phosphatase	Colourless Pale purple Pale orange	Purple
0.5	PyrA	Pyroglutamic acid- β-naphthylamide	0.0128	Pyrrolidonyl Arylamidase	Colourless Pale orange	Orange
0.6	NOVO	Novobiocin	0.0018	Resistance		
0.7	SAC	D-saccharose (sucrose)	0.56	Fermentation	Red	Yellow
0.8	NAG	N-acetyl-glucosamine	0.56	Fermentation	red-orange	Yellow-orange
0.9	TUR	D-turanose	0.56	Fermentation		
0.A	ARA	L-arabinose	0.56	Fermentation		
0.B	βGUR	4-nitrophenyl-βD-glucuronide	0.0158	B Glucuronidase	Colourless	Yellow
0.C 0.D 0.E 0.F	Empty cupules					

Figure 3 - 1 API results sheet



3.2.5 Antibiograms

Antibiograms were performed using the BSAC (British Society for Antimicrobial Chemotherapy) standardised disc diffusion method.

3.2.5.1 Control strains

Control strains of *S. aureus* NCTC 6571 and *S. aureus* ATCC 25923 were included with antibiogram testing to quality control the media, antibiotic discs and incubation conditions.

3.2.5.2 Microbiological media employed

For sensitivity testing of Staphylococcal species, Iso-sensitest agar (ISA) (Biomerieux, UK) was employed for all antibiotics with the exception of flucloxacillin testing, for which Columbia agar containing 2% (w/v) sodium chloride (Biomerieux, UK) was used.

3.2.5.3 Inoculum preparation

A bacterial suspension equivalent to 0.5 MacFarland was achieved by inoculation of test microorganisms into 3 ml of tryptone water, followed by adjustment of the suspension in accordance with a turbidometer.

3.2.5.4 Plate inoculation

Two microlitres of each bacterial suspension was streaked across an ISA plate with a sterile loop. For flucloxacillin testing, a sterile swab was dipped into the suspension, pressed lightly against the inside of the tube to remove excess fluid and streaked in 3 directions across a Columbia plate containing sodium chloride.

3.2.5.5 Application of antibiotic discs

Antibiotic discs were applied to the ISA plates within 15 min of inoculation with an antibiotic multipoint dispenser. The plates were then incubated within 15 min of applying the discs for 18 hr at 37°C in air. Two panels of antibiotics discs were used, the antibiotics in each panel are outlined in table 3-3.

For flucloxacillin susceptibility testing, a 1µg oxacillin disc was placed on the surface of an inoculated Columbia plate. The plate was then incubated at 30°C in air for 18 hr.

Table 3-3 Antibiotics included in the antibiograms

Panel	Antibiotic	Concentration in disc (μg)
A	Erythromycin	5
	Tetracycline	10
	Rifampicin	2
	Trimethoprim	5
	Fusidin	10
	Clindamycin*	2
B	Mupirocin – low*	5
	Mupirocin – high†	200
	Gentamicin	10
	Linezolid*	10
	Quinupristin/Dalfopristin*	15
	Vancomycin	5

* CoNS only,

† *S. aureus* only

3.2.5.6 Reading and Interpretation of the zones of inhibition

Before zones of inhibition were measured and interpreted it was confirmed that all cultures were pure and semi-confluent. A ruler was used to measure the zones of inhibition for each antibiotic to the nearest millimetre. The BSAC guidelines were referred to in order to determine whether each strain was sensitive or resistant to each antibiotic (see table 3-4).

Table 3-4 Interpretation of zones of inhibition

Antibiotic	Resistant ≤ mm	Sensitive ≥ mm
Erythromycin	19	20
Tetracycline	19	20
Rifampicin	29	30
Trimethoprim	19	20
Fusidin	29	30
Clindamycin	25	26
Mupirocin – low	21	22
Mupirocin – high	9	10
Gentamicin	19	20
Linezolid	19	20
Quinupristin/Dalfopristin	19	20
Vancomycin	11	12
Flucloxacillin	14	15

3.2.6 Genotypic characterisation of staphylococci by pulsed-field gel electrophoresis (PFGE)

All isolates were genotypically characterised in duplicate by *SmaI* chromosome macrorestriction profiling using PFGE and analysed using the GelCompar® computer software (chapter 2, section 2.2.9). A control reference strain, *S. aureus* NCTC 8325 was also included.

3.2.7 Genotypic characterisation of staphylococci by randomly amplified polymorphic DNA (RAPD)

3.2.7.1 *Standard stock solutions*

3.2.7.1.1 50 X TAE buffer (Tris, EDTA and Glacial acetic acid)

One litre of 50 X concentration TAE buffer was prepared by firstly making up 100 ml 0.5M EDTA pH 8 (see section 2.2.2.9.1.2). This was then added to 2M Tris base solution which was prepared by dissolving 242g Tris base in 800mls of distilled water. Finally, within a fume cupboard, 57.1ml of glacial acetic acid (Fisher Scientific, UK) was added to the Tris and EDTA mixture and the volume was made up to 1L with distilled water. To make the working solution, 20ml of 50X concentrated TAE buffer was added to 980ml distilled water. This solution is equal to; 40mM Tris, 1mM EDTA and 0.1% (v/v) glacial acetic acid.

3.2.7.2 *Preparation of template DNA*

Colonies of staphylococci recovered from the study patients were subcultured onto 7% horse blood agar and incubated overnight at 37°C in air. A reference strain of *S. epidermidis* NCTC 11047 and *S. aureus* NCTC 8325 were also subcultured for inclusion into the study. Each staphylococcal isolate was characterised by RAPD in duplicate.

Three to 5 colonies of staphylococci were suspended in 1ml of sterile, double-distilled water and centrifuged for 4 min at 9000 X *g*. The supernatant was then discarded and the step repeated twice. These washed bacterial cells were then resuspended in 1ml of sterile double distilled water and aliquoted into a sterile 1 ml plastic cuvette. The absorbance of the suspension was measured at 600nm using a spectrophotometer and adjusted, to an optical density of 1.7.

One-hundred microlitres of this suspension was then placed into a sterile eppendorf and incubated at 95°C for 12 min in a heating block (Techne, UK). Following this, the

suspension was centrifuged again at 9000 X *g*. The supernatant now contained the extracted DNA and was cooled to room temperature for use as a template. This template DNA was stored at -20°C until required for use.

3.2.7.3 *Polymerase chain reaction (PCR)*

All laboratory consumables used to produce the PCR reaction solutions were sterilised by placement in an ultraviolet light cabinet (Thistle Scientific, UK) for 30 min. The ultraviolet light was then switched off and all reaction solutions were prepared in this cabinet.

A PCR reaction was performed using a 10-mer primer with the sequence; 5'–AGC GTC ACT G-3' (MWG-Biotech AG, Germany) (Pereira and colleagues, 2002). The reaction was carried out in a 25µl reaction volume which contained 18.55µl sterile distilled water, 0.5µl 10mM dNTPs (10mM of each of the 4 dNTPs) (Promega, UK), 0.25µl of 5units/µl *Taq* DNA polymerase (Promega, UK), 1.2µl of 10 pM primer, 2µl of DNA template and 2.5µl 10 x PCR buffer 1 (Table 3-5; Opti-Prime kit, Stratagene, La Jolla, CA, USA).

Table 3-5 Stratagene buffer matrix of opti-prime kit (Schoettlin *et al.*, 1994)

10 mmol l^{-1} Tris-HCl	$MgCl_2$	25 mmol l^{-1} KCl	75 mmol l^{-1} KCl
pH 8.3	1.5 mmol l^{-1}	Buffer 1	Buffer 2
pH 8.3	3.5 mmol l^{-1}	Buffer 3	Buffer 4
pH 8.8	1.5 mmol l^{-1}	Buffer 5	Buffer 6
pH 8.8	3.5 mmol l^{-1}	Buffer 7	Buffer 8
pH 9.2	1.5 mmol l^{-1}	Buffer 9	Buffer 10
pH 9.2	3.5 mmol l^{-1}	Buffer 11	Buffer 12

The amplification procedure was performed in a PTC thermocycler (MJ Research Inc, Waltham, MA, USA) with ramping as follows: 1 cycle of denaturation for 4.5 min at 94°C followed by 5 low stringency cycles comprising 30 sec of denaturation at 94°C, 2 min of annealing at 20°C, 2 min of extension at 72°C. This was followed by 35 high stringency cycles of 30 sec of denaturation at 94°C, 1 min of annealing at 32°C and 2 min of extension at 72°C. The cycling was completed with 5 min of extension at 72°C. The reactions were stored at -20°C until required for separation by gel electrophoresis.

For each PCR reaction preparation, positive and negative controls were prepared; the positive control using the strains outlined in section 3.2.7.2 and the negative control by using sterile water in place of the DNA extractions.

3.2.7.4 *Gel electrophoresis*

3.2.7.4.1 Preparation of the agarose gel

The PCR amplification products were separated by electrophoresis in a 2% (w/v) agarose gel. The gel mount (Flowgen, UK) was prepared by placing autoclave tape around the open sides and adding a 12 - well comb (Flowgen, UK). The gel was prepared in a flask by firstly adding 1g of agarose (Agarose for the separation of GeneAmp™ PCR products, Applied Biosystems, USA) to 50ml of 1 X TAE buffer and weighing the mixture. The solution was then heated in a microwave to dissolve the agarose. The flasks were then reweighed and sterile water was added to the agarose until the weight was equal to the pre-heated solution. The gel was then cooled to approximately 50°C by holding the flask under cold running water. One µg/ml of ethidium bromide was then added to the 50 ml of gel (i.e. 5µl of 10mg/ml ethidium bromide). The gel was poured into the prepared mount, and any bubbles removed with a sterile pipette tip. The gel was then left to set for 30 min at room temperature.

3.2.7.4.2 Preparation of the electrophoresis tank

The electrophoresis tank (Flowgen, UK) was prepared by filling it with 1 X TAE buffer. The solidified agarose gel within the mount was then placed into the buffer and the tank topped up with 1 X TAE buffer to completely cover the gel. The gel was then ready for loading of the PCR amplification products.

3.2.7.4.3 PCR product preparation and gel loading

Frozen PCR products were defrosted at room temperature and 5µl of 6 X loading buffer added to each sample to give a 1 in 6 dilution. The loading buffer consisted of 0.25% (w/v) bromophenol blue (Sigma, UK), 0.25% (w/v) xylene cyanol (Sigma, UK), 30% (v/v) glycerol (Sigma, UK) and 69.5% sterile distilled water. After vortexing, 15µls was loaded into a designated well in the gel. Five microlitres of a 1-kb DNA molecular mass ladder (Hyperladder IV, Bioline, UK) was also loaded into a well in the gel.

3.2.7.4.4 Electrophoretic conditions

The samples were run at 100 volts for 1 hr.

3.2.7.4.5 Visualisation and Analysis of the RAPD gels

The RAPD gels were visualised and analysed using the same apparatus and conditions as for PFGE, outlined in sections 2.2.3.7 - 2.2.3.8. However, the size of the amplicons were determined by comparison with the DNA molecular mass ladder described in section 3.2.7.4.3. Only amplicons of 200bp or larger were analysed, and any gels demonstrating bands present within the negative control lane were not included in the analysis.

3.2.8 Determination of the typeability and discriminatory power of each typing method

The typeability of each typing method was calculated and expressed as simple percentages (Hunter and Gaston, 1988).

The discriminatory ability of each typing method was determined using Simpson's index of diversity. A single numerical index of discrimination (DI) is given by:

$$DI = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} nj\,(nj-1)$$

N = the total number of strains in the test population
S = the total number of types described
Nj = the number of strains belonging to the jth type

This equation is based on the fact that the probability of a randomly sampled single strain will belong to the jth group is nj/N, and the probability the 2 random strains will belong to that group is $nj\ (nj - 1)\ /\ N\ (N\text{-}1)$. All of these probabilities are summed to give the probability that any 2 strains will belong to the same group. This final figure is subtracted from 1 to give the index value (DI) ((Hunter and Gaston, 1988).

3.3 Results

3.3.1 Patients

3.3.1.1 Sternal surgical site infection due to S. aureus in cardiothoracic surgery patients

Six cardiothoracic surgery patients on the cardiac surgery critical care unit had a SSI in their sternal wound due to *S. aureus.* A total of 22 pure cultures of *S. aureus* were obtained from these patients following microbial culture of their clinical specimens. Patient demographics are given in table 3-6.

Table 3-6 - Patient demographics, surgical procedures and infection type of patients with sternal SSI

	Patients with sternal SSI
Mean age (years)	64
Age range (years)	44-75
Male	5
Female	1
Surgical procedure: CABG	5
MVR	1
Infection type:	
Superficial	2
Deep	4

3.3.1.2 CR-BSI due to CoNS in stem cell transplant patients

Thirty-three SCT patients had a clinical diagnosis of CR-BSI due to CoNS acquired during their stay on the BMT unit. These patients had a total of 36 episodes of CR-BSI due to CoNS from which 49 pure cultures were obtained. Patient demographics, clinical presentation and therapy type are given in table 3-7.

Table 3-7 – Patient demographics, CVC type, clinical presentation and type of transplant undergone by patients with CR-BSI

	Patients with CR-BSI
Mean age (years)	42
Age range (years)	18-71
Male	20
Female	13
CVC type:	
Hickman line	26
PICC	4
Short-term non-tunnelled C/L	3
Clinical presentation	
Acute lymphoblastic leukaemia (ALL)	1
Acute myeloid leukaemia (AML)	6
Chronic myeloid leukaemia (CML)	3
Myelodysplasia (MDS)	3
Non-Hodgkins lymphoma (NHL)	11
Hodgkins lymphoma (HL)	3
Multiple myeloma (MM)	4
Ewings sarcoma	1
Polyneuropathy, organomegaly, endocrinopathy, M protein and skin changes (POEMS).	1
Stem cell transplant type	
Allogeneic sibling PBSC	5
Mini allogeneic sibling PBSC	4
Allogeneic (matched unrelated donor) PBSC	1
Mini allogeneic (matched unrelated donor) PBSC	2
Allogeneic (matched unrelated donor) BMT	5
Autologous PBSC	16

3.3.2 Biotyping

3.3.2.1 Sternal surgical site infection due to S. aureus in cardiothoracic surgery patients

All bacterial isolates examined in this section of the study were confirmed as *S. aureus* by the API ID32 STAPH system. Of the 22 isolates of *S. aureus*, 8 distinct biotypes were identified, these biotypes and the frequency at which they occurred are shown in table 3-8.

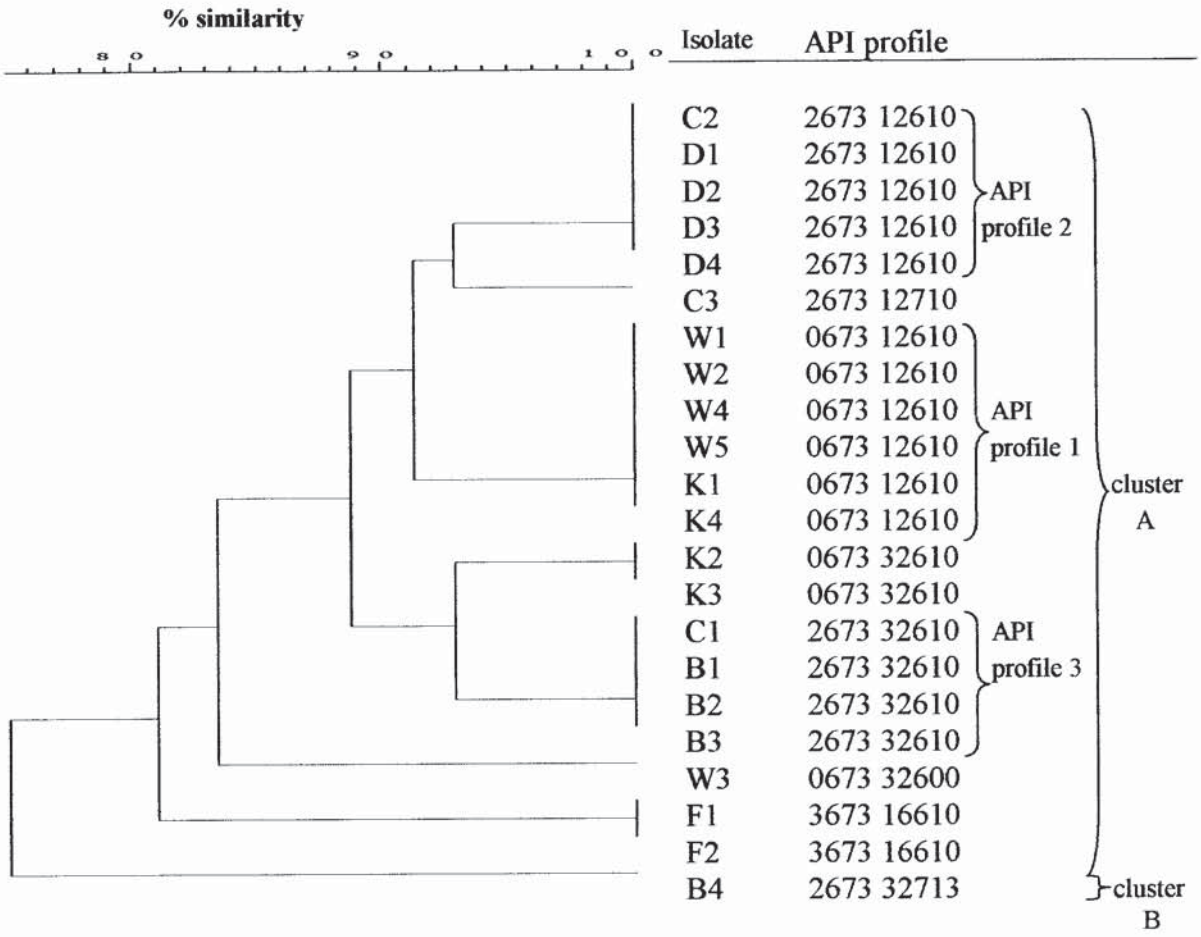
The level of similarity of the 22 isolates is illustrated by means of a dendrogram (Figure 3-2). Each patient is represented by the letter B, C, D, F, K or W. Only 2 out of 6 patients had biochemically identical isolates from multiple clinical specimens. Twenty-one out of 22 isolates were grouped into 1 cluster (A) which were 81% similar biochemically, the remaining isolate, B4 had 75% similarity to this cluster (B). On 3 occasions, isolates from different patients were identified as being identical (API profiles 1, 2 and 3).

All 22 (100%) isolates of *S. aureus* studied were typeable by API ID32 STAPH and achieved a DI of 0.86 from patients with sternal SSI.

Table 3-8 Biochemical profile numbers of 22 isolates of *S. aureus* determined by API ID32 STAPH and their frequency of occurrence

API profile number	API ID32 STAPH profile	Frequency of occurrence (%)
1	0673 12610	6 (27)
2	2673 12610	5 (23)
3	2673 32610	4 (18)
4	0673 32610	2 (9)
5	3673 16610	2 (9)
6	2673 12710	1 (5)
7	0673 32600	1 (5)
8	2673 32713	1 (5)

Figure 3-2 UPGMA dendrogram analysis of API biotyping profiles achieved on multiple isolates of *S. aureus* recovered from 6 patients (B, C, D, F, K, W) with sternal surgical site infection



3.3.2.2 CR-BSI due to CoNS in stem cell transplant patients

Five different species of CoNS were identified from SCT patients with a diagnosis of CR-BSI. Of the 49 isolates, 48 (98%) were typeable by API ID32 STAPH. Thirty-six distinct biotypes were identified. The species and biotypes identified and the frequency at which they occurred are shown in table 3-9.

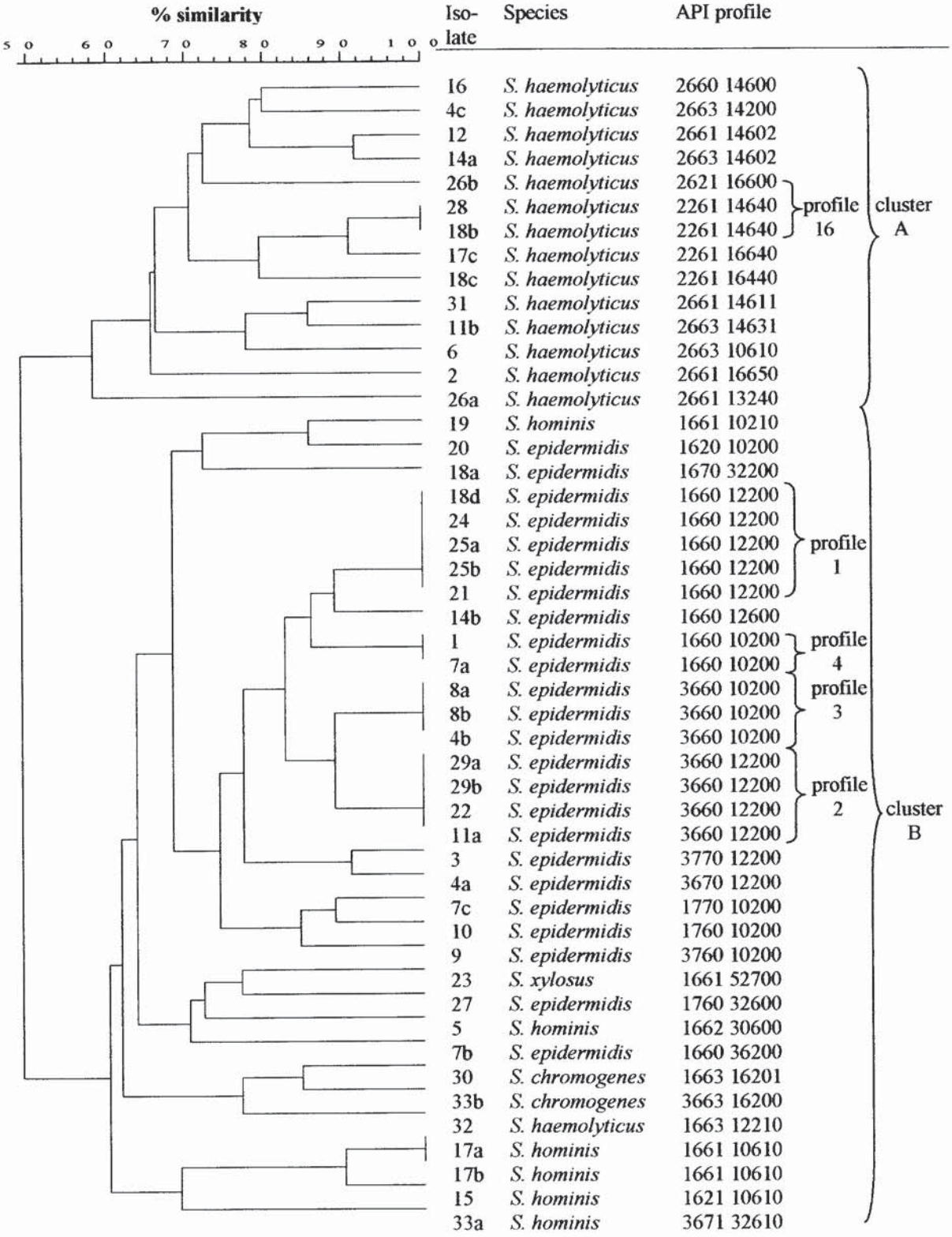
The relatedness of the 48 isolates is illustrated by means of a dendrogram (figure 3-3). Each patient is represented by numbers 1 to 33. The overall biochemical similarity of the isolates of CoNS from this patient group was 49%. Two major clusters were generated, cluster A at 59% similarity and cluster B at 60% similarity. UPGMA dendrogram analysis effectively grouped together strains from the same species. Indeed, all strains in cluster A were identified as *S. haemolyticus* and cluster B comprised of mainly *S. epidermidis*. Of the 11 patients with multiple episodes of CR-BSI, patients 8, 17, 25 and 29 had strains from 2 episodes identified as being identical (figure 3-3). UPGMA dendrogram analysis also identified 5 API profile groups, which contained isolates from different patients (API profile numbers 1-4 and 16,) (table 3-9).

Biotyping of CoNS from SCT patients with CR-BSI achieved a DI of 0.98.

Table 3-9 Species and biochemical profile numbers of 48 isolates of CoNS determined by API ID32 STAPH and their frequency of occurrence

Species	Overall frequency (%)	API profile number	API ID32 STAPH profile	Overall frequency (%)
S. epidermidis	24 (50)	1	1660 12200	5 (10)
		2	3660 12200	4 (8)
		3	3660 10200	3 (6)
		4	1660 10200	2 (4)
		5	1660 10200	2 (4)
		6	1620 10200	1 (2)
		7	1670 32200	1 (2)
		8	1660 12600	1 (2)
		9	3770 12200	1 (2)
		10	3670 12200	1 (2)
		11	1770 10200	1 (2)
		12	1760 10200	1 (2)
		13	3760 10200	1 (2)
		14	1760 32600	1 (2)
		15	1660 36200	1 (2)
S. haemolyticus	15 (31)	16	2261 14640	2 (4)
		17	2660 14600	1 (2)
		18	2663 14200	1 (2)
		19	2661 14602	1 (2)
		20	2663 14602	1 (2)
		21	2621 16600	1 (2)
		22	2261 16640	1 (2)
		23	2261 16440	1 (2)
		24	2661 14611	1 (2)
		25	2663 14631	1 (2)
		26	2663 10610	1 (2)
		27	2661 16650	1 (2)
		28	2661 13240	1 (2)
		29	1663 12210	1 (2)
S. hominis	6 (13)	30	1661 10610	2 (4)
		31	1661 10210	1 (2)
		32	1662 30600	1 (2)
		33	1621 10610	1 (2)
		34	3671 32610	1 (2)
S. chromogenes	2 (4)	35	1663 16201	1 (2)
		36	3663 16200	1 (2)
S. xylosus	1 (2)	37	1661 52700	1 (2)

Figure 3-3 UPGMA dendrogram analysis of API biotyping profiles achieved on 48 isolates of CoNS recovered from SCT patients with CR-BSI



3.3.3 Antibiograms

The standard BSAC disc diffusion method was used to determine the antibiograms of Staphylococcal isolates and is illustrated in figure 3-4.

Figure 3-4 Disc diffusion sensitivity testing by the BSAC method

3.3.3.1 Sternal surgical site infection due to S. aureus in cardiothoracic surgery patients

The antibiotic resistance patterns of the 22 isolates of *S. aureus* recovered from specimens from patients with sternal SSI and the percentage occurrence are shown in table 3-10. Five distinct antibiograms of *S. aureus* were identified by antibiotic sensitivity testing. The frequency of resistance of the 22 isolates to each of the antibiotics tested is shown in table 3-11. The level of similarity between the isolates based on antibiograms is illustrated by means of a dendrogram (figure 3-5). Three out of 6 (50%) patients had microorganisms yielding identical antibiogram profiles from all multiple specimens. Isolates from 5 patients (16 isolates) had antibiogram type 1. The remaining strains generated a cluster with an antibiogram similarity of 38%. All 22 (100%) strains of *S. aureus* were typeable by this method. The DI achieved for the use of this antibiogram panel, on this set of microorganisms was 0.47.

Table 3-10 Antibiograms of 22 isolates of *S. aureus* and their frequency of occurrence

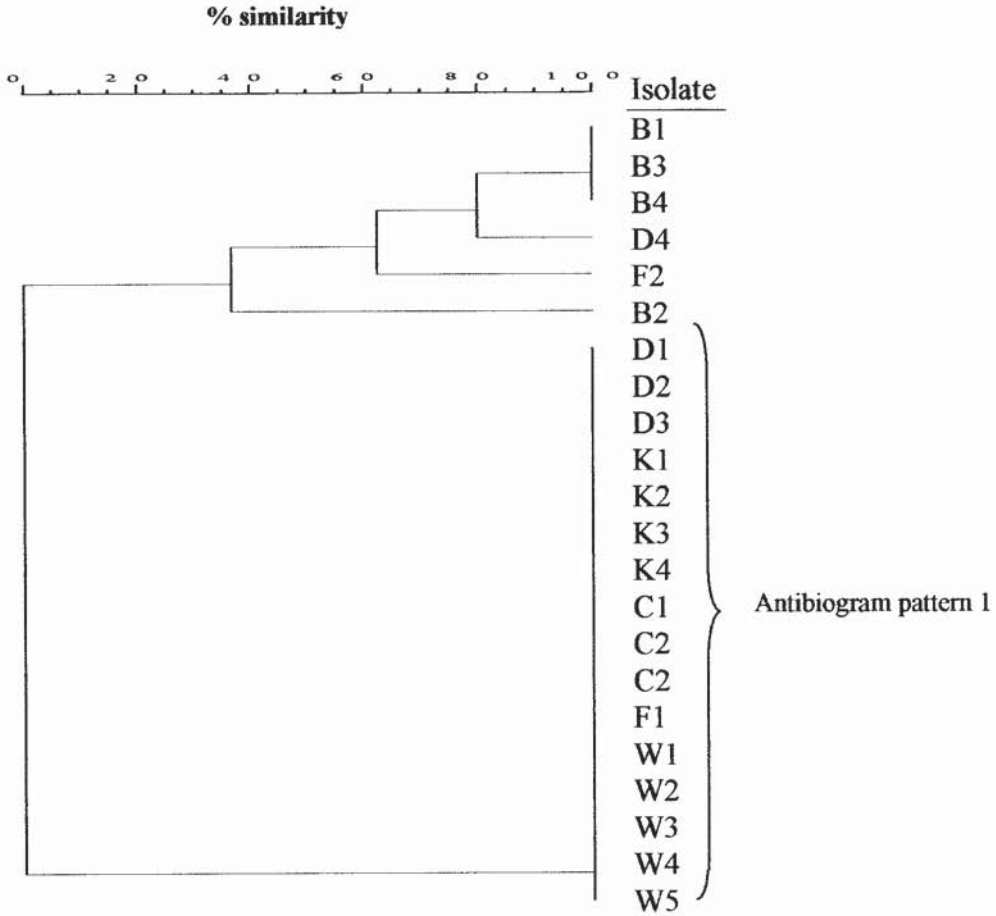
Antibiogram pattern number	Antibiotics									Frequency (%)	Strains
	FLU	ERY	TET	RIF	TRI	FUS	MUP	GEN	VAN		
1	S	S	S	S	S	S	S	S	S	16 (73%)	D1, D2, D3, K1, K2, K3, K4, C1, C2, C3, F1, W1, W2, W3, W4, W5.
2	R	R	S	S	S	S	S	S	S	3 (14%)	B1, B3, B4.
3	S	R	S	S	S	S	S	S	S	1 (5%)	B2
4	R	R	R	S	S	S	S	S	S	1 (5%)	D4
5	R	S	S	S	S	S	S	S	S	1 (5%)	F2

Key: FLU = flucloxacillin, ERY = erythromycin, TET = tetracycline, RIF = rifampicin, TRI = trimethoprim, FUS = fusidin, MUP = mupirocin, GEN = gentamicin, VAN = vancomycin.

Table 3-11 Percentage of strains of *S. aureus* resistant to the antibiotic used in the test panel

Antibiotic	Number of isolates of *S. aureus* resistant (%)
Flucloxacillin	5 (23)
Erythromycin	5 (23)
Tetracycline	1 (5)
Rifampicin	0 (0)
Trimethoprim	0 (0)
Fusidin	0 (0)
Mupirocin	0 (0)
Gentamicin	0 (0)
Vancomycin	0 (0)

Figure 3-5 UPGMA dendrogram analysis of antibiogram types achieved on multiple isolates of *S. aureus* recovered from 6 patients with sternal surgical site infection



3.3.3.2 CR-BSI due to CoNS in stem cell transplant patients

The percentage occurrence of antibiotic resistance patterns of the 49 isolates of CoNS recovered from SCT patients with CR-BSI are shown in table 3-12. Twenty-six distinct antibiograms of CoNS were identified by sensitivity testing. The frequency of resistance of the 49 isolates to each of the antibiotics tested is shown in table 3-13. The level of similarity between the isolates based on antibiograms is illustrated by means of a dendrogram (figure 3-6).

Table 3-12 Antibiograms of 49 isolates of CoNS and their frequency of occurrence

Antibiogram pattern number	Antibiotics												Frequency (%)	Strains
	FLU	ERY	TET	RIF	TRI	FUS	CLI	MUP	GEN	LIN	Q/D	VAN		
1	R	R	S	S	R	R	R	S	R	S	S	S	6 (12)	4a, 4b, 4c, 11a, 11b, 18d
2	R	R	S	S	R	R	R	R	S	S	S	S	3 (6)	2, 3, 14b
3	R	R	S	S	S	S	R	S	S	S	S	S	3 (6)	7a, 7b, 25b
4	R	R	S	S	R	R	R	S	S	S	S	S	3 (6)	9, 15, 22
5	R	R	S	S	R	S	R	R	S	S	S	S	3 (6)	13,19,29a
6	R	R	S	S	S	R	R	S	S	S	S	S	3 (6)	20, 23, 25a
7	R	R	R	S	R	R	R	R	S	S	S	S	3 (6)	26a, 26b, 33a
8	R	R	R	S	R	S	R	S	R	S	S	S	2 (4)	6, 14a
9	R	R	R	R	S	S	R	S	S	S	S	S	2 (4)	8a, 8b
10	R	R	S	S	R	S	R	S	R	S	S	S	2 (4)	12, 18b
11	R	R	R	S	R	R	R	R	R	S	S	S	2 (4)	16, 31
12	R	R	R	S	R	S	R	S	S	S	S	S	2 (4)	17c, 28
13	R	R	R	S	R	R	R	S	S	S	S	S	2 (4)	18c, 24
14	R	R	S	S	S	S	S	S	S	S	S	S	1 (2)	7c
15	R	S	S	S	R	S	S	S	S	S	S	S	1 (2)	5
16	S	R	S	S	R	S	R	S	R	S	S	S	1 (2)	10
17	R	S	R	S	R	R	S	S	R	S	S	S	1 (2)	17a
18	R	S	R	S	R	R	S	S	S	S	S	S	1 (2)	17b
19	S	R	S	S	R	R	R	S	R	S	S	S	1 (2)	18a
20	S	R	R	S	S	S	R	S	S	S	S	S	1 (2)	1
21	R	S	R	S	R	R	R	R	S	S	S	S	1 (2)	21
22	R	R	S	S	S	S	R	S	R	S	S	S	1 (2)	27
23	S	R	S	S	R	S	R	R	S	S	S	S	1 (2)	29b
24	R	R	R	S	R	R	R	S	R	S	S	S	1 (2)	30
25	S	R	S	S	R	R	R	R	R	S	S	S	1 (2)	32
26	S	R	S	S	R	R	R	R	R	S	S	S	1 (2)	33b

Key: FLU = flucloxacillin, ERY = erythromycin, TET = tetracycline, RIF = rifampicin, TRI = trimethoprim, FUS = fusidin, CLI = clindamycin, MUP = mupirocin, GEN = gentamicin, LIN = linezolid, Q/D = quinupristin/dalfopristin, VAN = vancomycin.

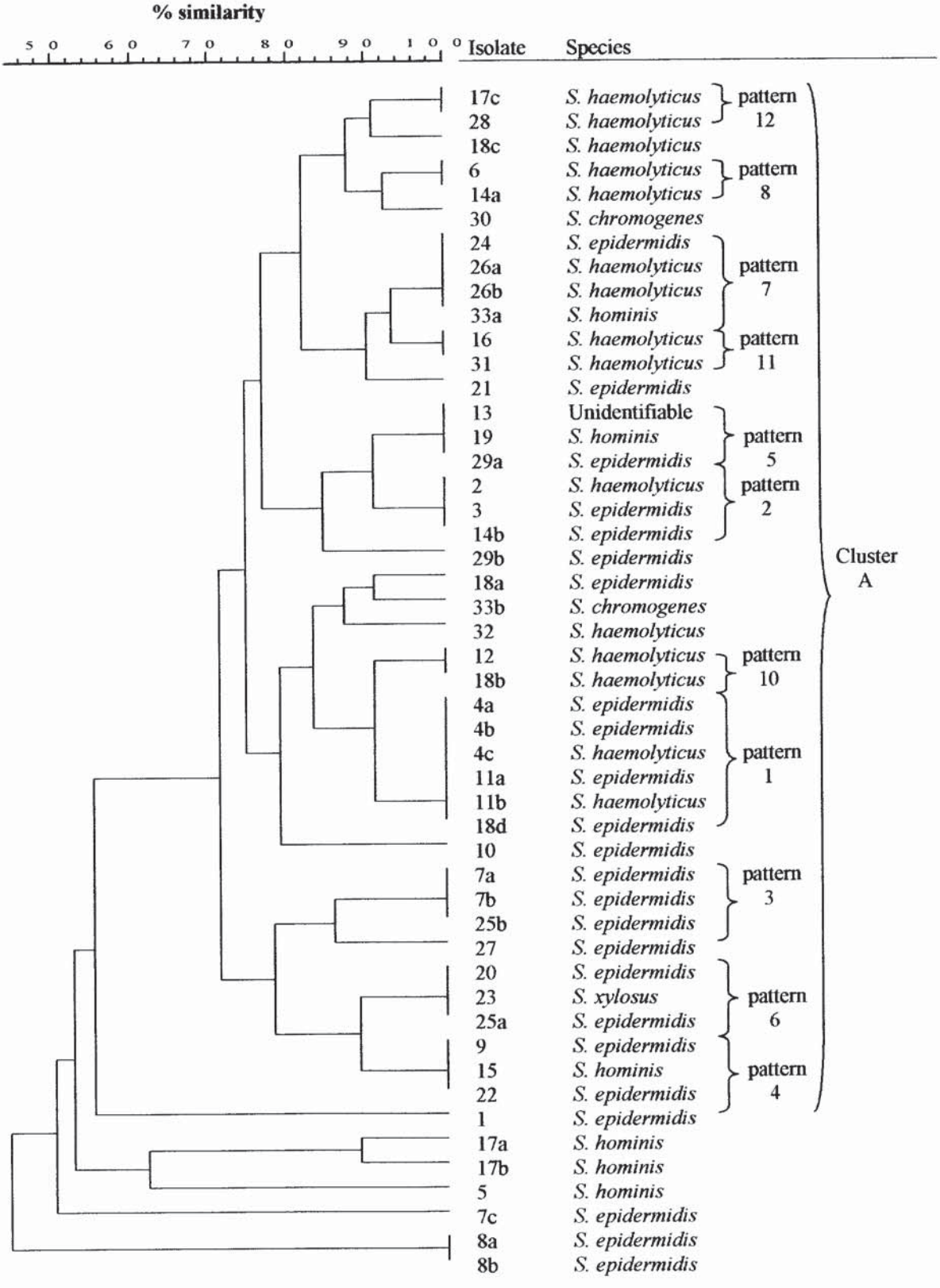
Table 3-13 Percentage of strains of CoNS resistant to the antibiotics used in the test panel

Antibiotic	Number of isolates of CoNS resistant (%)
Flucloxacillin	88%
Erythromycin	92%
Tetracycline	37%
Rifampicin	1%
Trimethoprim	78%
Fusidin	59%
Clindamycin	92%
Mupirocin	31%
Gentamicin	39%
Linezolid	0%
Quinupristin/dalfopristin	0%
Vancomycin	0%

The overall similarity of all isolates from this patient group was 46%. Forty-two out of the 49 (86%) isolates typed generated a major cluster at 72% similarity (cluster A) (figure 3-6). However, strains of the same species did not always cluster together. In fact, within each of the antibiogram patterns 1-2 and 4-7, different species of CoNS were identified (figure 3-6). Using this typing method, 5 out of 11 (45%) patients (patients 4, 7, 8, 11 and 26) with multiple episodes of CR-BSI had strains with identical antibiograms from at least 2 separate infectious episodes. Conversely, 11 different antibiogram groups (patterns 1-8 and 10-12) contained strains from different patients.

Antibiogram analysis of these patients resulted in a 100% typeability rate and a DI of 0.96.

Figure 3-6 UPGMA dendrogram analysis of antibiograms achieved on isolates of CoNS recovered from SCT patients with CR-BSI

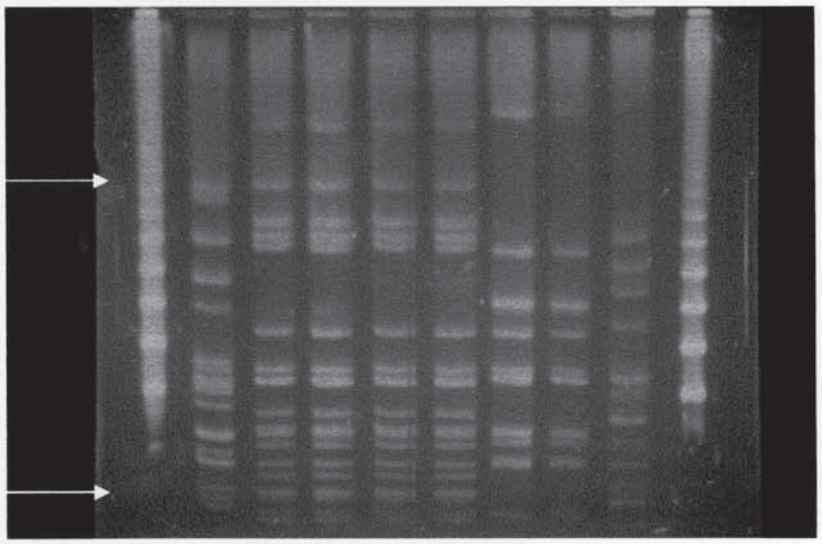


3.3.4 Pulsed-field gel electrophoresis

3.3.4.1 Sternal surgical site infection due to S. aureus in cardiothoracic surgery patients

Twenty-one out of 22 (95%) isolates of *S. aureus* implicated in sternal SSI were typeable by PFGE. Macrorestriction analysis of the 21 isolates produced a range of genetic profiles. All of the isolates yielded 7 to 11 fragments by PFGE within the 48.5 to 485kb range. Figure 3-7 shows the macrorestriction profiles of isolates from patients C and K.

Figure 3-7 Macrorestriction profiles of *S. aureus* isolates recovered from patients C and K with sternal surgical site infection

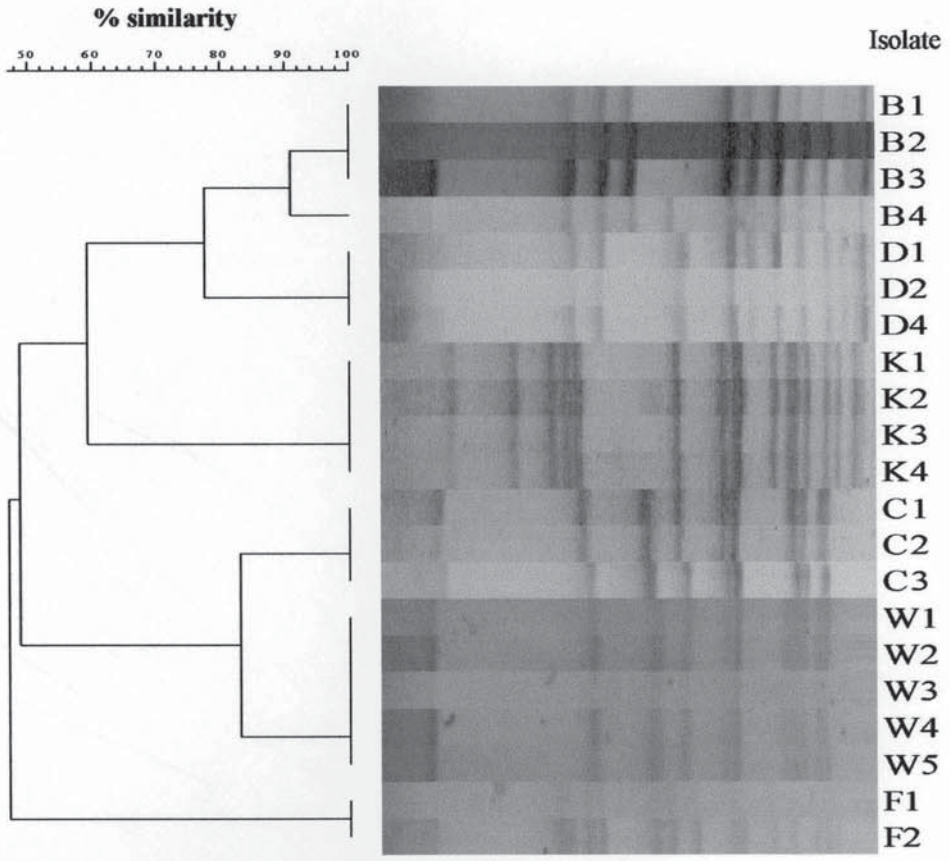


Lane number	1	2	3	4	5	6	7	8	9	10
Isolate number			K1	K2	K3	K4	C1	C2	B4	
Relatedness			*	*	*	*	†	†		

Lanes 1 and 10 = molecular weight size standard, lane 2 = control strain NCTC 11047. Identical 'relatedness' symbols represent identical genotypes.

The level of genetic similarity between the isolates of *S. aureus* based on macrorestriction profiles is illustrated by means of a dendrogram (figure 3-8). Overall, 7 distinct genotypes were identified at a genetic similarity of 47%. Characterisation by PFGE revealed that multiple isolates from all patients were found to be identical with the exception of patient B, whereby isolate B4 was 91% similar to isolates B1, B2 and B3 (2 bands difference). There was no common genotype when comparing isolates from different patients. However, isolates B1, B2 and B3 had only 2 bands difference when compared to isolates from patient D (78% similarity). There was also only 2 bands different between isolates from patients C and W (83% similarity). PFGE analysis of strains of *S. aureus* recovered from patients with sternal SSI resulted in a DI of 0.88.

Figure 3-8 UPGMA dendrogram analysis of macrorestriction profiles achieved from multiple isolates of *S. aureus* recovered from 6 patients with sternal surgical site infection

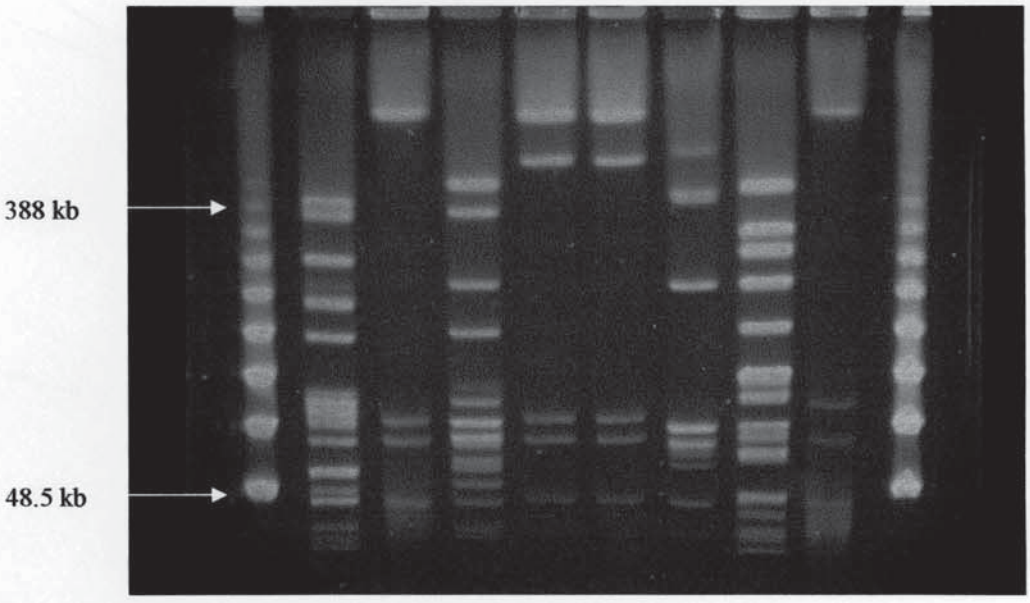


Multiple isolates were recovered from patients who were represented by the letters B, C, D, F, K and W.

3.3.4.2 CR-BSI due to CoNS in stem cell transplant patients

Forty-seven out of 49 (96%) isolates of CoNS implicated in CR-BSI were typeable by PFGE. One of the non-typeable isolates was also non-typeable by API ID32 STAPH (isolate 13). Macrorestriction fragment analysis of the 23 typeable isolates of *S. epidermidis* produced a range of restriction profiles all of which had 10 to 13 fragments within the 48.5 to 485 kb range. The 15 isolates of *S. haemolyticus* produced macrorestriction profiles with between 7 and 14 fragments within this molecular weight designation. All of the 6 isolates of *S. hominis* identified produced profiles with 3 to 8 fragments within the 48.5 to 485 kb range. Finally, all isolates of *S. chromogenes* and *S. xylosus* produced 10 restriction fragments within these molecular weight limits. Figure 3-9 shows the macrorestriction fragment profiles of CoNS isolates implicated in CR-BSI, lanes 3, 5 and 6 are *S. hominis*, lanes 4, 7 and 9 are *S. haemolyticus* and lanes 2 and 8 are *S. epidermidis*.

Figure 3-9 Macrorestriction profiles of CoNS isolates implicated in CR-BSI



Lane number	1	2	3	4	5	6	7	8	9	10
Isolate number			15	16	17a	17b	17c	18a	19	
Relatedness					*	*				

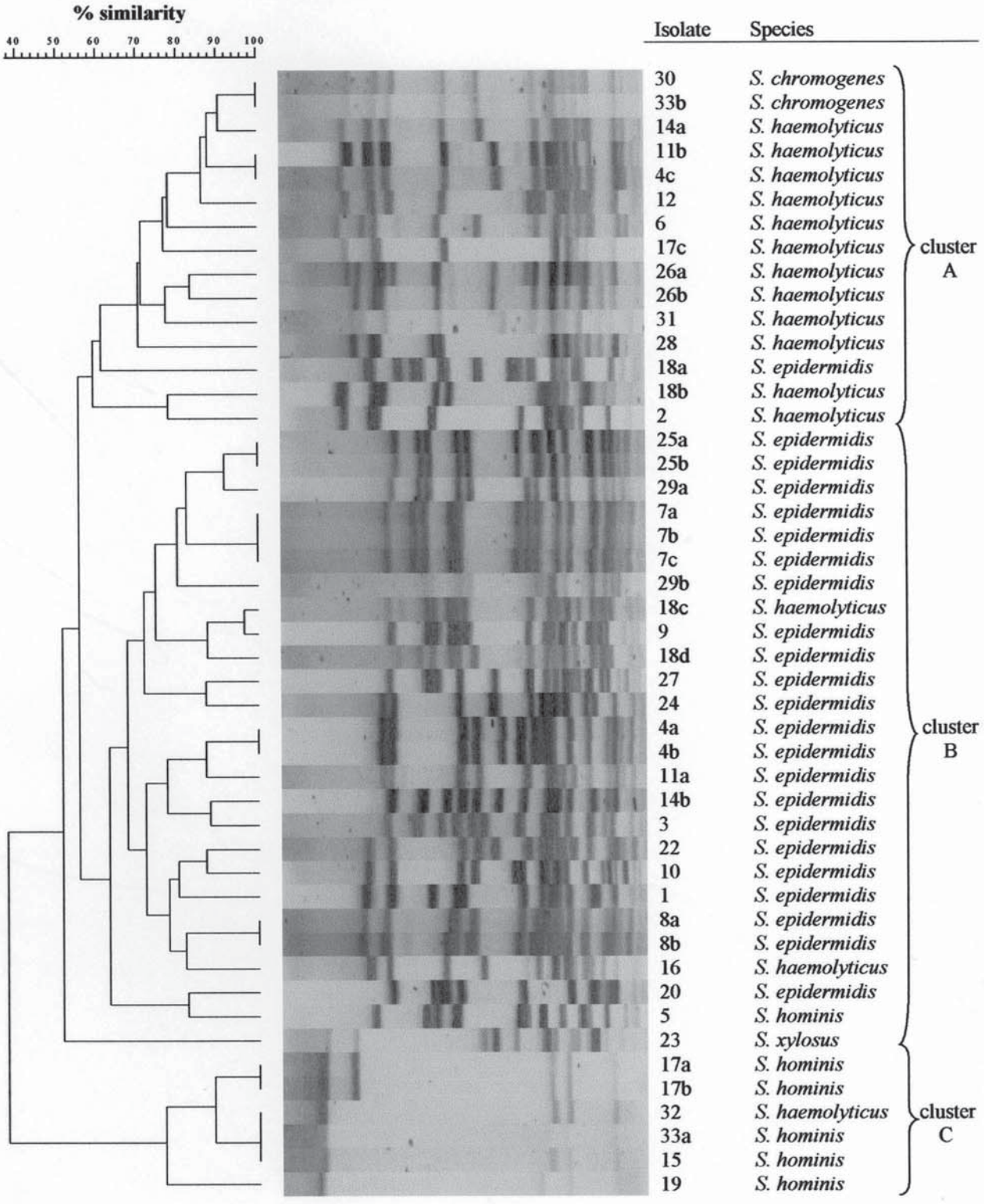
Lanes 1 and 10 = molecular weight size standard, lane 2 = control strain NCTC 11047. Identical 'relatedness' symbols represent identical genotypes.

The level of similarity between the isolates of CoNS based on these macrorestriction fragment profiles is illustrated by means of a dendrogram (figure 3-10). This dendrogram not only demonstrates genetic heterogeneity between different species of CoNS but also the heterogeneous nature of isolates within *S. epidermidis*, *S. haemolyticus* and *S. hominis*, respectively. Overall, 37 distinct genotypes were identified with a genetic similarity of 38%. At 62% similarity, 3 main clusters were generated, cluster A containing primarily *S. haemolyticus*, cluster B containing mainly *S. epidermidis* and cluster C containing mostly *S. hominis*. Cluster C also contained genetically identical species of CoNS (isolates 32, 33a and 15) with differing biotypes as determined by API ID32 STAPH.

Using PFGE, 5 out of 11 (45%) patients (patients 4, 7, 8, 17 and 25) who presented with multiple episodes of CR-BSI had isolates with identical macrorestriction fragment profiles from at least 2 separate infectious episodes. Conversely, 3 different genotypes contained isolates from different patients.

PFGE typing of isolates from this patient group resulted in a DI of 0.99.

Figure 3-10 UPGMA dendrogram analysis of macrorestriction profiles achieved from isolates of CoNS recovered from SCT patients with CR-BSI

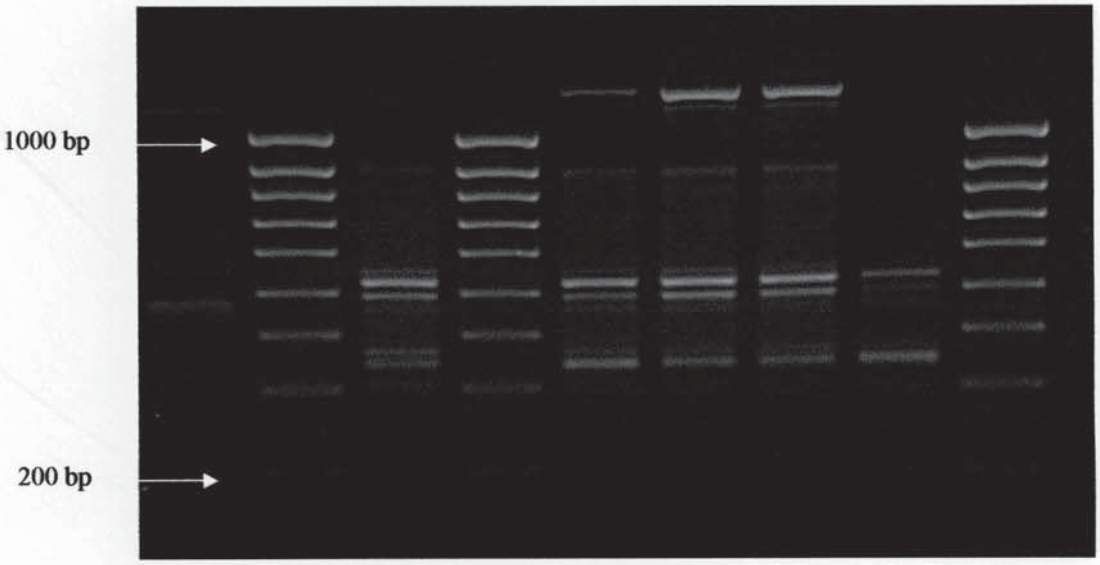


3.3.5 Randomly amplified polymorphic DNA

3.3.5.1 Sternal surgical site infection due to S. aureus in cardiothoracic surgery patients

All 22 isolates of *S. aureus* implicated in sternal SSI were typeable by RAPD. A range of RAPD profiles was produced, all of which yielded 4 to 9 amplicons within the 200 to 1500bp range. Figure 3-11 shows RAPD profiles of *S. aureus* recovered from patient D.

Figure 3-11 RAPD amplicon profiles of *S. aureus* isolates implicated in sternal surgical site infection

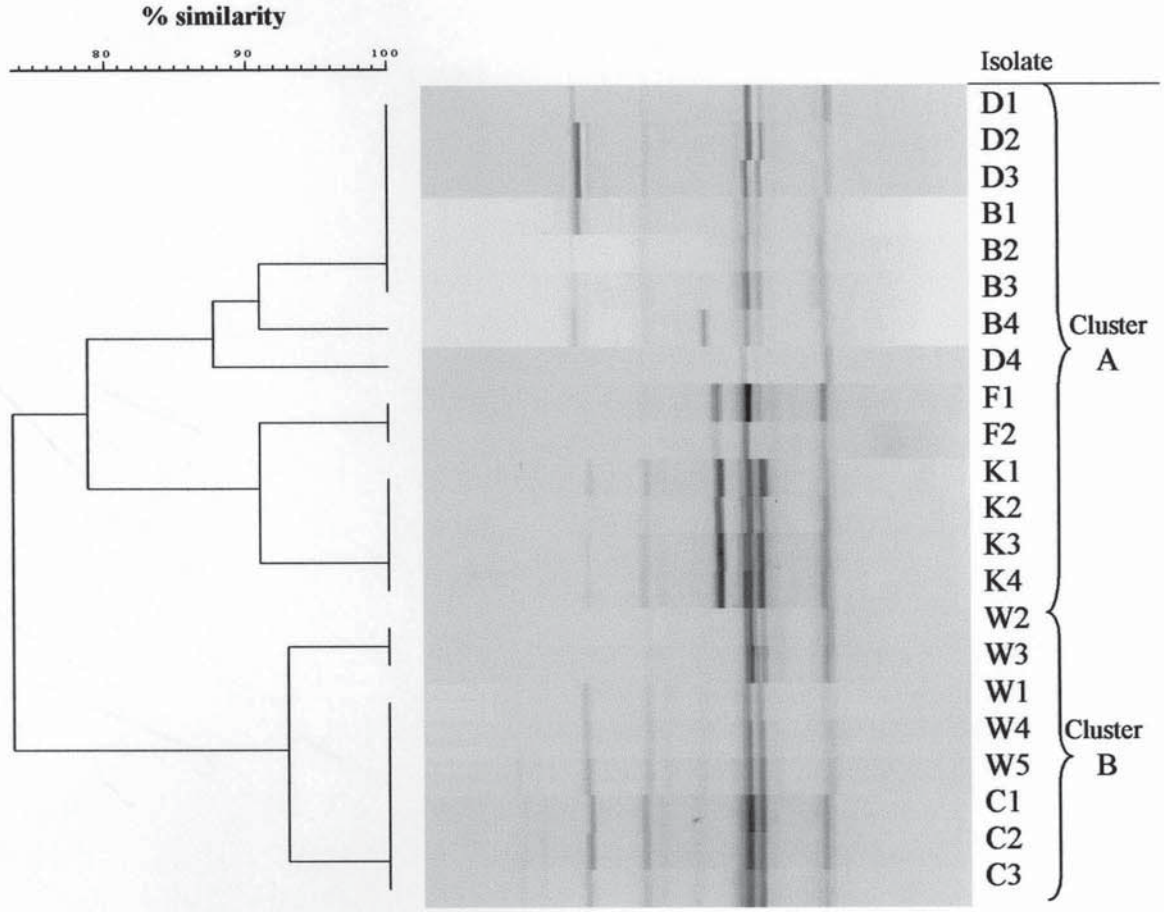


Lane no	1	2	3	4	5	6	7	8	9
Isolate					D1	D2	D4	D5	
Relatedness					*	*	*		

Lane 1 = negative control, lanes 2, 4 and 9 = molecular weight size standard, lane 3 = control strain NCTC 8325.
Identical 'relatedness' symbols represent identical genotypes.

The relationship between *S. aureus* profiles was determined by means of UPGMA dendrogram analysis (figure 3-12). Overall, 7 distinct genotypes were identified at a genetic similarity of 74%. Two main clusters were generated, cluster A at a similarity of 77% and cluster B at 93% similarity. When using RAPD, multiple isolates from individual patients were found to be identical in patients C, F and K. Patients B, D and W had isolates belonging to 2 different genotypes (91%, 88% and 94% similarities respectively). Isolates D1-3 shared the same profile as isolates B1-4, as did isolates W1, 4 and 5 with C1-3. RAPD analysis of these isolates resulted in a DI of 0.84.

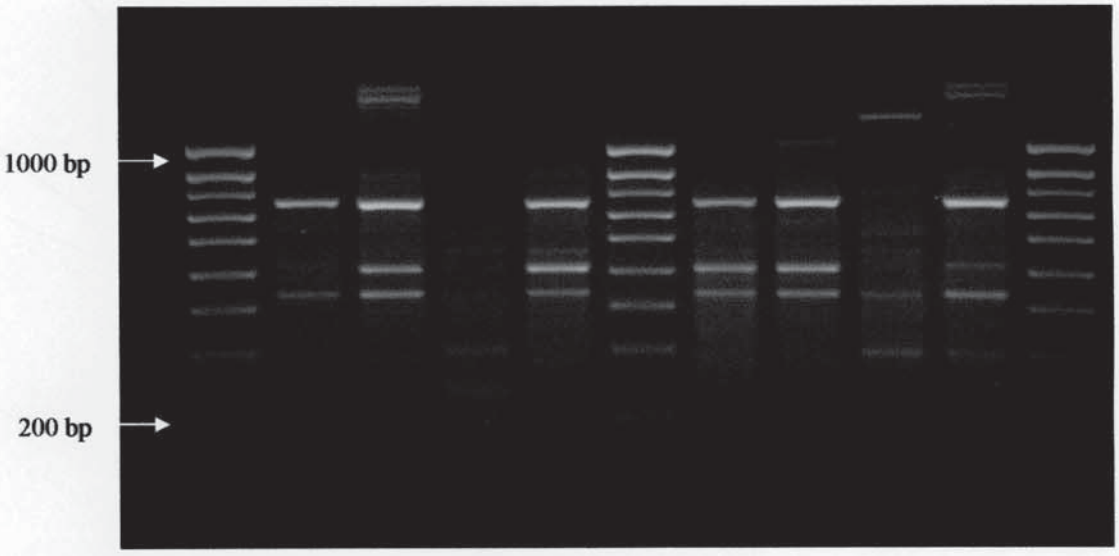
Figure 3-12 UPGMA dendrogram analysis of RAPD profiles achieved from multiple isolates of *S. aureus* recovered from 6 patients with sternal surgical site infection



3.3.5.2 CR-BSI due to CoNS in stem cell transplant patients

Forty-seven out of 49 (96%) isolates of CoNS implicated in CR-BSI were typeable by RAPD. The 2 non-typeable isolates were *S. hominis* and *S. chromogenes*. RAPD analysis of the 24 isolates of *S. epidermidis* produced a range of profiles, all of which had 3 to 6 amplicons within the 200 to 1500bp range. Within the same molecular weight range, the 14 isolates of *S. haemolyticus* had 3 to 11 amplicons. Six to 11 amplicons were achieved from the 6 isolates of *S. hominis* and 7 and 5 amplicons from *S. chromogenes* and *S. xylosus*, respectively. Figure 3-13 shows the RAPD profiles of CoNS isolates implicated in CR-BSI. Lanes 4, 6, 8 and 9 are *S. epidermidis*, lanes 5 and 11 are *S. hominis* and lane 10 is an isolate of *S. haemolyticus*.

Figure 3-13 RAPD amplicon profiles of CoNS isolates implicated in CR-BSI



Lane no	1	2	3	4	5	6	7	8	9	10	11	12
Isolate				1	2	3		4a	4b	4c	5	
Relatedness				*				*			*	

Lane 1 = negative control, lanes 2, 7 and 12 = molecular weight size standard, lane 3 = control strain NCTC 11047.
Identical 'relatedness' symbols represent identical genotypes.

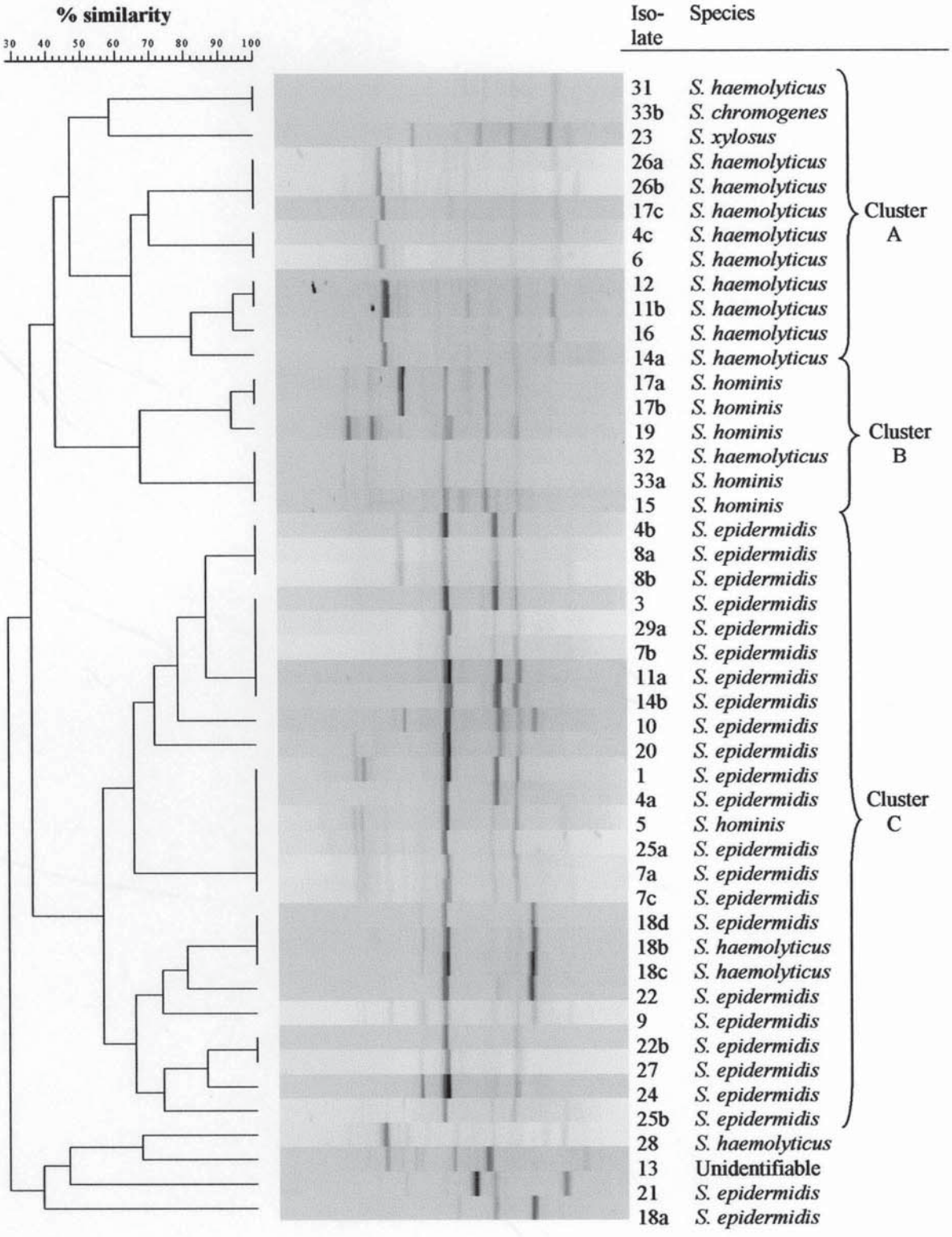
The similarity between CoNS RAPD profiles was determined by UPGMA dendrogram analysis (figure 3-14). The dendrogram demonstrates genetic heterogeneity between both different species of CoNS and also within each species itself as identified by API ID32 STAPH. Overall, 25 distinct profiles were identified at a similarity of 30%. Isolates of *S. haemolyticus* were represented in a cluster at 46% similarity (cluster A). Five out of 6 (83%) isolates of *S. hominis* were clustered at a similarity of 67% (cluster B) and cluster C (58% similarity) comprised mainly *S. epidermidis* isolates. On 4 occasions, isolates from

different species of CoNS (as determined by API ID32 STAPH) were found within the same genotype.

Using RAPD, 5 out of 11 patients (patients 7, 8, 17, 18 and 26) who presented with multiple episodes of CR-BSI had isolates with identical RAPD profiles from at least 2 separate infectious episodes. Conversely, 9 different genotypes contained isolates from different patients.

RAPD typing of isolates from this patient group resulted in a DI of 0.96.

Figure 3-14 UPGMA dendrogram analysis of RAPD profiles achieved from isolates of CoNS recovered from SCT patients with CR-BSI



3.4 Discussion

The aim of this work was to evaluate phenotypic and genotypic typing methods to assess the relatedness of isolates of *S. aureus* from patients with sternal SSI and isolates of CoNS from SCT patients with CR-BSI. In both patient groups the microorganisms typed during this study are the most frequently implicated in each respective infection (Mossad *et al.*, 1997; Coello *et al.*, 2003).

The results of biotyping isolates of *S. aureus* from 6 patients with sternal SSI showed that only 2 patients had all their isolates from each specimen belonging to 1 biotype. This may be due to the sternal SSI in 4 of the patients being due to multiple strains of *S. aureus*. Alternatively, the differences in biotype could be due to subjectivity and difficulty in the interpretation of the biochemical reactions of the API strip resulting in several sub-types (Geary *et al.*, 1997). On 3 occasions, isolates from different patients were identified as being biochemically identical, thus suggesting cross-transmission of *S. aureus* on the cardiac surgery critical care unit during the study period. However, the DI of biotyping *S. aureus* by API ID32 STAPH was only 0.86. Hunter and Gaston (1988) suggested that an index value greater than 0.90 is desirable if the typing results are to be interpreted with confidence. Based on this guideline, it is unlikely that the isolates of *S. aureus* were identical, thus suggesting that cross-transmission between these patients had not occurred. The biotyping of *S. aureus* isolates by API ID 32 STAPH however, had an excellent typeability rate of 100%.

The typeability of isolates of CoNS from SCT patients with CR-BSI by biotyping was also high (98%). The fact that 2% of isolates were not typeable by this method may reflect the wide heterogeneity of clinical isolates of CoNS compared to *S. aureus*. Indeed, the API ID32 STAPH system may not be sufficiently discriminatory to identify those strains of CoNS less frequently encountered in clinical practice. However, this biotyping technique proved to be more discriminative for CoNS (DI=0.98) than for *S. aureus*. This may suggest that patients who suffered multiple episodes of CR-BSI and had isolates recovered from more than 1 episode identified as being identical by this system may, indeed have had a recurrent infection caused by the same microorganism. Reinfection may have occurred due to inadequate antimicrobial therapy or due to the infected CVC not being removed. The high discriminatory power of biotyping CoNS may also suggest that CoNS with identical API profiles recovered from different patients with CR-BSI may have originated

from a common source. Biotyping, however has traditionally produced poor discriminatory results (Weller, 2000). The subjective interpretation of biochemical reaction as previously reported by Geary and co-workers (1997), in this instance may have resulted in the generation of too many sub-types to effectively cluster outbreak-related strains of CoNS. This phenomenon has previously been observed in a study conveyed by Tenover and colleagues (1994) whereby biotyping was employed to type isolates of *S. aureus*.

Antimicrobial sensitivity testing against a panel of 9 antibiotics divided the 22 isolates of *S. aureus* into just 5 antibiogram types. Isolates of *S. aureus* from 3 patients had identical antibiogram profiles from multiple specimens and isolates from 5 patients belonged to 1 antibiogram type. These results suggest that antibiogram typing is less discriminatory than biotyping. Indeed, for *S. aureus* a DI of only 0.47 was achieved using this typing method. This concurs with results from previous studies using antibiogram typing to characterise *S. aureus* (Tenover *et al.*, 1994). It is believed that the low discriminatory power of this method is, in part due to the abundance of antibiotic resistance genes in the clinical environment (Lang *et al.*, 1999). However, in this study, discriminatory power may be low as the isolates of *S. aureus* were sensitive to the antibiotics in the panel used. Indeed, 73% of *S. aureus* isolates were sensitive to all antibiotics tested. The discriminatory power may be improved if a larger panel of antibiotics were included.

The discriminatory power of antimicrobial sensitivity testing for CoNS during this study was found to be considerably higher than for *S. aureus* (0.96). This may be in part due to the increased number of antibiotics tested against CoNS compared to *S. aureus*. However, this enhanced DI value for CoNS may also reflect the heterogeneic nature of the bacterial population studied. The high DI may provide evidence for 5 patients who presented with multiple episodes of CR-BSI having being due to a recurrent infection caused by the same microorganism. Furthermore, results generated by antibiograms suggest that cross-transmission of CoNS between patients may have occurred on 11 occasions. However, the fact that antibiogram typing did not cluster together members of the same species and members of different species were grouped together in specific antibiograms suggests that either the discriminatory power is not as high as the DI value suggests or the API identifications were inaccurate. Previous studies have also found that typing based on antibiotic sensitivity patterns for CoNS has produced results with poor discriminatory value (Geary *et al.*, 1997; Herbert *et al.*, 1988). However, since antibiogram typing has an excellent typeability record which was reflected with a 100% rate during this study and is

the cheapest typing method, it may have a valid use in a routine clinical microbiology laboratory with limited resources (Tenover *et al.*, 1994). To improve the discriminatory power of antibiogram typing it is suggested that a quantitative approach based on measurements of zones of inhibition is used (Tenover *et al.*, 1994; Weller, 2000). Care needs to be taken when interpreting antibiograms based on a limited number of antibiotics and where local common patterns of resistance predominate.

Genotyping of *S. aureus* by PFGE identified more distinct types than with the 2 phenotypic typing techniques. Indeed, a DI of 0.88 was achieved during this section of the study. This reflects results generated in previous studies (Weller, 2000). When using this typing technique, multiple isolates recovered from individual patients were genotypically identical with the exception of 1 patient. Furthermore, there was no common genotype of *S. aureus* recovered from different patients, indicating that cross-transmission did not occur during the study period. However, if the criteria of Tenover and colleagues (1995) is applied for interpreting chromosomal DNA restriction patterns produced by PFGE, some strains appear more related. The criteria states that strains with 2-3 band differences are classified as 'closely related' as they are consistent with a single genetic event (a point mutation or an insertion or deletion of DNA). Application of this criteria demonstrates that isolates from patient B and D and those from C and W are closely related genetically which may suggest that a common genotype of *S. aureus* predominates on the cardiac surgery critical care unit. Despite PFGE having a typeability rate of 100% for *S. aureus* isolates, during this study, 1 isolate (5%) was non-typeable (Tenover *et al.*, 1994).

The discriminatory power of PFGE for typing CoNS (0.99) during this study was higher than that achieved for *S. aureus* and also both phenotypic methods assessed. Again, as with *S. aureus*, the superiority of PFGE compared to phenotypic techniques for typing CoNS is well-documented. Indeed, Toldos and co-workers (1997) concluded that had only the biotype and antibiogram type been determined in an investigation into bacteraemias caused by CoNS in immunocompromised patients, several bacteraemic episodes would have been incorrectly assessed. In addition, PFGE has been found to have a superior discriminatory power when compared to other genotypic techniques (Wang *et al.*, 2003). During this section of the study, isolates of the same species were effectively clustered together by UPGMA analysis. On only 1 occasion were 2 isolates from different species (as determined by API) identified as the same genotype. However, this may, be due to the subjective interpretation of biochemical reactions which generate the biotype profile number. The high discriminative power of PFGE for typing CoNS achieved during this

study would allow a confident suggestion that 5 patients presented with reinfection due to the same microorganism, and that on 3 occasions cross-transmission of CoNS may have occurred. Despite this, the heterogeneic nature of the CoNS population is demonstrated when using PFGE. Strains of *S. epidermidis* and *S. haemolyticus*, however, exhibited greater genomic diversity compared to *S. hominis* whose macrorestriction profiles contained less DNA fragments. Pulsed-field gel electrophoresis is the gold standard for genotyping many species of microorganisms. However, Van Belkum and colleagues (1998) suggested that standardisation of PFGE methodologies is required to effectively compare isolates from different laboratories. Guidelines for the implementation of standardisation of this technique for MRSA has now been described (Murchan *et al.*, 2003). The disadvantages of using PFGE for characterisation of microorganisms is that it is laborious, time consuming, results are frequently retrospective and specialised equipment is necessary (Matushek *et al.*, 1996). However, several approaches have been investigated to improve the methodology (Matushek *et al.*, 1996; Chang and Chui, 1998; Leonard and Carroll, 1997).

The typeability of *S. aureus* by RAPD conditions outlined in this study was 100%. Multiple isolates of *S. aureus* from 3 patients with sternal SSI were genetically identical. Isolates from the remaining 3 patients may suggest that the infections were caused by more than 1 strain of *S. aureus* or that these microorganisms were subjected to a single genetic event (from a common progenitor) which may be reflected in their high percentage of similarity. On 2 occasions, 2 different patients yielded genetically identical isolates of *S. aureus* as characterised by RAPD suggesting cross-transmission of *S. aureus* between patients. However, the genetic profiles should be interpreted with care as the DI for RAPD for typing *S. aureus* isolates from patients with sternal SSI was only 0.84. RAPD was less discriminatory than biotyping which is surprising as previous studies have generated contrasting results (Myllys *et al.*, 1997). However, during this study, RAPD was more discriminative than antibiogram typing. This was also illustrated in a previous study conveyed by Tambic and colleagues (1999) whereby MRSA isolates sharing the same antibiotic sensitivity pattern were divided into 4 RAPD profiles. The discriminatory power of RAPD was inferior to that of PFGE which has also been previously reported (Saulnier *et al.*, 1993). However, the discriminatory power of RAPD may be further improved by the use of more than 1 primer (Hopkins and Hilton, 2001) or by performing restriction enzyme analysis (REA) on specific fragments generated by RAPD (Hopkins and Hilton, 2001).

More recent work by Fung and colleagues (2001) demonstrated that RAPD compared well with PFGE when investigating an outbreak of MRSA in a cardiovascular surgery unit.

The typeability of CoNS by RAPD during this study was 96% as 2 isolates were non-typeable. This may indicate that the primer used is less suitable for typing strains of *S. hominis* and *S. chromogenes* and consideration should be given for alternative primers in future work. Genetic heterogeneity amongst CoNS was again evident when using RAPD as a typing method, however, strains of the same species of CoNS (as determined by API) were found within the same RAPD genotype. Using RAPD, results suggest that 5 patients who presented with multiple episodes of CR-BSI had at least 2 episodes caused by the same microorganism. Furthermore, on 9 occasions, cross-transmission of CoNS may have occurred on the BMT unit. Indeed, as RAPD had a DI of 0.96 for this set of isolates, these events may be assumed with confidence. During this section of the study, RAPD was less discriminatory than biotyping and yielded the same discriminative power as antibiogram typing when characterising CoNS. This was not reflected in a study by Bingen and colleagues (1995) who found RAPD to have a higher discriminatory value than biotyping and antibiogram typing in a study of paediatric bacteraemia caused by methicillin-resistant CoNS. The discriminatory power of RAPD was also lower than PFGE for typing CoNS which again, has previously been documented (Klutymans *et al.*, 1998). Nevertheless, the DI achieved by RAPD during this study was high enough to use the technique with confidence. Indeed, 2 studies which typed CoNS from haemato-oncology departments demonstrated that RAPD profile groupings often reflect those seen with PFGE (van Belkum *et al.*, 1996; Nouwen *et al.*, 1998). RAPD has also exhibited discriminatory superiority over other PCR-based typing techniques. For example, in a recent study typing methicillin-resistant CoNS from a neonatal ICU, RAPD was shown to have a superior power to discriminate amongst isolates when compared to the use of repetitive-element PCR (rep-PCR) (Bogado *et al.*, 2002).

Despite the encouraging results with trials evaluating the use of RAPD to genotype microorganisms, this technique has other disadvantages in addition to its reported poor discriminatory power. For example, RAPD is commonly associated with a poor intra-laboratory reproducibility record (van Belkum *et al.*, 1995). The results generated by RAPD may be affected by buffer composition, PCR conditions, the DNA extraction method used, batch to batch variation in primer synthesis, the ratio of DNA template concentration to primer concentration, the model of thermocycler used, and the supplier and concentration of *Taq* DNA polymerase (Tyler *et al.*, 1997). One fundamental variable

is the composition of the buffer employed. Variation of $MgCl_2$ concentration affects primer annealing and template denaturation in addition to enzyme activity and fidelity (Schoettlin *et al.*, 1994). Excess Mg^{2+} may result in the accumulation of non-specific amplification products whereas insufficient Mg^{2+} may reduce the yield (Saiki, 1989). In addition, KCl_2 and other salts affect the required denaturing and annealing temperatures as well as the enzyme activity (Schoettlin *et al.*, 1994). It has been reported that variations in buffer pH may also affect the profiles obtained (Hilton *et al.*, 1997; Perry *et al.*, 2003). However, the time consuming process of optimising PCR conditions can be simplified with the use of the Stratagene Opti-Prime PCR optimisation kit (Schoettlin *et al.*, 1994; Hilton *et al.*, 1997). The poor reproducibility of RAPD may also be due to inconsistent interpretation of the significance of weak bands (Saulnier *et al.*, 1993; Struelens *et al.*, 1992). Interpretation of RAPD profiles is also hampered by the fact that no guidelines for the interpretation of such profiles have been produced as for restriction enzyme analysis (Tenover *et al.*, 1995).

However, RAPD has produced some promising results, is relatively simple to perform and is a rapid method of obtaining results, therefore it is a typing method which exhibits potential in epidemiological studies and further investigation is warranted.

During this study, the GelCompar™ computer software was used for analysis of all profiles. This programme has been reported to produce analyses which are comparable with to those generated by other programmes such as the BioImage™ package (BioImage corporation, USA) (Rementeria *et al.*, 2001). Despite implementation of computer packages making standardised decisions, both of these programmes have been criticised for the fact that the user has the capacity to; a) check for artefacts, b) check for missed banding allocations and c) manually edit analyses (Gerner-Smidt *et al.*, 1998).

Comparisons of discriminatory capabilities of typing techniques, despite being standardised by the Simpson's index of diversity must also be treated with caution. The index aids comparisons between typing systems with most value in large and non-local collections of isolates (Hunter and Gaston, 1988). Since an appropriate minimum number of strains has not been outlined, values achieved during this study in particular, during characterisation of strains from sternal SSI should be interpreted with caution due to the low numbers of isolates characterised.

In this study, no correlation between the phenotype and genotype of isolates as determined by PFGE, RAPD, biotyping and antibiogram typing was demonstrated in either patient group. Indeed, on numerous occasions, isolates with identical genotypes were found to have differing biotypes and antibiogram types. This may reflect the variable expression of phenotypic traits within a strain which has been demonstrated in studies by Geary and co-workers (1997) and Tegnell and colleagues (2002).

Furthermore, genetic profiles of staphylococci obtained by PFGE and RAPD for both patient groups studied were not comparable. Indeed, PFGE further sub-divided genotypes generated using RAPD and vice versa, a phenomenon which was also demonstrated in a study by van Belkum and colleagues (1995).

In summary, findings from genotypic typing during this current study suggested that although there appeared to be no single causative strain of *S. aureus* responsible for all cases of sternal SSI, the isolates associated with infection in patients B and D were closely related, as were isolates recovered from patients C and W. Genotypic analysis of CoNS isolates from SCT patients with CR-BSI illustrated that whilst small clusters of infection may be caused by identical or closely related strains, no persistent common strain was identified within the BMT unit during this time period. Instead, the heterogeneic nature of isolates implicated in these infections was illustrated. These typing techniques did, however identify relapse of infection within individuals rather than re-infection with a different strain. Results from this study also suggest that epidemiological information on infections caused by staphylococci cannot be obtained from phenotypic methods alone. This is in part due to their poor discriminatory values and the fact that phenotypic characteristics of identical genotypes have been illustrated to vary during the course of an infection (Mickelsen *et al.*, 1985; Etienne *et al.*, 1990). In conclusion, genotypic methods of characterisation are needed to investigate microorganisms associated with infection and to undertake epidemiological investigations. However, due to the expensive and time consuming nature of techniques such as PFGE, more rapid, inexpensive genotypic typing methods such as RAPD need to be further developed.

4. An investigation to determine if characterisation of coagulase-negative staphylococci by routine microbiological techniques misleads the diagnosis of catheter-related bloodstream infection

4.1 Introduction

The laboratory diagnosis of CR-BSI due to CoNS by current microbiological methods is complicated, since CoNS recovered from clinical samples may represent contamination from the skin surface as well as true infection. As CoNS are the most frequent cause of CR-BSI, accurate identification of strains recovered from CVC tip and blood cultures is essential to ensure accurate diagnosis.

Microbiological diagnosis of CR-BSI is based on the recovery of identical microorganisms (species and antibiogram) from the catheter tip and blood cultures of patients with suspected infection (O'Grady *et al.*,2002). However, recent reports suggest that cultures of colonially identical CoNS may contain multiple different strains (Viedma *et al.*, 2000; Kloos and Bannerman, 1994). Furthermore, it has been implied that multiple strains of CoNS may be associated with CR-BSI (Viedma *et al.*, 2000; Rijinders *et al.*, 2001).

Characterisation and confirmation of the identity of CoNS recovered from clinical samples including CVC tips and blood cultures in the routine microbiology laboratory is commonly based on only Gram-stain, coagulase test, biochemical profiles and antibiogram. However, previous work presented in this thesis (chapter 3) has demonstrated that phenotypically indistinguishable strains of CoNS are genetically unrelated when characterised by PFGE which is highly discriminatory.

The aims of this current investigation were to;

a) Characterise strains of CoNS associated with CR-BSI by phenotypic methods including biotyping, antibiograms and PFGE.
b) To determine if routine identification of CoNS by current phenotypic methods is sufficient to facilitate the accurate diagnosis of CR-BSI.
c) Investigate if multiple strains of CoNS are associated with CR-BSI.

4.2 Materials and Methods

4.2.1 Patients

Stem cell transplant (SCT) patients in the BMT unit, University Hospital Birmingham NHS Trust who had a Hickman CVC *in situ* and a clinical diagnosis of CR-BSI due to CoNS were recruited onto the study. The clinical diagnosis of CR-BSI was confirmed microbiologically by recovery of identical microorganisms (based on species and antibiogram) from the CVC tip and blood culture (O'Grady *et al.*, 2002).

4.2.2 Routine isolation and identification of CoNS

4.2.2.1 Routine culture of blood and catheter tip cultures

Blood and catheter tip cultures were performed in accordance with routine laboratory standard operating procedures (sections 2.2.2.5 and 3.2.2.3).

4.2.2.2 Routine identification of CoNS

Single colonies from morphologically pure cultures of CoNS recovered from blood cultures and catheter tips of each patient were selected for identification. Microorganisms were identified as CoNS by routine microbiological methods including Gram stain and the coagulase test (section 2.2.2.8).

4.2.2.3 Antibiograms

Antibiograms were performed in accordance with BSAC standardised disc diffusion guidelines (section 3.2.5). The panel of antibiotics employed were; flucloxacillin 1µg (FLU), erythromycin 5µg (ERY), trimethoprim 5µg (TRI), fusidin 10µg (FUS), rifampicin 2µg (RIF), mupirocin 5µg and 200µg (low and high concentration MU1 and MU2 respectively), tetracycline 10µg (TET), gentamicin 10µg (GEN), clindamycin 2µg (CLI), vancomycin 5µg (VAN), synercid 15µg (SYN) and linezolid 10µg (LIN).

4.2.3 Further characterisation of CoNS recovered from blood cultures and CVC tips

For each patient, a further eight representative colonial types of CoNS (three from the blood culture and five from the catheter tip) were subcultured onto blood agar and incubated at 37° C for 24 hr in air to obtain pure cultures for phenotypic and genotypic characterisation.

4.2.3.1 Antibiograms

Eight isolates from each patient were characterised by antibiogram in accordance with the BSAC standardised disc diffusion guidelines (section 2.3.5). The panel of antibiotics employed were; 1μg FLU, 5μg ERY, 5μg TRI, 10μg FUS, 2μg RIF, 5μg and 200μg MU, 10μg TET, 10μg GEN, 2μg CLI, 5μg VAN, 15μg SYN, 10μg LIN and 30μg TEIC.

4.2.3.2 Biotyping

All isolates were biotyped by the API ID 32 STAPH (Biomerieux, France) (section 3.2.4).

4.2.3.3 Molecular characterisation of CoNS by pulsed-field gel electrophoresis

All isolates were characterised genotypically by *SmaI* chromosome macrorestriction profiling using pulsed-field gel electrophoresis (PFGE) and analysed using the GelCompar® computer software (section 2.2.3).

4.3 Results

4.3.1 Patients

Eight patients were recruited into the study. Patient demographics and clinical presentation are given in table 4-1.

Table 4-1 – Patient demographics and clinical presentation

Mean age (years) Age range (years)	50 (18-77)
Male Female	5 3
Clinical presentation Acute myeloid leukaemia (AML) Chronic myeloid leukaemia (CML) Acute lymphoblastic leukaemia (ALL) Non-Hodgkin's lymphoma (NHL)	 4 1 1 2

4.3.2 Biotyping

Of the 64 isolates of CoNS characterised by biotyping, 10 different API profiles and three species of CoNS were obtained (tables 4-2 to 4-9).

4.3.3 Antibiograms

Of the 64 isolates of CoNS characterised by antibiogram types, 10 different antibiogram profiles were obtained (tables 4-2 to 4-9).

4.3.4 Pulsed-field gel electrophoresis

Of the 64 isolates of CoNS characterised by PFGE, 21 distinct genotypes were obtained (figures 4-1 to 4-8).

4.3.5 Comparison of phenotypic and genotypic typing methods

Analysis of the phenotypic and genotypic characterisation of 64 isolates of CoNS investigated demonstrated that PFGE distinguished more strains of CoNS compared to biotyping or antibiograms (21, 10 and 10 respectively). Indeed, for all isolates characterised, PFGE achieved a DI of 0.93, biotyping a DI of 0.84 and antibiogram typing a DI of 0.87. Antibiograms and biochemical profiles obtained from individual strains of

CoNS from the same patient did not always correlate. Furthermore, phenotypic and genotypic characteristics of individual strains studied correlated in only 4 out of 8 (50%) patients (patients 1-4). Genotypic and phenotypic characteristics of CoNS recovered from the remaining 4 patients did not correlate (patients 5-8).

4.3.6 The diagnosis of CR-BSI

The number of identical types of CoNS obtained through phenotypic and genotypic characterisation of multiple colonies recovered from the blood cultures and CVC tips of patients with CR-BSI is given in table 4-10.

Phenotypic characterisation of multiple colonies of CoNS recovered from the blood cultures and catheter tips by biotyping alone, revealed that 4 out of 8 (50%) patients (patients 1-3, 5) had identical microorganisms in both samples thus supporting the clinical diagnosis of CR-BSI. However, limited isolates of CoNS recovered from the tip were identical to particular isolates recovered from the blood cultures in a further 2 patients (patients 6 and 8).

Characterisation of these samples by antibiogram typing demonstrated that only 3 out of 8 patients (38%) patients (patients 1-3) had identical microorganisms from both types of clinical sample thus supporting the clinical diagnosis of CR-BSI. However, in a further 4 patients (patients 5-8), limited isolates of CoNS recovered from the tip had identical antibiograms to particular isolates recovered from the blood cultures.

Genotypic characterisation of multiple colonies of CoNS recovered from the blood cultures and catheter tips by PFGE, revealed that 3 out of 8 (38%) patients (patients 1-3) had identical microorganisms in both samples thus supporting the diagnosis of CR-BSI. However, in a further 2 patients (patients 5 and 6) specific isolates of CoNS recovered from the tip had identical macrorestriction profiles to certain isolates recovered from the blood cultures.

Table 4-2 – Antibiograms and biotypes of 8 isolates of CoNS recovered from patient 1

	Antibiogram														API profile number	API species identification
	FLU 1µg	ERY 5µg	TRI 5µg	FUS 10µg	RIF 2µg	MU1 5µg	MU2 200µg	TET 10µg	GEN 10µg	CLI 2µg	VAN 5µg	TEIC 30µg	SYN 15µg	LIN 10µg		
BC1	R	R	R	R	R	S	S	S	R	R	S	S	S	S	3673 14251	*S. simulans*
BC2	R	R	R	R	R	S	S	S	R	R	S	S	S	S	3673 14251	*S. simulans*
BC3	R	R	R	R	R	S	S	S	R	R	S	S	S	S	3673 14251	*S. simulans*
TIP1	R	R	R	R	R	S	S	S	R	R	S	S	S	S	3673 14251	*S. simulans*
TIP2	R	R	R	R	R	S	S	S	R	R	S	S	S	S	3673 14251	*S. simulans*
TIP3	R	R	R	R	R	S	S	S	R	R	S	S	S	S	3673 14251	*S. simulans*
TIP4	R	R	R	R	R	S	S	S	R	R	S	S	S	S	3673 14251	*S. simulans*
TIP5	R	R	R	R	R	S	S	S	R	R	S	S	S	S	3673 14251	*S. simulans*

BC = blood culture, TIP = catheter tip, FLU = flucloxacillin, ERY = erythromycin, TRI = trimethoprim, FUS = fusidin, RIF = rifampicin, MU1 = mupirocin (low concentration), MU2 = mupirocin (high concentration), TET = tetracycline, GEN = gentamicin, CLI = clindamycin, VAN = vancomycin, TEIC = teicoplanin, SYN = synercid, LIN = linezolid.

Figure 4-1 – Dendrogram of *SmaI* macrorestriction profiles of 8 isolates of CoNS recovered from patient 1

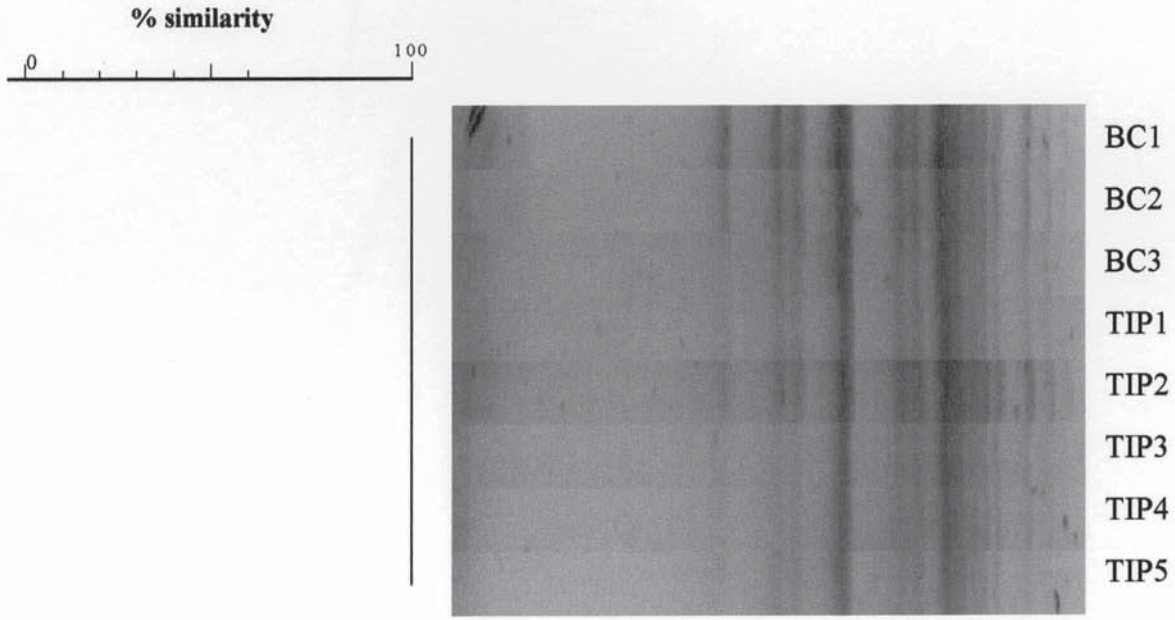


Table 4-3 – Antibiograms and biotypes of 8 isolates of CoNS recovered from patient 2

	Antibiogram														API profile number	API species identification
	FLU 1µg	ERY 5µg	TRI 5µg	FUS 10µg	RIF 2µg	MU1 5µg	MU2 200µg	TET 10µg	GEN 10µg	CLI 2µg	VAN 5µg	TEIC 30µg	SYN 15µg	LIN 10µg		
BC1	R	R	R	S	S	S	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
BC2	R	R	R	S	S	S	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
BC3	R	R	R	S	S	S	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
TIP1	R	R	R	S	S	S	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
TIP2	R	R	R	S	S	S	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
TIP3	R	R	R	S	S	S	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
TIP4	R	R	R	S	S	S	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
TIP5	R	R	R	S	S	S	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*

BC = blood culture, TIP = catheter tip, FLU = flucloxacillin, ERY = erythromycin, TRI = trimethoprim, FUS = fusidin, RIF = rifampicin, MU1 = mupirocin (low concentration), MU2 = mupirocin (high concentration), TET = tetracycline, GEN = gentamicin, CLI = clindamycin, VAN = vancomycin, TEIC = teicoplanin, SYN = synercid, LIN = linezolid.

Figure 4-2 – Dendrogram of *SmaI* macrorestriction profiles of 8 isolates of CoNS recovered from patient 2

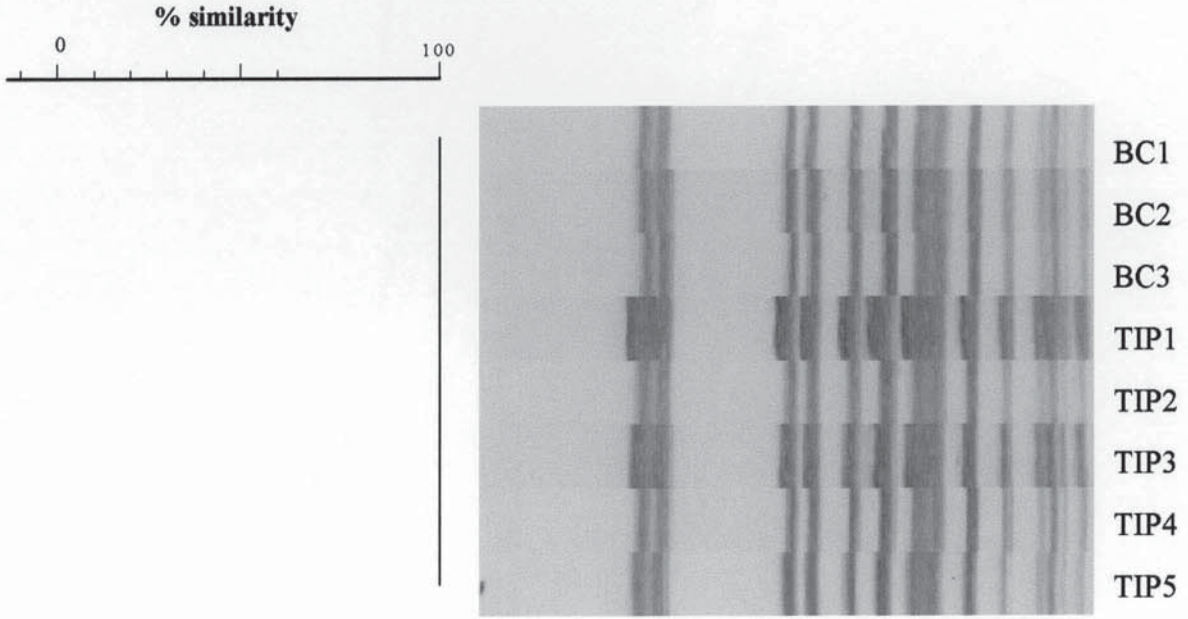


Table 4-4 – Antibiograms and biotypes of 8 isolates of CoNS recovered from patient 3

	Antibiogram														API profile number	API species identification
	FLU 1µg	ERY 5µg	TRI 5µg	FUS 10µg	RIF 2µg	MU1 5µg	MU2 200µg	TET 10µg	GEN 10µg	CLI 2µg	VAN 5µg	TEIC 30µg	SYN 15µg	LIN 10µg		
BC1	R	S	R	S	S	S	S	S	R	S	S	S	S	S	2661 14240	*S.haemolyticus*
BC2	R	S	R	S	S	S	S	S	R	S	S	S	S	S	2661 14240	*S.haemolyticus*
BC3	R	S	R	S	S	S	S	S	R	S	S	S	S	S	2661 14240	*S.haemolyticus*
TIP1	R	S	R	S	S	S	S	S	R	S	S	S	S	S	2661 14240	*S.haemolyticus*
TIP2	R	S	R	S	S	S	S	S	R	S	S	S	S	S	2661 14240	*S.haemolyticus*
TIP3	R	S	R	S	S	S	S	S	R	S	S	S	S	S	2661 14240	*S.haemolyticus*
TIP4	R	S	R	S	S	S	S	S	R	S	S	S	S	S	2661 14240	*S.haemolyticus*
TIP5	R	S	R	S	S	S	S	S	R	S	S	S	S	S	2661 14240	*S.haemolyticus*

BC = blood culture, TIP = catheter tip, FLU = flucloxacillin, ERY = erythromycin, TRI = trimethoprim, FUS = fusidin, RIF = rifampicin, MU1 = mupirocin (low concentration), MU2 = mupirocin (high concentration), TET = tetracycline, GEN = gentamicin, CLI = clindamycin, VAN = vancomycin, TEIC = teicoplanin, SYN = synercid, LIN = linezolid.

Figure 4-3 – Dendrogram of *SmaI* macrorestriction profiles of 7 isolates of CoNS recovered from patient 3

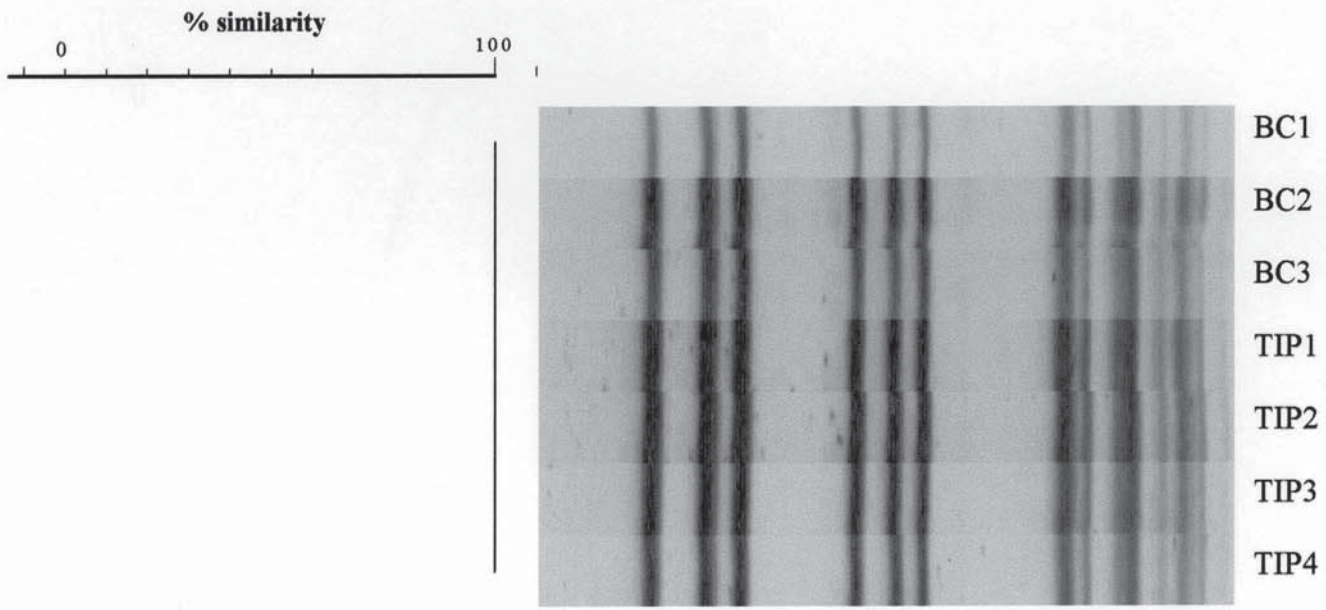


Table 4-5 – Antibiograms and biotypes of 8 isolates of CoNS recovered from patient 4

	Antibiogram														API profile number	API species identification
	FLU 1µg	ERY 5µg	TRI 5µg	FUS 10µg	RIF 2µg	MU1 5µg	MU2 200µg	TET 10µg	GEN 10µg	CLI 2µg	VAN 5µg	TEIC 30µg	SYN 15µg	LIN 10µg		
BC1	R	R	S	S	S	S	S	S	R	S	S	S	S	S	3660 12200	*S. epidermidis*
BC2	R	R	S	S	S	S	S	S	R	S	S	S	S	S	3660 12200	*S. epidermidis*
BC3	R	R	S	S	S	S	S	S	R	S	S	S	S	S	3660 12200	*S. epidermidis*
TIP1	R	R	R	R	R	R	S	R	R	R	S	S	S	S	2661 14610	*S.haemolyticus*
TIP2	R	R	R	R	R	R	S	R	R	R	S	S	S	S	2661 14610	*S.haemolyticus*
TIP3	R	R	R	R	R	R	S	R	R	R	S	S	S	S	2661 14610	*S.haemolyticus*
TIP4	R	R	R	R	R	R	S	R	R	R	S	S	S	S	2661 14610	*S.haemolyticus*
TIP5	R	R	R	R	R	R	S	R	R	R	S	S	S	S	2661 14610	*S.haemolyticus*

BC = blood culture, TIP = catheter tip, FLU = flucloxacillin, ERY = erythromycin, TRI = trimethoprim, FUS = fusidin, RIF = rifampicin, MU1 = mupirocin (low concentration), MU2 = mupirocin (high concentration), TET = tetracycline, GEN = gentamicin, CLI = clindamycin, VAN = vancomycin, TEIC = teicoplanin, SYN = synercid, LIN = linezolid.

Figure 4-4 – Dendrogram of *SmaI* macrorestriction profiles of 8 isolates of CoNS recovered from patient 4

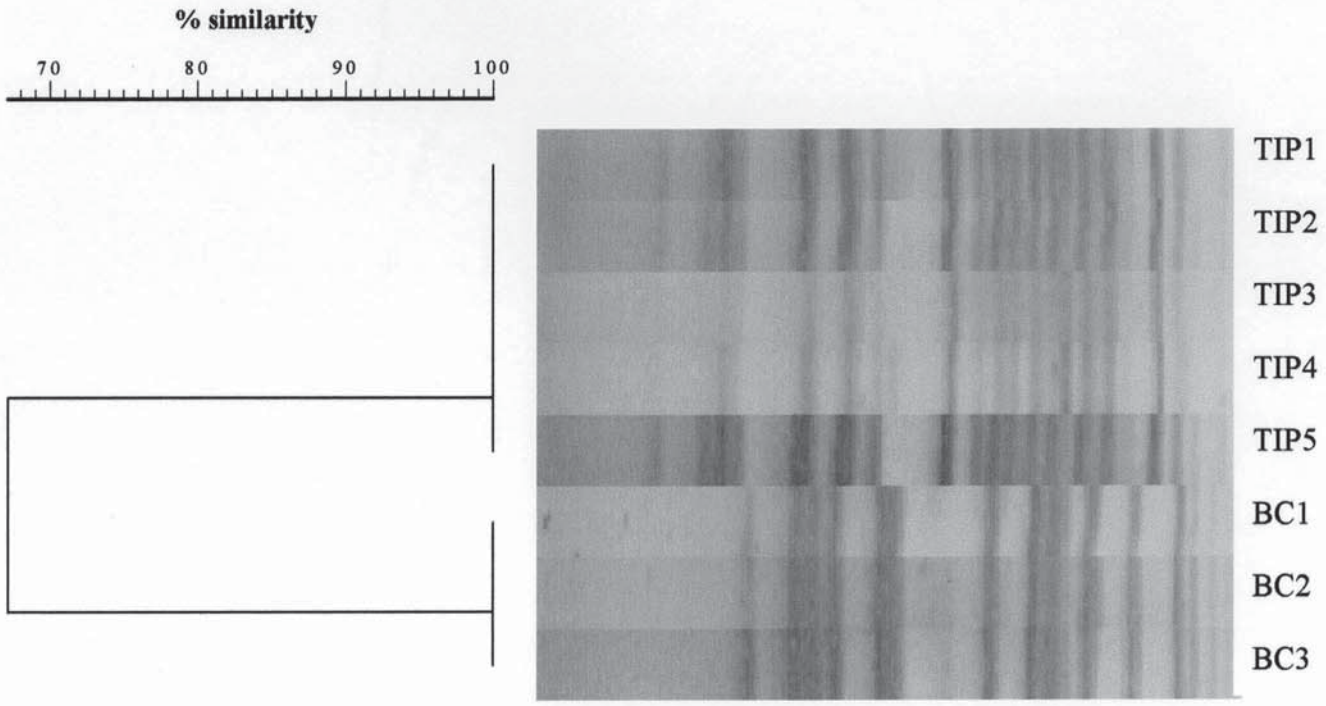


Table 4-6 – Antibiograms and biotypes of 8 isolates of CoNS recovered from patient 5

	Antibiogram														API profile number	API species identification
	FLU 1µg	ERY 5µg	TRI 5µg	FUS 10µg	RIF 2µg	MU1 5µg	MU2 200µg	TET 10µg	GEN 10µg	CLI 2µg	VAN 5µg	TEIC 30µg	SYN 15µg	LIN 10µg		
BC1	R	R	R	S	S	S	S	S	R	R	S	S	S	S	3660 12200	*S. epidermidis*
BC2	R	R	R	S	S	S	S	S	R	R	S	S	S	S	3660 12200	*S. epidermidis*
BC3	R	R	R	S	S	S	S	S	R	R	S	S	S	S	3660 12200	*S. epidermidis*
TIP1	R	R	R	S	S	S	S	S	R	R	S	S	S	S	3660 12200	*S. epidermidis*
TIP2	S	R	R	S	S	S	S	S	R	R	S	S	S	S	3660 12200	*S. epidermidis*
TIP3	R	R	R	S	S	S	S	S	R	R	S	S	S	S	3660 12200	*S. epidermidis*
TIP4	R	R	R	S	S	S	S	S	R	R	S	S	S	S	3660 12200	*S. epidermidis*
TIP5	R	R	R	S	S	S	S	S	R	R	S	S	S	S	3660 12200	*S. epidermidis*

BC = blood culture, TIP = catheter tip, FLU = flucloxacillin, ERY = erythromycin, TRI = trimethoprim, FUS = fusidin, RIF = rifampicin, MU1 = mupirocin (low concentration), MU2 = mupirocin (high concentration), TET = tetracycline, GEN = gentamicin, CLI = clindamycin, VAN = vancomycin, TEIC = teicoplanin, SYN = synercid, LIN = linezolid.

Figure 4-5 – Dendrogram of *SmaI* macrorestriction profiles of 8 isolates of CoNS from patient 5

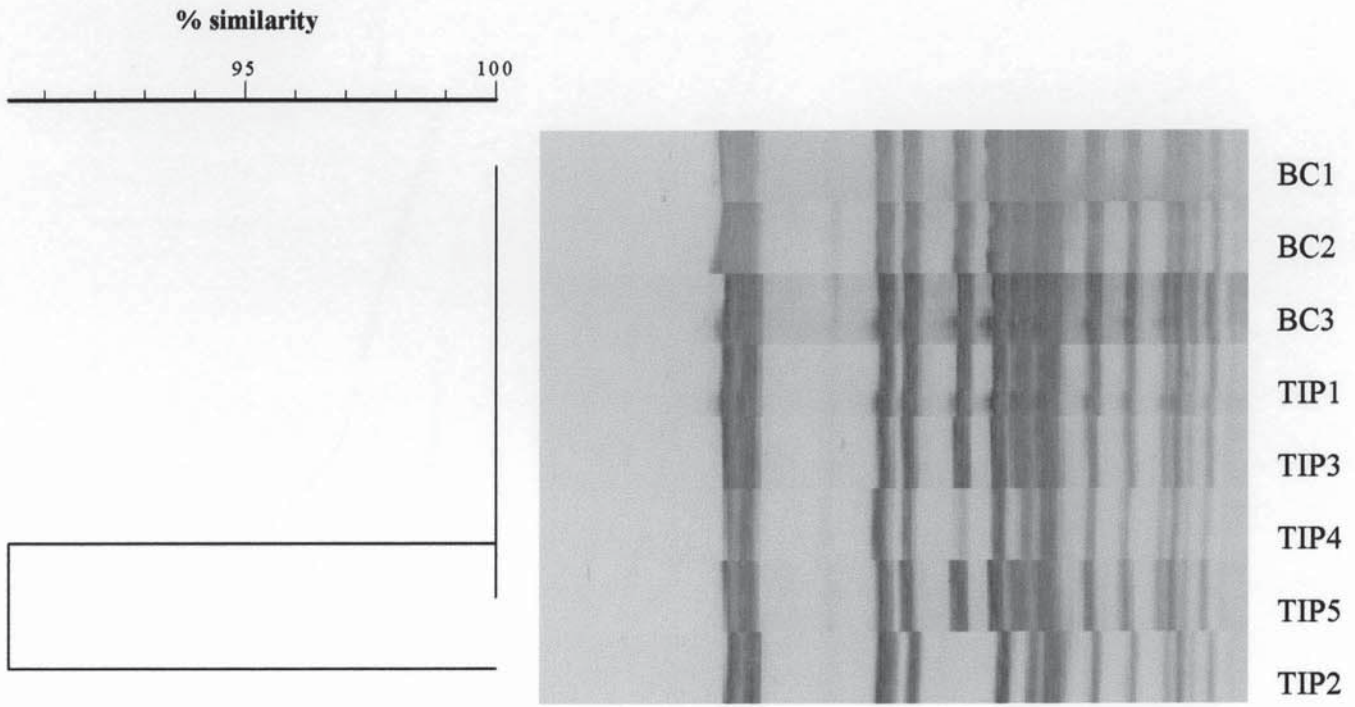


Table 4-7 – Antibiograms and biotypes of 8 isolates of CoNS recovered from patient 6

	Antibiogram														API profile number	API species identification
	FLU 1µg	ERY 5µg	TRI 5µg	FUS 10µg	RIF 2µg	MU1 5µg	MU2 200µg	TET 10µg	GEN 10µg	CLI 2µg	VAN 5µg	TEIC 30µg	SYN 15µg	LIN 10µg		
BC1	R	R	R	R	R	R	S	S	R	R	S	S	S	S	3660 12210	*S. epidermidis*
BC2	R	R	R	R	R	R	S	S	R	R	S	S	S	S	3660 12210	*S. epidermidis*
BC3	R	R	R	R	R	R	S	S	R	R	S	S	S	S	3660 12210	*S. epidermidis*
TIP1	R	S	S	S	S	S	S	S	S	S	S	S	S	S	3660 12210	*S. epidermidis*
TIP2	R	R	R	R	R	R	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
TIP3	R	S	S	S	S	S	S	S	S	S	S	S	S	S	3660 12210	*S. epidermidis*
TIP4	R	R	R	R	R	R	S	S	R	R	S	S	S	S	3660 12210	*S. epidermidis*
TIP5	R	R	R	R	R	R	S	S	R	R	S	S	S	S	3660 12210	*S. epidermidis*

BC = blood culture, TIP = catheter tip, FLU = flucloxacillin, ERY = erythromycin, TRI = trimethoprim, FUS = fusidin, RIF = rifampicin, MU1 = mupirocin (low concentration), MU2 = mupirocin (high concentration), TET = tetracycline, GEN = gentamicin, CLI = clindamycin, VAN = vancomycin, TEIC = teicoplanin, SYN = synercid, LIN = linezolid.

Figure 4-6 – Dendrogram of *SmaI* macrorestriction profiles of 7 isolates of CoNS recovered from patient 6

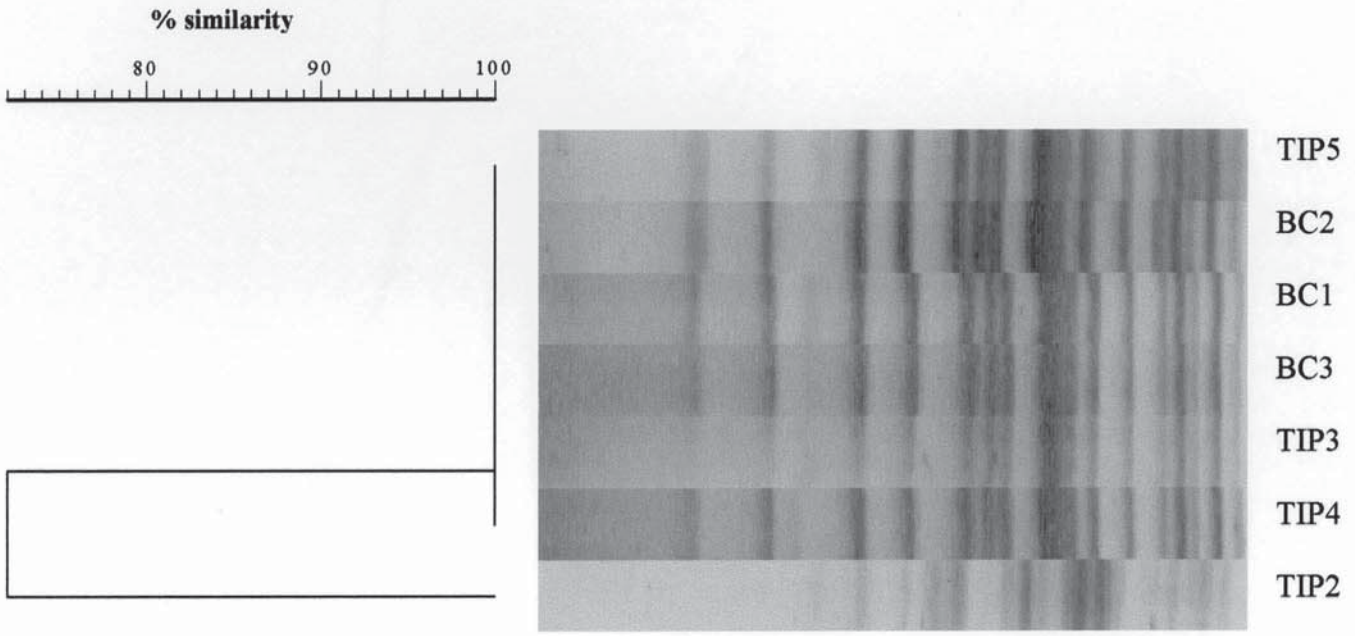


Table 4-8 – Antibiograms and biotypes of 8 isolates of CoNS recovered from patient 7

	Antibiogram														API profile number	API species identification
	FLU 1µg	ERY 5µg	TRI 5µg	FUS 10µg	RIF 2µg	MU1 5µg	MU2 200µg	TET 10µg	GEN 10µg	CLI 2µg	VAN 5µg	TEIC 30µg	SYN 15µg	LIN 10µg		
BC1	R	R	R	R	R	R	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
BC2	R	R	R	R	R	R	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
BC3	R	R	R	R	R	R	S	S	R	R	S	S	S	S	1760 02200	*S. epidermidis*
TIP1	R	S	S	S	S	S	S	S	S	S	S	S	S	S	3660 12200	*S. epidermidis*
TIP2	R	R	R	R	R	R	S	S	R	R	S	S	S	S	1760 02200	*S. epidermidis*
TIP3	R	S	S	S	S	S	S	S	S	S	S	S	S	S	3660 12200	*S. epidermidis*
TIP4	R	R	R	R	R	R	S	S	R	R	S	S	S	S	3660 12200	*S. epidermidis*
TIP5	R	R	R	R	R	R	S	S	R	R	S	S	S	S	3660 12200	*S. epidermidis*

BC = blood culture, TIP = catheter tip, FLU = flucloxacillin, ERY = erythromycin, TRI = trimethoprim, FUS = fusidin, RIF = rifampicin, MU1 = mupirocin (low concentration), MU2 = mupirocin (high concentration), TET = tetracycline, GEN = gentamicin, CLI = clindamycin, VAN = vancomycin, TEIC = teicoplanin, SYN = synercid, LIN = linezolid.

Figure 4-7 – Dendrogram of *SmaI* macrorestriction profiles of 8 isolates of CoNS recovered from patient 7

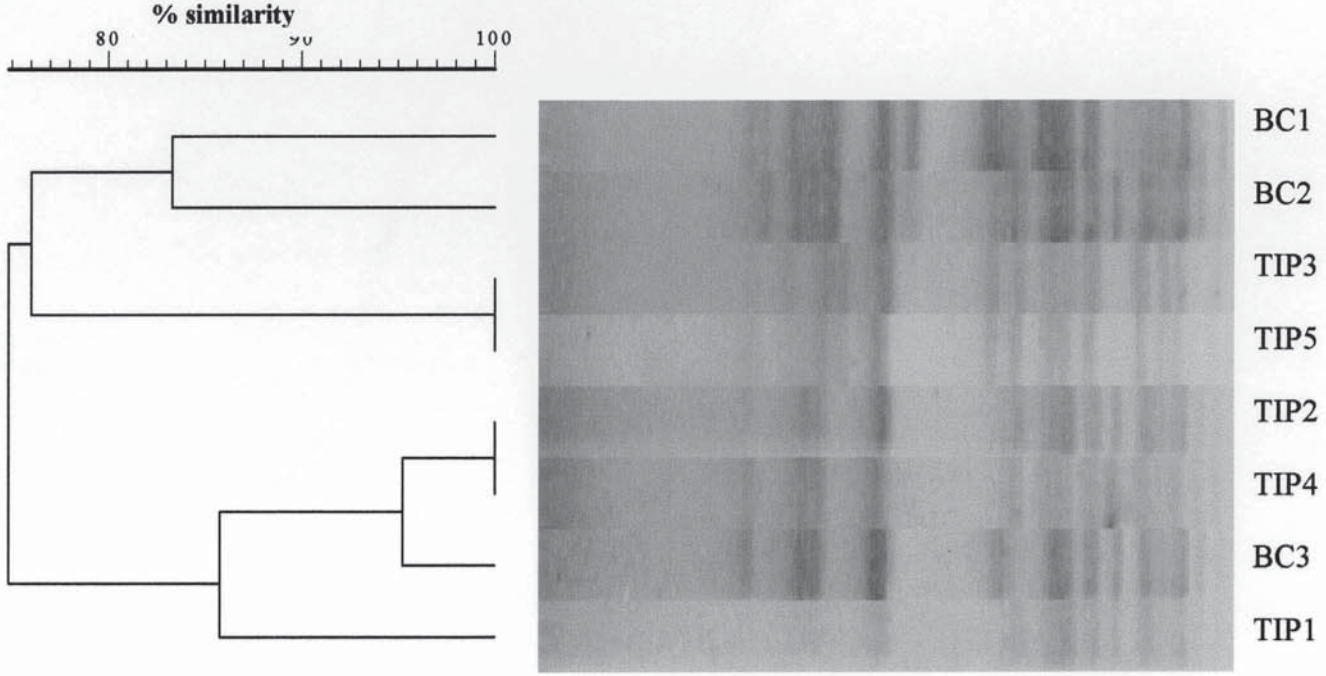


Table 4-9 – Antibiograms and biotypes of 8 isolates of CoNS recovered from patient 8

	Antibiogram														API profile number	API species identification
	FLU 1µg	ERY 5µg	TRI 5µg	FUS 10µg	RIF 2µg	MU1 5µg	MU2 200µg	TET 10µg	GEN 10µg	CLI 2µg	VAN 5µg	TEIC 30µg	SYN 15µg	LIN 10µg		
BC1	R	R	R	R	S	R	R	S	R	R	S	S	S	S	2663 14200	*S.haemolyticus*
BC2	R	R	R	R	S	R	R	S	R	R	S	S	S	S	1660 10200	*S. epidermidis*
BC3	S	S	S	S	S	S	S	S	S	S	S	S	S	S	3660 12200	*S. epidermidis*
TIP1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	3630 02200	*S. epidermidis*
TIP2	R	R	R	R	S	R	S	S	R	R	S	S	S	S	3660 12200	*S. epidermidis*
TIP3	R	R	R	R	S	R	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
TIP4	R	R	R	R	S	R	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*
TIP5	R	R	R	R	S	R	S	S	R	R	S	S	S	S	1660 12200	*S. epidermidis*

BC = blood culture, TIP = catheter tip, FLU = flucloxacillin, ERY = erythromycin, TRI = trimethoprim, FUS = fusidin, RIF = rifampicin, MU1 = mupirocin (low concentration), MU2 = mupirocin (high concentration), TET = tetracycline, GEN = gentamicin, CLI = clindamycin, VAN = vancomycin, TEIC = teicoplanin, SYN = synercid, LIN = linezolid.

Figure 4-8 – Dendrogram of *SmaI* macrorestriction profiles of 8 isolates of CoNS recovered from patient 8

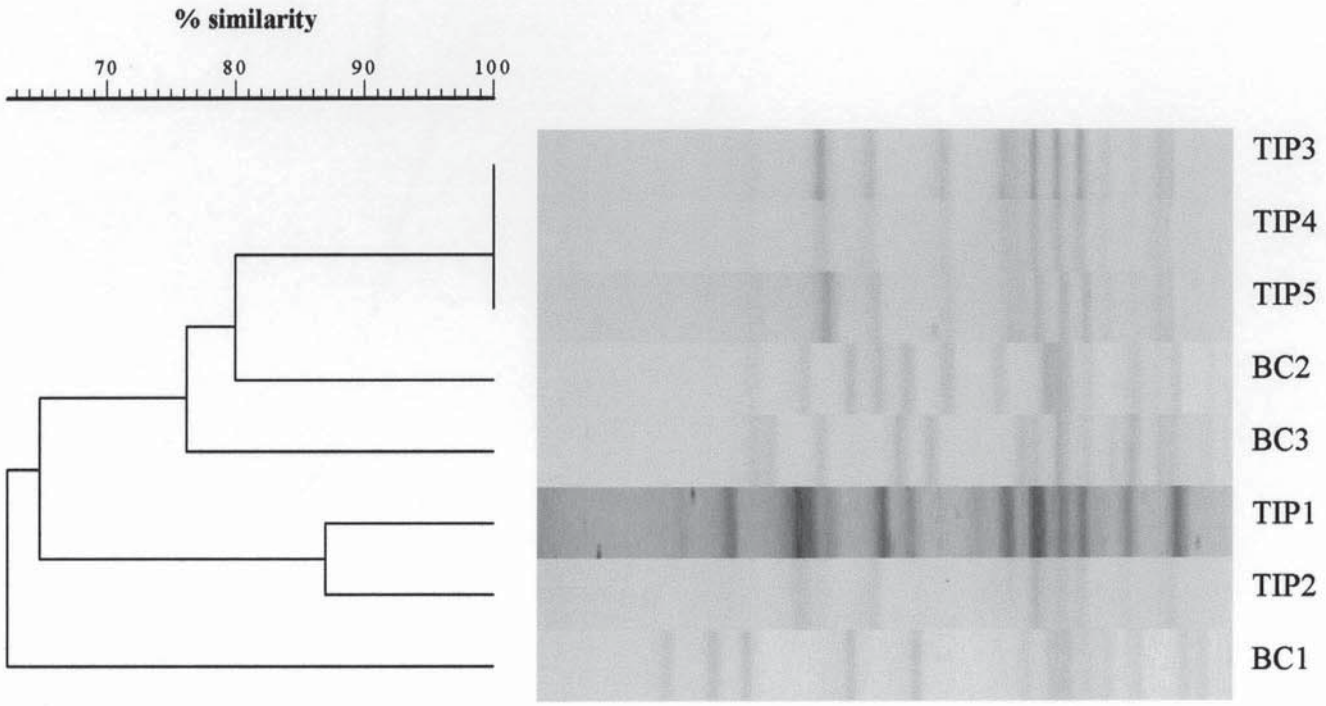


Table 4-10 – Number of types of CoNS identified following characterisation of multiple colonies by biotyping, antibiogram typing and pulsed-field gel electrophoresis

		Biotyping			Antibiogram typing			PFGE types		
Patient number	Sample (and no of isolates tested)	No of isolates typeable	No of types obtained	Identical microorganisms in both samples?	No of isolates typeable	No of types obtained	Identical microorganisms in both samples?	No of isolates typeable	No of types obtained	Identical microorganisms in both samples?
1	TIP (5)	5	1	Yes	5	1	Yes	5	1	Yes
	BC (3)	3	1		3	1		3	1	
2	TIP (5)	5	1	Yes	5	1	Yes	5	1	Yes
	BC (3)	3	1		3	1		3	1	
3	TIP (5)	5	1	Yes	5	1	Yes	4	1	Yes
	BC (3)	3	1		3	1		3	1	
4	TIP (5)	5	1	No	5	1	No	5	1	No
	BC (3)	3	1		3	1		3	1	
5	TIP (5)	5	1	Yes	5	2	Yes*	5	2	Yes*
	BC (3)	3	1		3	1		3	1	
6	TIP (5)	5	2	Yes*	5	2	Yes*	4	2	Yes*
	BC (3)	3	1		3	1		3	1	
7	TIP (5)	5	2	No	5	2	Yes*	5	3	No
	BC (3)	3	2		3	1		3	3	
8	TIP (5)	5	3	Yes*	5	2	Yes*	5	3	No
	BC (3)	3	3		3	2		3	3	

TIP = CVC tip, BC = blood culture

*** = limited isolates of CoNS from the TIP were identical to particular isolates from the blood culture (all multiple isolates are not identical)**

4.4 Discussion

In this current study, the ability of phenotypic and genotypic methods to characterise CoNS and facilitate the diagnosis of CR-BSI was assessed. The comparative discriminatory power of biotyping, antibiogram typing and PFGE utilised during this study were similar to the results obtained in chapter 3. Indeed, PFGE was again found to be the most discriminative method for typing CoNS during this study (DI=0.93) as it further distinguished between isolates of CoNS which the phenotypic methods were unable to do. However, DI values for biotyping and antibiogram typing during this study were lower than those obtained in the previous chapter (0.84 and 0.87, respectively). These results concur with those achieved during previous studies which demonstrated that genotypic typing techniques are more discriminative than phenotypic typing methods (Tenover *et al.*, 1994; Weller, 2000; Toldos *et al.*, 1997).

Many cultures of CoNS obtained from patients are considered by the routine microbiology laboratory to be pure as there may be no variation in colonial morphology of the CoNS following 24 hr incubation. Consequently, identification of CoNS and antibiotic sensitivity testing is undertaken on single colonies from blood and tip cultures. The results of this study demonstrate that cases of CR-BSI may be misdiagnosed if single colonies are selected at random as several genotypes may be present on an agar plate. Indeed, heterogeneity amongst multiple isolates of CoNS selected at random was demonstrated with only some of the isolates recovered from the tip and blood cultures being identical. This may be indicative of CR-BSI, however, if a smaller number of isolates or a single isolate was selected for analysis, the microbiological diagnosis of CR-BSI may not have been confirmed. Indeed, in a recent study by Dobbins and colleagues (2002) 5 out of 21 CR-BSI cases were not confirmed by PFGE further supporting the need to test multiple colonies of CoNS to make an accurate diagnosis of CR-BSI.

It has been suggested that a 24 hr incubation period is inadequate to observe differences in colony morphology in CoNS cultures (Kloos and Bannerman, 1994). In a recent study by Viedma and colleagues (2000), it was found that CoNS recovered from 9 catheter tip cultures were morphologically identical after 24 hr incubation, however, following 72 hr clear differences in colonial morphology were observed. This study also demonstrated that there were multiple strains present in morphologically pure populations identified following 72 hr incubation. In cases whereby multiple strains of a particular microorganism are present in culture, it may be difficult to determine which is associated

with infection. For example, some strains of CoNS, may represent colonisation or contamination of a CVC rather than true infection. A recent study suggests that lower colony counts would be observed in culture in contaminated samples compared to infection (Viedma *et al.*, 2000). However, it may not be possible to identify contaminating colonies after 24 hr incubation which makes quantification problematic.

In this current study, polyclonal infection due to CoNS in patients with CR-BSI was not demonstrated (i.e. ≥2 strains of CoNS from the blood culture with ≥2 corresponding identical strains from the tip culture was not demonstrated). This may be due to an insufficient number of patients being studied and/or an insufficient number of colonies being selected for investigation. However, a number of studies demonstrating polyclonal CoNS infection have been reported. Rijinders and co-workers (2001), discovered that polyclonality of catheter infection is not an exceptional occurrence. The polyclonality of CoNS responsible for infection has also been demonstrated in patients with homograft and prosthetic valve infectious endocarditis (Van Eldere *et al.*, 2000; Van Wijngaerden *et al.*, 1997). Indeed, Van Eldere and colleagues (2000) illustrated the possibility of genetic variation in infections of prosthetic heart valves. Also, recently the polyclonal nature of strains of *S. epidermidis* responsible for prosthetic joint infections was demonstrated (Galdbart *et al.*, 1999). Whilst the diagnosis of CR-BSI may be complicated due to the high level of polyclonality in CoNS, the same level of variation has not been demonstrated in populations of *S. aureus* (Khatib *et al.*, 2003).

If polyclonal infection in patients with CR-BSI is not identified, this may lead to therapeutical problems. Catheter-related bloodstream infections are often difficult to treat which may occasionally be in part due to their polyclonality. Antibiotics such as a glycopeptide could be employed to treat all CR-BSI due to CoNS. However this may further contribute to staphylococcal glycopeptide resistance, especially since some infections may be caused by CoNS which are sensitive to antistaphylococcal penicillins (Rijinders *et al.*, 2001). Furthermore, if a polyclonal infection is inadequately treated, the recovery of a clone of a particular microorganism which is different to the strain originally isolated may be mis-diagnosed as reinfection rather than relapse of the original infection especially if a limited number of colonies are characterised (Rijinders *et al.*, 2001). The findings from this study indicate that that epidemiological studies of CoNS infection (as in chapter 3) may not form realistic pictures in outbreak strain investigations since multiple colonies from each culture are not routinely selected for characterisation.

The presence of the same strain of microorganism on the tip and in the blood is indicative of CR-BSI, however, it must be considered that cultures may be polyclonal and not all strains may be represented in the analysis. The selection of too few colonies for characterisation may suggest that current published rates of CR-BSI due to CoNS are understated. However, conversely, CR-BSI rates may be overstated if indiscriminative phenotypic typing methods are used to make this diagnosis. While it is not feasible to characterise all colonies found in culture, since variation in antibiotic sensitivities are found within such populations, a representative number of colonies should be evaluated in order to ensure that an appropriate antibiotic regime is employed.

In conclusion, the results from the current study highlight that; genotypic typing techniques such as PFGE are more discriminatory that phenotypic typing methods, the routine identification and characterisation of CoNS by phenotypic techniques lacks discrimination and may mislead the diagnosis of CR-BSI based on current guidelines and multiple colonies of CoNS should be selected for characterisation to facilitate the diagnosis of infection, despite the fact that multiple strains of CoNS were not associated with CR-BSI during the study period. Since it is unlikely that expensive, time-consuming genotypic techniques such as PFGE will be incorporated in the routine microbiology laboratory, rapid, inexpensive genotypic methods such as RAPD need to be developed.

5. Serodiagnosis of catheter-related bloodstream infection and sternal surgical site infection by lipid S enzyme-linked immunosorbent assay

5.1 Introduction

Catheter-related bloodstream infection (CR-BSI) and surgical site infection (SSI) are responsible for a large proportion of hospital-acquired infections. The morbidity, economic costs and difficulties associated with the diagnosis of CR-BSI are discussed in chapter 1.

Surgical site infection (SSI) following median sternotomy is an uncommon but potentially fatal complication of cardiac surgery. The reported incidence of sternal SSI varies widely and ranges between 0.9% and 20% (Ulicny and Hiratzka, 1991). Furthermore, the attributable costs of sternal SSI following median sternotomy are high. Indeed, patients with a deep chest wall SSI following coronary artery bypass grafting (CABG) have a 20 day increase in hospitalisation with additional costs of 20,000 US dollars and a mortality rate of 22% (Hollenbeak *et al.*, 2000; Hollenbeak *et al.*, 2002).

Early and accurate diagnosis of sternal SSI is essential for successful treatment. Grossi and colleagues (1985) suggested that 80% of sternal SSI may be eradicated by simple surgical debridement and/or closed antibiotic irrigation if the diagnosis is established within 20 days. However if diagnosis is delayed, surgical debridement followed by muscle flap reconstruction may be required (Grossi *et al.*, 1985). Sternal SSI is often diagnosed by the presence of symptoms of local inflammation including; purulent exudate, erythema, tenderness, pyrexia, elevated levels of serum inflammatory markers (Marggraf *et al.*, 1999) and by an abnormal computerised tomography (CT) scan (Browdie *et al.*, 1991). Several other methods have been evaluated to diagnose sternal wound SSI including; sternal puncture (Benlolo *et al.*, 2003), infrared thermography of peristernal skin (Robicsek *et al.*, 1984), plain radiography (Cooper *et al.*, 1992) and radionucleotide imaging techniques using; Indium-111 labelled leukocytes (Marggraf *et al.*, 1999), Technetium-99m- labelled leukocytes and monoclonal antigranulocyte antibodies (Browdie *et al.*, 1991). However, all these current diagnostic techniques are often time consuming, expensive and lack sensitivity.

Diagnosis of sternal wound infections is difficult in the early postoperative period due to the general inflammatory response that follows extracorporeal circulation (Bitkover *et al.*, 1996). Use of inflammatory markers to aid diagnosis during this period is therefore of limited value (Baykut *et al.*, 2000; Rothenburger *et al.*, 1999). Indeed, patients presenting with late infection due to CoNS (>3 weeks postoperatively) may have reduced levels of serum inflammatory markers including C-reactive protein (CRP) and peripheral white blood cell count (WBC) than those patients presenting with early infection (Tegnell *et al.*, 2000). Most imaging techniques which are used for the diagnosis of sternal SSI are based on the visualisation of anatomical structures and are limited in their diagnostic value as infection is indistinguishable from surgical artefacts and the presence of oedema, haematoma or haemorrhage which are often present in normal patients recovering from cardiac surgery (Bell *et al.*, 1978).

The microorganisms most commonly recovered from sternal SSI are *Staphylococcus aureus* and CoNS which account for 32% and 23% of sternal SSI respectively (Mossad *et al.*, 1997). Microbiological culture of clinical material recovered from the sternal SSI is required to confirm the clinical diagnosis. However this may be complicated by the use of prophylactic antibiotics used for cardiac surgery. In addition, interpretation of sternal cultures yielding CoNS, a major cause of sternal SSI, is complicated as the isolated microorganisms may be disregarded as skin contamination.

Interpretation of microbiological culture results for the diagnosis of infections including CR-BSI and sternal SSI is often complex as positive cultures due to staphylococci, in particular CoNS, may be representative of contamination or colonisation rather than true infection. Serological diagnosis of infection provides a useful adjunct to microbiological culture as levels of specific antibodies or antigens may be estimated in patients which may facilitate the diagnosis of infection or aid in the interpretation of positive cultures.

Many serological tests have been developed for the diagnosis of staphylococcal infection (chapter 1, sections 1.5.2.2.6–1.5.2.2.7) which account for a large proportion of hospital-acquired infections. However, the main method of serological diagnosis of staphylococcal infection in routine clinical microbiology laboratories is the anti-staphylolysin test (ASTA) whereby levels of antibody to α-haemolysin produced by *S. aureus* are estimated. Although widely used, the assay lacks sensitivity and is used for the diagnosis of infection due to *S. aureus* only and not CoNS, the major cause of CR-BSI (Larinkari, 1982).

A rapid, indirect enzyme-linked immunosorbent assay (ELISA) incorporating lipid S, a novel exocellular lipoteichoic antigen produced by staphylococci has been recently developed to facilitate the diagnosis of deep-seated infection caused by both CoNS and *S. aureus* (Worthington *et al.*, 2002; Elliott *et al.*, 2000; Lambert *et al*, 2000). The ELISA has previously been used to successfully facilitate the diagnosis of CR-BSI predominantly in immunocompetent patients (Worthington *et al.*, 2002; Elliott *et al.*, 2000).

The aims of this study were to:

a) Determine if elevated serum levels of anti-lipid S IgG are obtained in cardiothoracic surgery patients with colonised CVC tips as defined by Maki and colleagues (1977) compared to patients yielding negative tip cultures.

b) Determine if the lipid S ELISA facilitates the diagnosis of CR-BSI in haematology patients.

c) Assess the potential of the lipid S ELISA in facilitating the serodiagnosis of sternal SSI infection in cardiothoracic surgery patients following median sternotomy.

5. 2 Materials and Methods

5.2.1 Patients

Local research ethical committee approval was sought prior to commencement of the study (appendix 1).

5.2.1.1 Patients with colonised CVC

Patients at the Cardiac Surgery Critical Care Unit, University Hospital, Birmingham, UK who required a quad-lumen CVC as part of their clinical management were entered into the study. Following aseptic removal of their CVC, the distal tip was cultured for microorganisms by the roll plate technique (Maki *et al.*, 1977). Patients with no evidence of infection but whose CVC distal tip yielded $\geq$ 15 cfu of CoNS were classified as patients with colonised CVC. Patients with no CVC tip colonisation who yielded negative cultures or no evidence of infectious complications were classified as control patients. All patients investigated had no evidence of infection within the previous 6 months. Clotted blood samples were obtained from colonised and control patients on the day of CVC removal for anti-lipid S IgG estimation. Total WBC and lymphocyte counts were also recorded for each patient on the day which the CVC was removed and the sera collected.

5.2.1.2 Patients with CR-BSI

Patients at the haematology and Bone Marrow Transplant (BMT) unit, University Hospital, Birmingham, UK who were diagnosed with a CR-BSI were entered into this part of the study. Haematology and SCT patients with no evidence of infectious complications for three weeks following CVC insertion were recruited as controls. All patients recruited with the exception of those with CR-BSI had no evidence of infection 6 months prior to the time of recruitment. Clotted blood samples were obtained for anti-lipid S IgG estimation seven days post-CVC insertion from control patients and from infected patients at the time CR-BSI was diagnosed microbiologically. Total WBC and lymphocyte counts were recorded for each patient on the day which infection was first diagnosed.

5.2.1.3 Patients with sternal SSI

Patients at the Cardiac Surgery Critical Care Unit, University Hospital, Birmingham, UK who were diagnosed as having a sternal SSI following elective cardiac surgery were entered into this part of the study. CDC guidelines were utilised to define sternal SSI (table 3-1) (Horan *et al.*, 1992). Randomly selected cardiac surgery patients with no evidence of infectious complications 7 days following surgery were recruited as controls.

All patients recruited with the exception of those with sternal SSI had no evidence of infection six months prior to recruitment. Clotted blood samples were obtained for anti-lipid S IgG estimation 5 days post-cardiac surgery from control patients and from infected patients at the time sternal SSI was diagnosed. Total WBC and lymphocyte counts were recorded for each patient on the day which infection was diagnosed.

5.2.2 Laboratory methods

5.2.2.1 Preparation of lipid S-coated plates

Lipid S antigen was prepared from a culture supernatant of seven strains of CoNS grown in 20mL of brain-heart infusion broth (Oxoid, Basingstoke, UK) at 37°C for 24 hr. All strains were isolated from the blood cultures of patients with proven CR-BSI. Antigen was recovered from the culture supernatants by gel permeation chromatography (superose 12) and diluted with 400 volumes of sodium carbonate/bicarbonate buffer (0.05M, pH 9.6) (Worthington *et al.*, 2002). One-hundred microlitres of this solution containing 0.125μg of lipid S antigen was used to coat each well of a microtitre plate (Immulon 2; Dynatech Laboratories, Chantilly, Virginia, USA) after which, they were incubated at 4°C for 18 hr to allow the antigen to bind. Excess antigen was then removed by washing with TBS-Tween (0.01M Tris-HCl, pH 7.4, NaCl 0.9% w/v, Tween-20 0.3% v/v). Unbound sites were blocked by incubation with fresh TBS-Tween buffer for 1 hr at 4°C. After removal of the buffer, the plates were dried and stored at -20°C until required.

5.2.2.2 Lipid S ELISA

ELISA plates were allowed to reach room temperature prior to performing the assay. Patients' sera were diluted 1 in 6400 in TBS/Tween buffer and 100μl was added to each well of the plate. All sera, including positive and negative controls were tested in duplicate. Positive control serum was obtained from a patient with a confirmed diagnosis of CRI with a titre of 1:100, 000, the negative control serum was normal human serum (Bradsure Biologicals, Loughborough, UK) (Elliott *et al.*, 2000). After incubation for 2 hr at 37°C, excess serum was removed and the plates were washed with TBS/Tween buffer. Antihuman IgG conjugate (Sigma, Poole, Dorset, UK); diluted 1 in 5000 in TBS/Tween buffer) was then added to each well and incubated for 1 hr at 37°C for the detection of bound IgG. After removal of the conjugate by washing with TBS/Tween, 100μl of chromogenic substrate was added to each well. The substrate contained 10 mg 3,3',5,5'-tetramethylbenzidine (TMB) (Sigma, Poole, Dorset, UK) dissolved in 1ml dimethyl sulphoxide (DMSO) and was diluted into 100ml sodium acetate/citrate buffer (0.1M, pH

6.0) containing 50 µl of H_2O_2 (5% v/v). After 25 min of incubation at 37°C, the reaction was stopped by the addition of 100µl of sulphuric acid (1M) after which, the optical density was read at 450nm. Enzyme immunoassay units (EIU) were calculated based on a previously described formula (Balfour and Harford, 1990).

5.3 Results

5.3.1 Patients

5.3.1.1 Patients with colonised CVC

Eighty-three cardiac surgery patients were recruited into the study; 34 patients whose CVC tips were colonised with CoNS and 49 patients whose CVC tips were culture-negative and served as controls. Patient demographics and surgical procedure performed are given in table 5-1.

Table 5-1 – Patient demographics and surgical procedures performed

	Control patients	Patients with colonised CVC
Mean age (years)	64	61
Age range (years)	21-84	29-79
Male	28	22
Female	21	12
Procedure: CABG	31	19
AVR	4	4
MVR	3	2
CABG + AVR	5	0
CABG + MVR	0	0
CABG + AVR + MVR	1	0
AVR + MVR	2	1
Aortic surgery	0	3
PVR	1	0
VSD closure/repair	1	0
ASD closure/repair	0	1
Cardiac transplant	1	4

CABG (Coronary artery bypass graft)
AVR (Aortic valve replacement)
MVR (Mitral valve replacement)
PVR (Pulmonary valve replacement)
VSD (Ventricular septal defect)
ASD (Atrial septal defect)

5.3.1.2 Patients with CR-BSI

Sixty-two haematology/BMT patients were recruited into this section of the study. Thirty-eight of these patients were diagnosed with CR-BSI (24 due to CoNS, 6 due to *S. aureus*, 4 due to *Enterococcus* spp. and 4 due to Gram-negative microorganisms). Twenty-four control patients with indwelling CVC but no clinical or microbiological symptoms of CR-BSI were recruited. Patient demographics, CVC type and clinical presentations are given in table 5-2. CR-BSI presented in patients a mean of 43 days post-CVC insertion (range = 4 to 253).

5.3.1.3 Patients with sternal SSI

Ninety-four patients were recruited into the study. Fifty-seven patients with SSI were investigated; 32 with sternal SSI due to *S. aureus*, 4 due to CoNS and 10 due to Gram-negative microorganisms (4 *Pseudomonas* spp, 3 *Klebsiella* spp, 2 *Serratia* spp and 1 *Enterobacter* spp.). Eleven patients whose microbiological results yielded mixed CoNS that were considered as non-significant skin microorganisms were also recruited. This patient group is referred to as those yielding 'skin' microorganisms. Thirty-seven patients with no microbiological or clinical evidence of infection were recruited as controls. Patients first presented with superficial sternal SSI a mean of 11 days post-operatively (range = 3 to 30). Patients were diagnosed with deep sternal SSI, a mean of 26 days post-operatively (range = 2 to 278). Patient demographics, surgical procedure and infection types are given in table 5-3.

Table 5-2 – Patient demographics, CVC type and clinical presentation of patients with CR-BSI and controls

Patient group	Controls (n=23)	CR-BSI due to CoNS (n=24)	CR-BSI due to *S. aureus* (n=6)	CR-BSI due to *Enterococcus* spp. (n=4)	CR-BSI due to Gram-negative microorganisms (n=4)
Mean age (years)	43	43	48	51	42
Age range (years)	26-76	19-63	23-66	25-66	31-56
Male	12	13	4	3	3
Female	11	11	2	1	1
CVC type:					
Hickman line	23	19	6	3	4
PICC	0	3	0	0	0
Short-term non-tunneled C/L	0	2	0	1	0
Clinical presentation					
Aplastic anaemia (AA)	0	0	1	0	0
Acute lymphoblastic leukaemia (ALL)	1	3	0	1	0
Acute myeloid leukaemia (AML)	6	5	1	1	2
Chronic myeloid leukaemia (CML)	2	0	0	0	1
Myelodysplasia (MDS)	2	2	0	0	0
Chronic lymphoblastic leukaemia (CLL)	1	0	0	0	0
Non-Hodgkins lymphoma (NHL)	7	11	3	2	0
Multiple myeloma (MM)	2	3	1	0	1
Waldenstroms macroglobulinaemia (WM)	1	0	0	0	0
Polyneuropathy, organomegaly, endocrinopathy, M protein and skin changes (POEMS).	1	0	0	0	0

Table 5-3 - Patient Demographics, surgical procedures and infection type of patients with sternal SSI and controls

	Control group (n=37)	SSI due to *S. aureus* (n=32)	SSI due to CoNS (n=4)	SSI due to 'skin' microorganisms (n=11)	Gram-negative SSI (n=10)
Mean age (years)	64	65	69	57	65
Age range (years)	42-82	48-82	59-79	43-76	43-79
Male	32	22	4	7	7
Female	5	10	0	3	3
Surgical procedure:					
CABG	31	23	3	7	7
AVR	6	3	0	1	1
MVR	1	2	0	2	0
CABG + AVR	0	2	1	0	0
CABG + MVR	0	1	0	0	1
Aortic surgery	0	1	0	1	1
Infection type:					
Superficial	-	13	1	11	4
Deep	-	19	3	0	6

5.3.2 Anti-lipid S IgG titres

5.3.2.1 Patients with colonised CVC

A scattergram demonstrating the range of serum IgG titres to lipid S is given in figure 5-1. Anti-lipid S IgG titres obtained from patients with colonised tips and controls are shown in table 5-4. There was no significant difference between the serum IgG titres of patients with CVC tips colonised with CoNS compared to patients yielding negative cultures (p=0.8, Mann-Whitney U Test).

Table 5-4 – Serum IgG titres to lipid S in patients with colonised and non-colonised CVC tips

	Control patients (non-colonised CVC tip)	Patients with colonised CVC tips
Total	49	34
Number with positive IgG titres (%)	1 (2)	2 (6)
Mean anti-lipid S IgG titre	180	501
Titre range	ND - 8816	ND – 15455

5.3.2.2 Patients with CR-BSI

A scatter graph demonstrating the range of serum IgG titres to lipid S in patients with CR-BSI is given in figure 5-2. Anti-lipid S IgG titres are shown in table 5-5. There was no significant difference in serum IgG titres between the controls and patients with CR-BSI (CoNS – p=0.07, *S. aureus* – p=0.28, *Enterococcus* spp – p=0.53, Gram-negative microorganisms – p=1.0, Mann-Whitney U Test). There was also no correlation between the time of onset of CR-BSI and the antibody titres in patients with CR-BSI due to Gram-positive microorganisms (p=0.2). The diagnostic parameters of the lipid S ELISA for the diagnosis of CR-BSI in haematology/SCT patients are given in table 5-6.

Figure 5-1 – Scattergram demonstrating the range of serum anti-lipid S IgG titres in patients with colonised CVC tips and controls

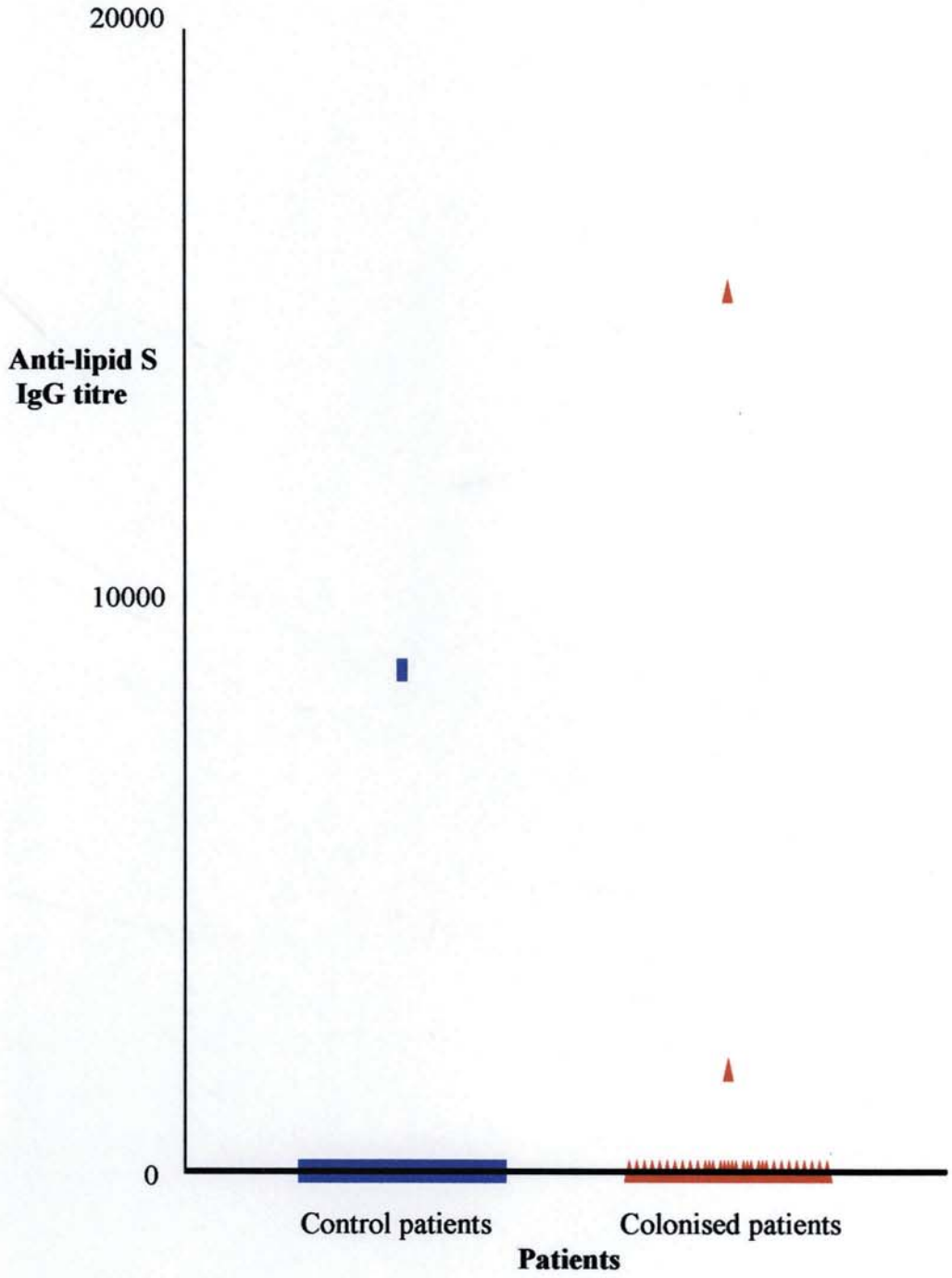


Figure 5-2 – Scattergram demonstrating the range of serum anti-lipid S IgG titres in patients with CR-BSI

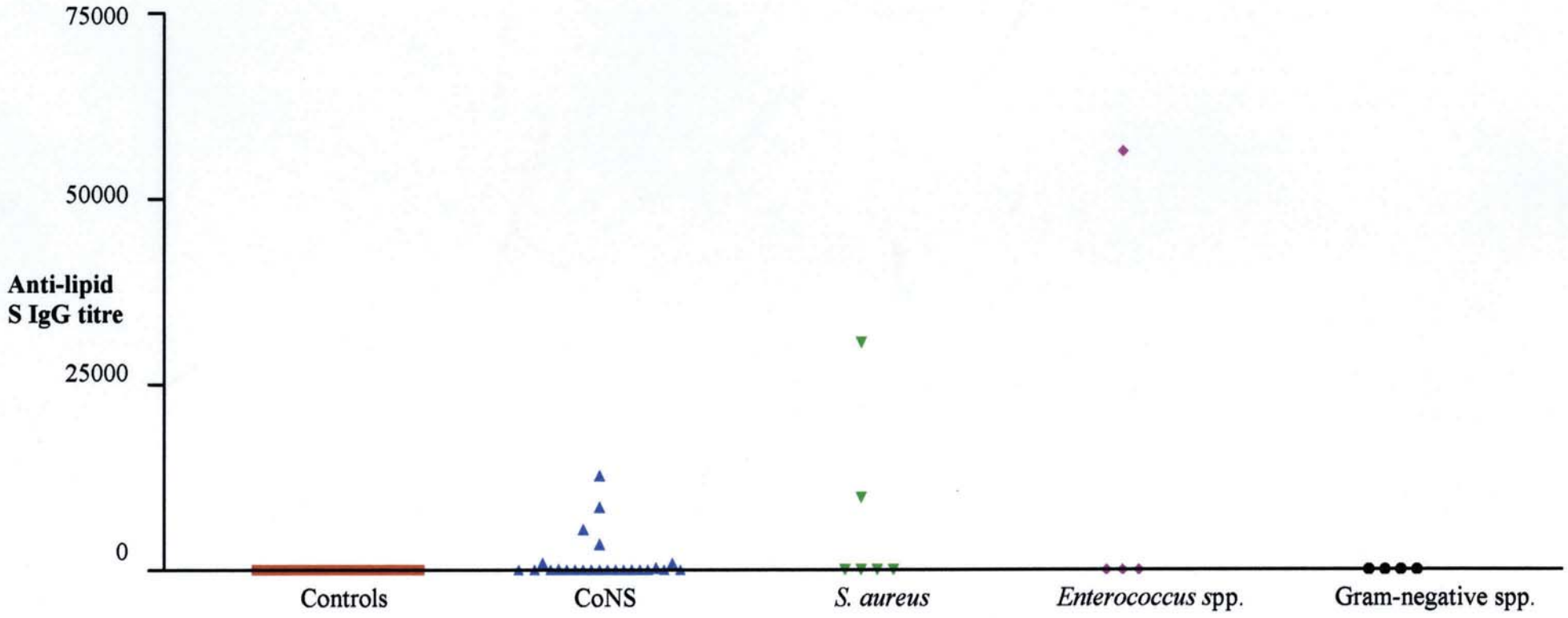


Table 5-5 – Serum IgG titres to lipid S in patients with CR-BSI

Causative microorganism	Controls	CoNS	*S. aureus*	*Enterococcus* spp.	Gram-negative spp.
Total	23	24	6	4	4
Number of patients with positive IgG titres (%)	0 (0)	8 (33)	2 (33)	1 (25)	0 (0)
Mean anti-lipid S IgG titre	ND	1365	6732	14088	ND
Titre range	ND	ND - 12761	ND - 30607	ND - 56352	ND

Table 5-6 – Diagnostic parameters of the lipid S ELISA for CR-BSI in haematology/SCT patients

	Sensitivity (%)	Specificity (%)	Positive predictive value (PPV) (%)	Negative predictive value (NPV) (%)	Accuracy (%)
CoNS	33	100	100	59	66
S. aureus	33	100	100	85	86
Enterococcus spp.	25	100	100	88	89

5.3.2.3 Patients with sternal SSI

A scattergram demonstrating the range of serum IgG titres to lipid S is given in figure 5-3. Anti-lipid S IgG titres are shown in table 5-7. Serum IgG titres of patients with sternal SSI due to *S. aureus*, CoNS and 'skin' microorganisms were all significantly higher than the non-infected control group ($p<0.0001$, $p=0.0001$ and $p=0.0025$ respectively, Mann-Whitney U Test). Patients with deep SSI due to *S. aureus* had significantly higher serum anti-lipid S IgG titres than those with superficial SSI ($p=0.04$, Mann-Whitney U Test). The serum IgG titres to lipid S in superficial and deep infection caused by *S. aureus* in shown in figure 5-4. There was no significant difference in serum levels of anti-lipid S IgG in patients with sternal SSI due to Gram-negative microorganisms and controls ($p=0.74$, Mann-Whitney U Test). There was no significant correlation between time of onset of sternal SSI due to Gram-positive microorganisms and serum IgG levels to lipid S (superficial: $p=0.7$, deep: $p=0.4$). The diagnostic parameters of the lipid S ELISA for the diagnosis of sternal SSI are shown in table 5-8.

Figure 5-3 – Scattergram demonstrating the range of serum anti-lipid S IgG titres in patients with sternal SSI

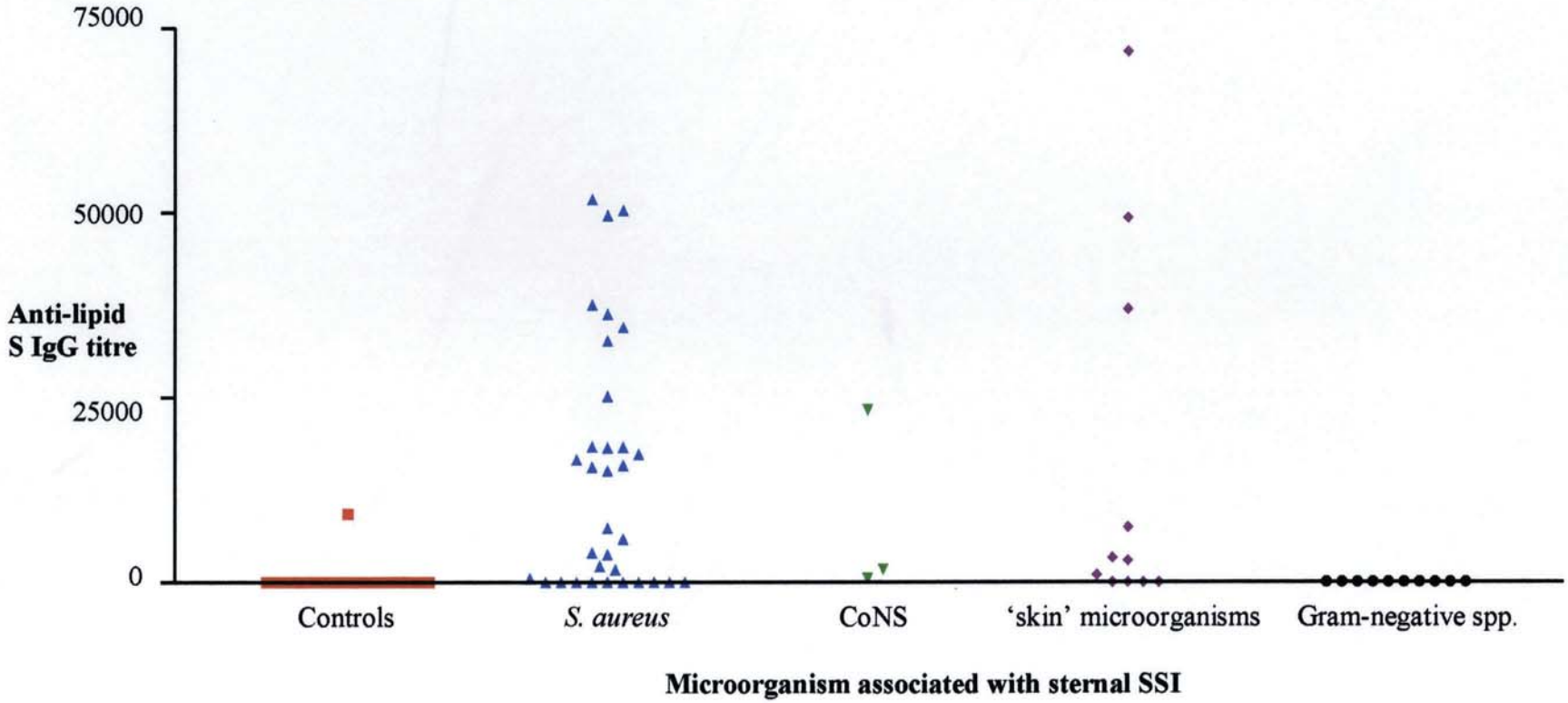


Table 5-7 - Serum IgG titres to lipid S in patients with superficial or deep sternal SSI

Patient group (causative microorganism)		Superficial incisional SSI	Deep incisional SSI	Total in patient group
Non-infected controls	Number	-	-	37
	Number with positive IgG titres (%)	-	-	1 (3)
	Mean anti-lipid S IgG titre	-	-	251
	Titre range	-	-	ND-51874
S. aureus	Number	13	19	32
	Number with positive IgG titres (%)	7 (54)	15 (79)	22 (69)
	Mean anti-lipid S IgG titre	7253	19841	14727
	Titre range	ND-34479	ND-51874	ND-51874
CoNS	Number	1	3	4
	Number with positive IgG titres (%)	1 (100)	3 (100)	4 (100)
	Mean anti-lipid S IgG titre	516	10827	8250
	Titre range	516	7372-23307	516-23307
'skin' microorganisms	Number	11	-	11
	Number with positive IgG titres (%)	7 (64)	-	7 (64)
	Mean anti-lipid S IgG titre	15725	-	15725
	Titre range	ND-71833	-	ND-71833
Gram-negative microorganisms	Number	4	6	10
	Number with positive IgG titres (%)	0 (0)	0 (0)	0 (0)
	Mean anti-lipid S IgG titre	ND	ND	ND
	Titre range	ND	ND	ND

Figure 5-4 – Scattergram demonstrating the range of serum anti-lipid S IgG titres in patients with superficial and deep sternal SSI due to *Staphylococcus aureus*

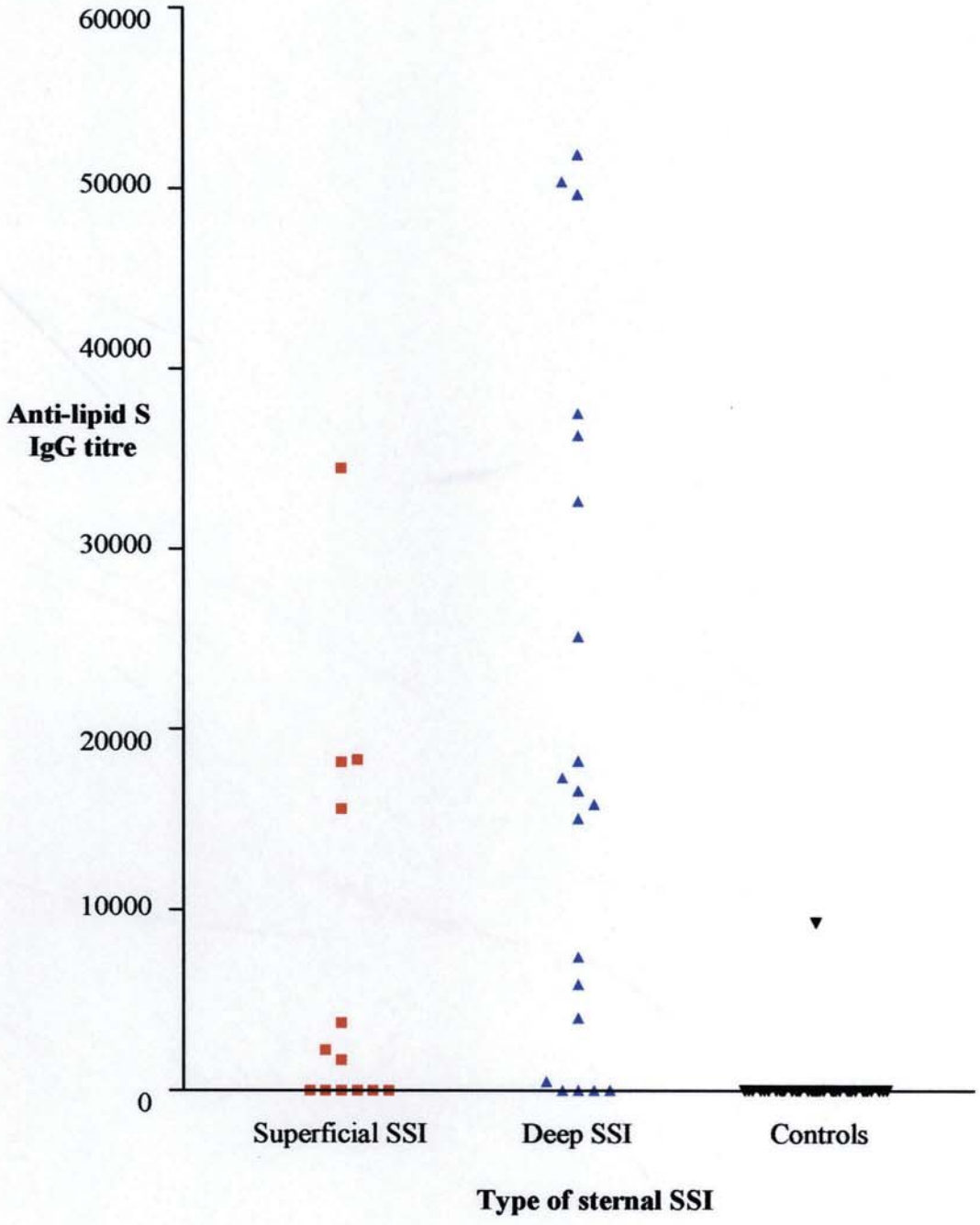


Table 5-8 – The diagnostic parameters of the lipid S ELISA for sternal SSI

Causative microorganism	Infection type	Sensitivity (%)	Specificity (%)	Positive predictive value (PPV) (%)	Negative predictive value (NPV) (%)	Accuracy (%)
S. aureus	Superficial	54	97	88	86	86
	Deep	79	97	94	90	91
	Overall	69	97	96	78	84
CoNS	Superficial	100	97	50	100	97
	Deep	100	97	75	100	98
	Overall	100	97	80	100	98
'skin' microorganisms	Superficial	64	97	88	90	90
	Deep	-	-	-	-	-
	Overall	64	97	88	90	90

5.3.3 Immunocompetency (WBC)

5.3.3.1 Patients with colonised CVC

The mean WBC and lymphocyte count of blood samples taken from patients on the day of CVC removal is summarised in table 5-9. All 3 (100%) patients with elevated anti-lipid S IgG titres had WBC and lymphocyte counts within the normal range.

5.3.3.2 Patients with CR-BSI

The mean WBC and lymphocyte count of blood samples taken from patients on the day of test sera collection is summarised in table 5-9. Of the 11 patients with elevated anti-lipid S IgG titres, only 2 (18%) had both normal WBC and lymphocyte counts. Two (18%) of the remaining patients had reduced lymphocyte counts and 7 (64%) had both reduced WBC and lymphocyte counts.

5.3.3.3 Patients with sternal SSI

The mean WBC and lymphocyte count from blood samples taken from patients on the day of test sera collection is summarised in table 5-9. Of the 33 patients with elevated anti-lipid S IgG titres, 25 (76%) had both normal WBC and lymphocyte counts. The remaining 8 (24%) patients had reduced lymphocyte counts.

Table 5-9 – Mean WBC and lymphocyte counts in patients with colonised CVC, CR-BSI and sternal SSI (and respective controls)

	CVC tip colonisation study		CR-BSI study		Sternal SSI study	
	Control patients	Test patients	Control patients	Test patients	Control patients	Test patients
Total	49	34	23	38	37	32
Mean WBC (and range) x 10^9/L	10.4 (3.5-37)	11.2 (2.4-20.6)	7.4 (0.2-35)	4.2 (0-51)	9.4 (5.2-16.1)	10.2 (5-27.6)
Number of patients with low WBC (%)	1 (2%)	1 (3%)	7 (30%)	24 (63%)	0 (0%)	0 (0%)
Mean lymphocyte (and range) x 10^9/L	1.5 (0.5-5.7)	1.4 (0.2-3.2)	0.9 (0.1-2.3)	1.3 (0-25.8)	1.5 (0.5-3.2)	1.5 (0.2-4.7)
Number of patients with low lymphocyte counts (%)	13 (27%)	10 (29%)	12 (52%)	30 (79%)	4 (11%)	12 (38%)

Normal healthy adult WBC = 4.0-11.0 x 10^9/L, lymphocyte count 1.5-3.5 x 10^9/L (Hoffbrand *et al.*, 2001)

5.4 Discussion

In this current investigation, the potential of the lipid S ELISA to facilitate the diagnosis of CR-BSI in haematology patients and SSI in cardiothoracic surgery patients was assessed. Furthermore, serum levels of anti-lipid S IgG were estimated in patients with colonised CVC due to CoNS to determine if elevated levels of antibody is associated with catheter colonisation.

The results of this current study highlight the potential of the lipid S ELISA as a rapid serological test to facilitate the diagnosis of sternal SSI due to staphylococci. The assay had sensitivities of 69% for infections due to *S. aureus*, 100% for CoNS and 64% for 'skin' microorganisms, which compares well to results from previous studies that assessed the lipid S ELISA for serodiagnosis of central venous CRI (Worthington *et al.*, 2002), endocarditis (Connaughton *et al.*, 2001) and prosthetic joint infection (Rafiq *et al.*, 2000). In addition, an increased assay sensitivity was achieved in patients with deep sternal SSI due to *S. aureus* compared to those with superficial infection (54% and 79% respectively), which may reflect the chronic nature of deep SSI. Indeed, the sensitivity of the lipid S ELISA has frequently been demonstrated to be higher in chronic infections including endocarditis (Connaughton *et al.*, 2001) and prosthetic joint infection (Rafiq *et al.*, 2000). Difficulties in determining whether or not CoNS are associated with SSI or are sample contaminants remains problematic for the Clinical Microbiologist. This is further complicated as patients infected with CoNS often exhibit delayed, discrete symptoms (Archer and Armstrong, 1983). The results of this study highlight the potential of the lipid S ELISA in aiding the diagnosis of true infection due to CoNS and differentiating them from patients with contaminated samples. Furthermore, CoNS are often regarded as commensal 'skin' microorganisms from many wound infections in routine Clinical Microbiology laboratories and are discarded without further antimicrobial sensitivity testing. Indeed, Tammelin and co-workers (2002) suggested that up to 30% of cases of postsurgical mediastinitis caused by CoNS may be misinterpreted as non-infected. In this study, 7 (64%) out of 11 patients whose SSI cultures yielded CoNS were regarded as non-significant had elevated levels of anti-lipid S IgG. Retrospective analysis of clinical information in accordance with CDC guidelines (table 3-1) confirmed that these patients had a clinically defined SSI. This test may therefore provide a useful adjunct when interpreting cultures yielding CoNS from patients with SSI. Rapid serological diagnosis of SSI by lipid S ELISA may therefore facilitate not only the establishment of the clinical

diagnosis but also facilitate the administration of appropriate antibiotic treatment thus improving patient outcome.

The lipid S ELISA failed to detect CR-BSI due to Gram-positive microorganisms in haematology patients with any significant success. Indeed, in these patients the test had sensitivity values of only 33% for CoNS, 33% for *S. aureus* and 25% for *Enterococcus* spp. The non-infected controls and patients with CR-BSI due to Gram-positive microorganisms all had negative anti-lipid S IgG titres. Previous work which has evaluated the lipid S ELISA has been predominantly in immunocompetent patients (Connaughton *et al.*, 2001; Rafiq *et al.*, 2000).

Serological analysis of anti-lipid S IgG titres in patients with colonised CVC compared with controls revealed that there was no significant difference between the 2 patient groups. The lipid S ELISA may therefore facilitate the interpretation of positive culture plates due to CoNS whereby >15 colonies are achieved, thus confirming or excluding the diagnosis of CR-BSI. This is clinically important in preventing the unnecessary administration of antibiotics to those patients whose tips were colonised with CoNS. Inappropriate use of antibiotics in the clinical setting has led to an increase in the number of resistant and multiresistant microorganisms including *S. aureus* and CoNS.

In these studies, 30% of patients with a diagnosis of sternal SSI and 68% of patients with CR-BSI and due to Gram-positive microorganisms had non-detectable serum levels of anti-lipid S IgG. There are several possible hypotheses as to why serum IgG titres were non-detectable in these patients. The time of onset of infection to collection of serum samples may not have been optimal to detect sufficient anti-lipid S IgG titres; the strain of microorganism associated with the infection may have produced little or no exocellular lipid S antigen; the infection may not have been caused by the microorganism recovered or the patient may have been incapable of initiating an immune response.

To assess the immunocompetency of the patients recruited, WBC and lymphocyte counts were recorded in order to determine if each patient had sufficient cells to produce antibodies to the lipid S antigen. Of those patients with colonised CVC and associated control patients the mean WBC and lymphocyte counts were within the normal range, however approximately 28% of patients overall had slightly reduced lymphocytes which may impair the patients' ability to mount a humoral immune response.

For patients with CR-BSI, the mean WBC and lymphocyte counts were, again within the normal range of healthy adults. However 49% of patients had a reduced total WBC count (overall, 69% had reduced lymphocyte counts) which suggests that these patients did not have the means to mount a humoral response with production of anti-lipid S IgG. These low blood counts may be due to patients receiving myeloablative therapy including chemotherapy, total body irradiation and immunosuppressants which constitute part of a stem cell transplantation course.

Of those patients with sternal SSI, the mean WBC and lymphocyte counts were within the normal range and only 23% of patients overall had reduced lymphocyte counts. However, this population of patients with reduced lymphocyte counts may explain why the lipid S assay did not achieve a higher sensitivity value when used for this patient group.

Interestingly, detectable levels of serum anti-lipid S IgG were observed in some immunocompromised individuals suggesting that the infection may have commenced earlier than diagnosed by the clinician or Microbiology laboratory and therefore anti-lipid S IgG antibodies were present before WBC were reduced by immunosuppressive therapy.

In conclusion, the lipid S ELISA is a sensitive and specific indirect ELISA for the rapid serodiagnosis of sternal SSI and CR-BSI due to Gram-positive microorganisms in immunocompetent patients which may aid in optimising patient management. Since this assay achieved poor sensitivity results for immunocompromised patients, other non-immunological diagnostic tests should be considered to support the diagnosis in this patient group. The assay may also provide a useful tool for microbiologists when interpreting cultures of CoNS recovered from microbiological specimens. However, further assessment of the lipid S ELISA for diagnosing these infections is required in the clinical setting. Potential study protocols should investigate; the optimum time in the infection for sera collection, whether the use of paired or multiple serum samples would improve diagnosis, the level of immunocompetency required for the test to be useful and the screening of a panel of Gram-positive microorganisms for their ability to produce the lipid S antigen.

6. Evaluation of the lipid S enzyme linked immunosorbent assay and acridine orange leucocyte cytospin test for the diagnosis of Hickman catheter-related bloodstream infection in haemato-oncology and stem cell transplant patients

6.1 Introduction

It was demonstrated in chapter 5 that the lipid S ELISA is a sensitive and specific test for the rapid serodiagnosis of CR-BSI due to staphylococci in immunocompetent patients. However, this assay achieved poor sensitivity results for immunocompromised patients. It was also demonstrated that the degree of immunocompetency influences results achieved in the lipid S ELISA and that other non-immunological diagnostic tests should be considered in such patients. The acridine orange leucocyte cytospin (AOLC) test is a non-immunological assay that has been evaluated for the diagnosis of CR-BSI in a number of studies (Bong *et al.*, 2003; Tighe *et al.*, 1996; von Baum *et al.*, 1998; Rushforth *et al.*, 1993; Kite *et al.*, 1988; Kite *et al.*, 1999; Tighe *et al.*, 1995). In addition, the AOLC has been recognised as a rapid test for the diagnosis of CR-BSI. Indeed, results may be obtained within 1 hr of sample collection compared to the 24-48 hr it takes to perform routine quantitative blood cultures (Rushforth *et al.*, 1993). Bong and colleagues (2003) also demonstrated that the use of the AOLC test avoids the unnecessary removal of CVC and the costs associated with treating a mis-diagnosed CR-BSI. However, as described in chapter 1 (section 1.5.2.2.5), the AOLC has been successfully utilised to diagnose CR-BSI in neonates rather than adult patients (Rushforth *et al.*, 1993; Tighe *et al.*, 1995). This may be due to the use of CVC with narrower internal lumen diameters in neonates, creating increased velocity and turbulence of blood flow through the CVC during aspiration liberating more microorganisms from the CVC lumen surface (Kite *et al.*, 1999). A further explanation is that neonatal bacteraemia is characterised by higher bacterial counts per ml of blood than in adult bacteraemia (Kite *et al.*, 1999).

The aims of this current study were to:

a) Evaluate the lipid S ELISA for the serodiagnosis of staphylococcal Hickman CR-BSI in haemato-oncology and stem cell transplant patients undergoing intensive myeloablative therapy.

b) Assess the efficacy of the AOLC in the diagnosis of CR-BSI in adult haemato-oncology and stem cell transplant patients with wide-lumen Hickman CVC.

c) Compare the lipid S ELISA and AOLC as rapid tests for the diagnosis of CR-BSI in haemato-oncology and stem cell transplant patients.

6.2 Materials and Methods

6.2.1 Patient selection

Local research ethical committee approval was sought for patients from the haematology and Bone Marrow Transplant units who require a central venous catheter as part of their clinical management to be entered into the study (appendix 2). Patients admitted for Hickman CVC insertion and subsequent SCT between May 2001 and January 2003 were prospectively recruited into the study. Informed consent was obtained from all patients recruited into the study (appendices 3 and 4). All patients recruited had no evidence of infection 6 months prior to the time of recruitment. Patient demographics were recorded on a standard case report form (appendix 5).

Inclusion criteria

1) Patients greater than 18 years of age

2) Patients undergoing treatment at the haematology and BMT units, University Hospital Birmingham NHS Trust, UK

Exclusion criteria

1) Patients with a psychiatric history or who are mentally handicapped

2) Breast feeding or pregnant women

6.2.2 Lipid S ELISA

Clotted blood samples were obtained from each patient at regular intervals from the time of Hickman CVC insertion to removal. The lipid S ELISA was performed on all serum samples as outlined in chapter 5, sections 5.2.2.1 to 5.2.2.2.

6.2.3 Acridine orange leucocyte cytospin test

Fifty microlitre samples were obtained from blood aspirated from the CVC at regular intervals and tested by the method outlined by Tighe and colleagues (1995). The blood sample was mixed for 30 sec with 1.2ml of hypotonic formal saline (0.146% NaCl in 4% formalin (Genta Medical, UK)) to lyse erythrocytes and fix leucocytes. Hypertonic saline (2.8ml) (1.168% NaCl) was then added and the sample was centrifuged at 2000 x *g* for 5 min. Once the supernatant had been decanted, the deposit was resuspended. The cytospin slide (Ultima, UK), filter card (Shandon Inc, PA, USA) and funnel (Shandon Inc, PA, USA) were assembled ready for use. Next, 200µl of the suspension was transferred to the funnel for cytospin at 1200rpm in a Shandon II cytospin (Shandon Inc, PA, USA). This

centrifugation process resulted in the formation of a cellular monolayer on the slide which was then stained with acridine orange (1/25000 w/v) (Pro-Lab Diagnostics, UK) for 30 sec and left to air dry.

6.2.4 Routine microbiological and haematological investigations

Blood and Hickman exit site swab cultures were performed by the routine microbiology laboratory to detect CRI as part of each patients' clinical management (chapter 3, sections 3.2.2.1 and 3.2.2.3). Any positive microbiological specimens obtained from study patients were recorded. Also, as an integral component of the patients' clinical management, regular WBC counts were performed by the routine haematology laboratory. The results of the total WBC and lymphocyte counts performed on days where specimens were collected for the lipid S ELISA and/or AOLC were recorded.

6.3 Results

6.3.1 Patients

Sixteen patients were recruited into the study. Patient demographics and clinical presentation are given in table 6-1.

Table 6-1 Patient demographics and clinical presentation

Mean age (years)	39
Age range (years)	23 - 54
Male	12
Female	6
Clinical presentation	
Non-Hodgkins lymphoma (NHL)	7
Acute Lymphoblastic leukaemia (ALL)	1
Myelodysplasia (MD)	2
Multiple myeloma (MM)	1
Waldenstrom's macroglobulinaemia (WM)	1
Hodgkins Lymphoma (HL)	1
Chronic Myeloid Leukaemia (CML)	1
Aplastic anaemia (AA)	1
Polyneuropathy, organomegaly, endocrinopathy, M protein and Skin changes (POEMS)	1

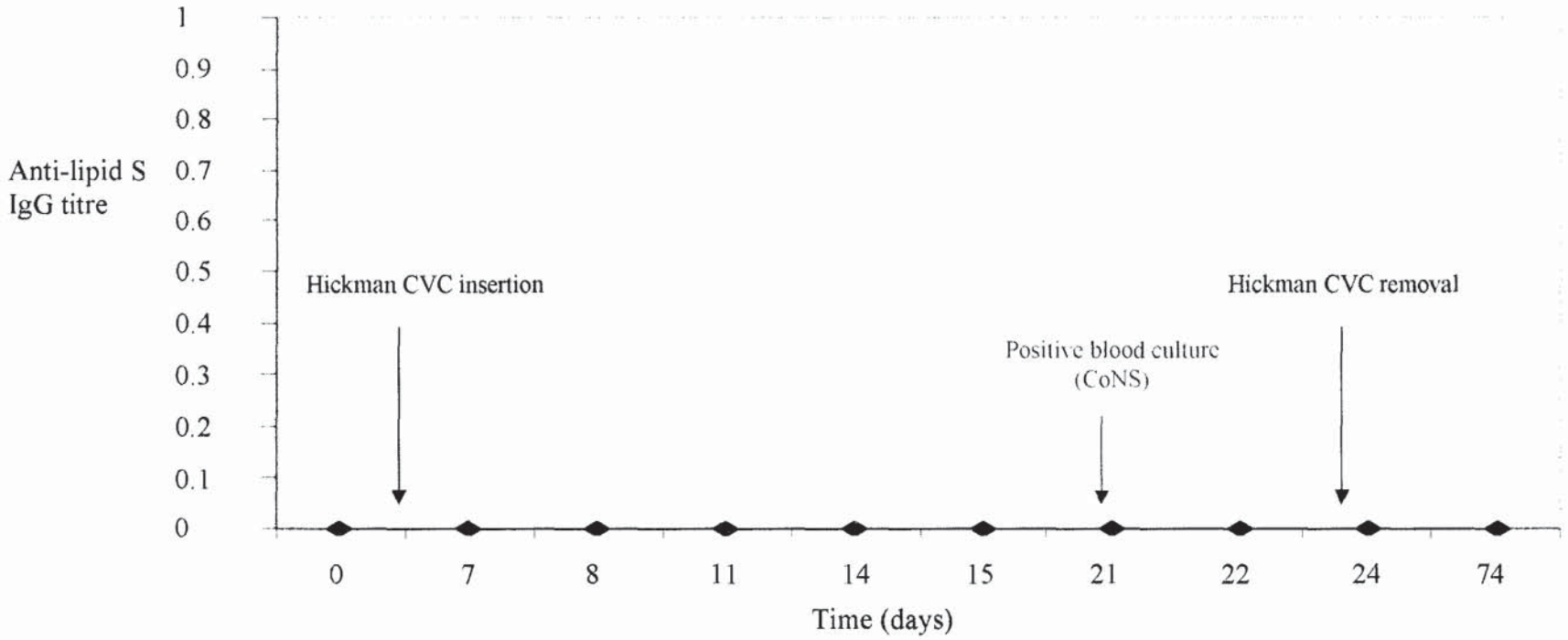
The anti-lipid S IgG titres, AOLC results, leucocyte counts and positive microbiological results for the 16 patients during the study period are given in figures 6-1 to 6-16.

6.3.2 Catheter-related infection in study patients

Three out of 16 (19%) of the patients recruited had no clinical or microbiological symptoms of CR-BSI during Hickman CVC placement (figures 6-5, 6-9 and 6-11).

Of the 13 remaining patients, 11 (85%) had episodes of CR-BSI due to CoNS (figures 6-1 to 6-4, 6-6 to 6-8 and 6-12 to 6-15), 3 (23%) due to Gram-negative microorganisms (figures 6-10, 6-12 and 6-13) and 1 (8%) due to *S. aureus* during Hickman CVC placement.

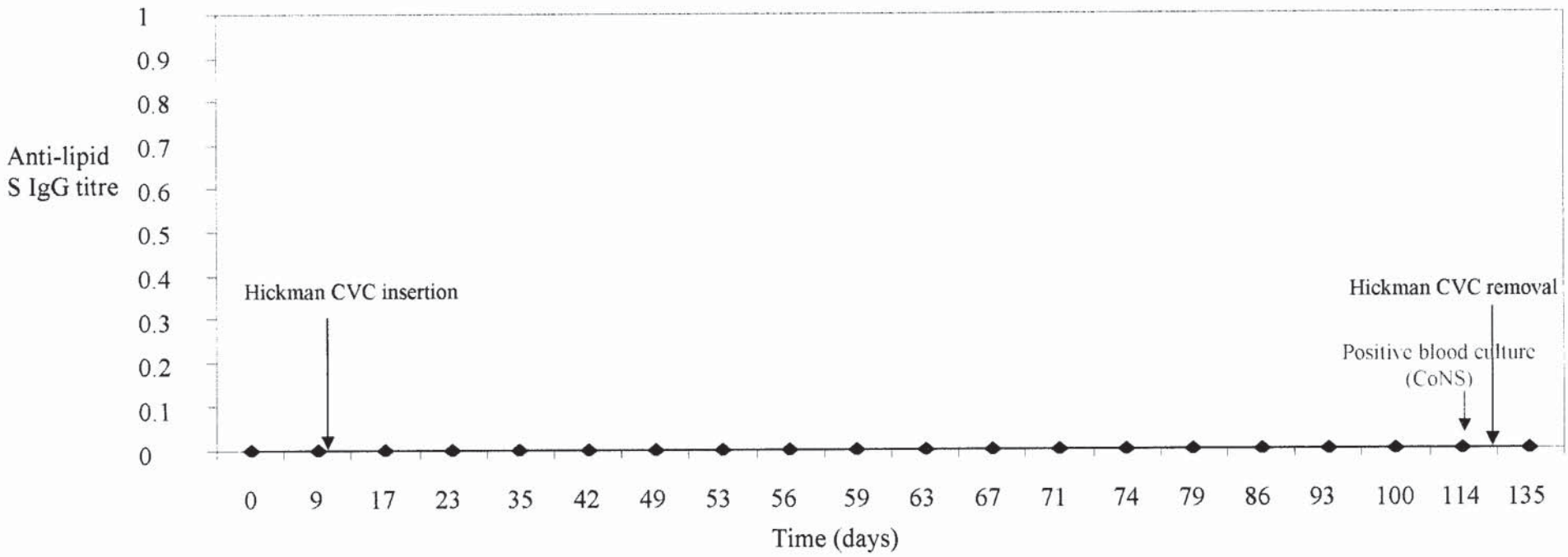
Figure 6-1 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 1



Day of blood collection	0	7	8	11	14	15	21	22	24	74
AOLC result	ND	ND	+	ND	+	ND	+	ND	ND	ND
WBC ($x10^9$/L)	4.7	9.3	8.2	7.2	5.6	3.4	4.0	6.3	10.8	2.1
Lymphocyte count ($x10^9$/L)	1.3	0.4	0.3	0.2	0.1	0.1	0.2	0.6	0.7	0.2

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

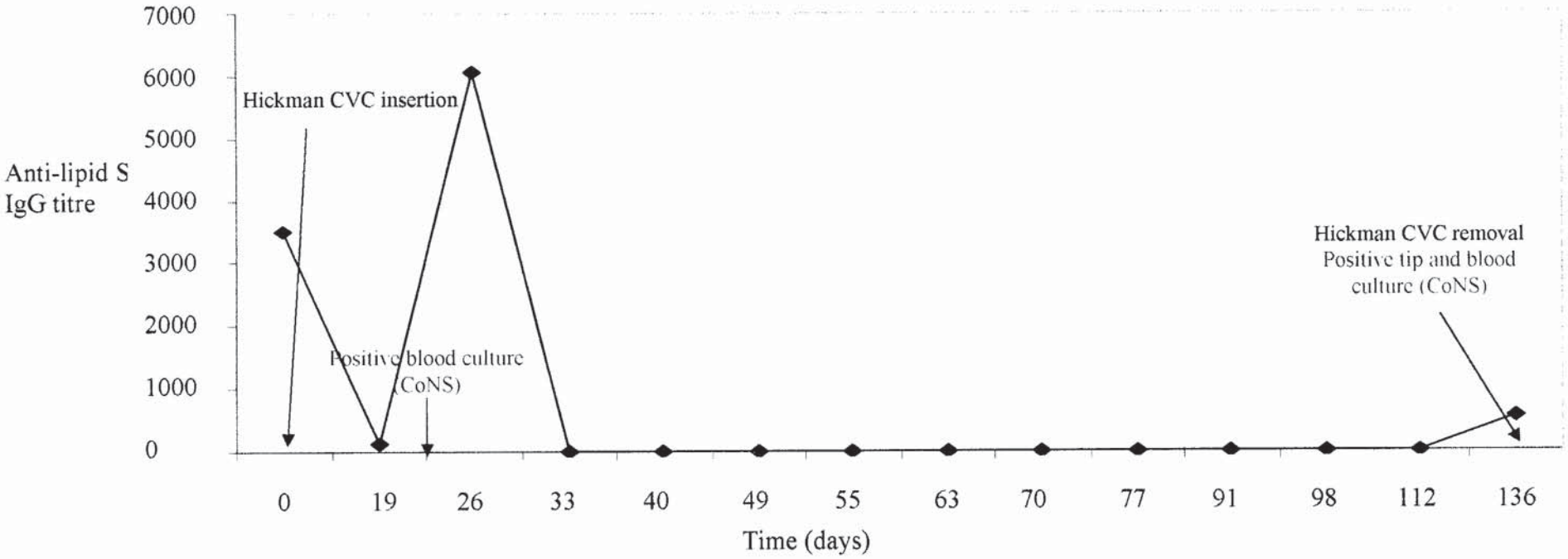
Figure 6-2 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 2



Day of blood collection	0	9	17	23	35	42	49	53	56	59	63	67	71	74	79	86	93	100	114	135
AOLC result	ND	ND	+	+	-	-	+	ND	+	ND	+	ND	+	ND	ND	-	-	-	+	ND
WBC ($x10^9$/L)	4	8.6	4.2	1.9	5.3	7.1	7.3	9.4	3.6	0.8	0	0.1	3.4	5.8	5.6	6.2	6.5	4.9	4.5	0.4
Lymphocyte count ($x10^9$/L)	1	1.9	0.7	0.8	1	1.6	1.9	0.1	0	0	0	0	0.2	0.3	0.5	0.8	1	1	0.9	0.1

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

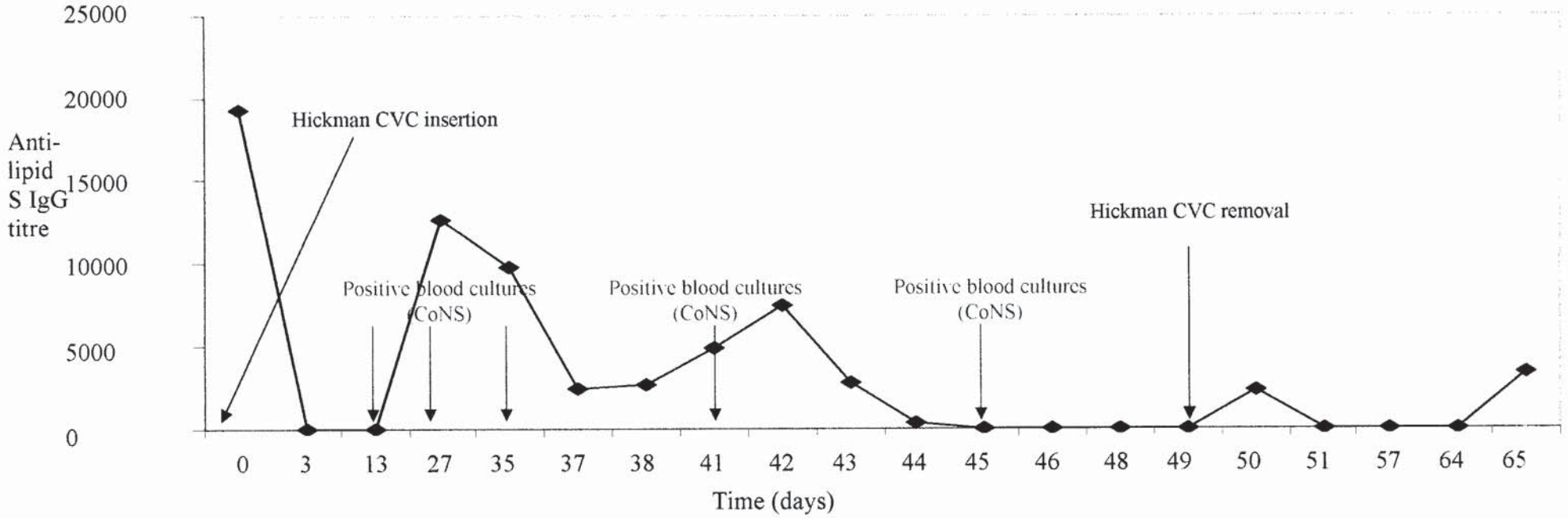
Figure 6-3 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 3



Day of blood collection	0	19	26	33	40	49	55	63	70	77	91	98	112	136
AOLC result	ND	+	+	+	+	-	+	+	-	+	-	-	+	+
WBC (x10^9/L)	4.5	4.3	4	4.2	0.5	6.1	8.7	7.2	7.5	7.9	5.1	4.1	4.5	7.7
Lymphocyte count (x10^9/L)	2.3	1.9	1.6	1.5	0.2	1.4	2.0	1.4	1.4	1	0.8	0.8	1.5	1.8

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

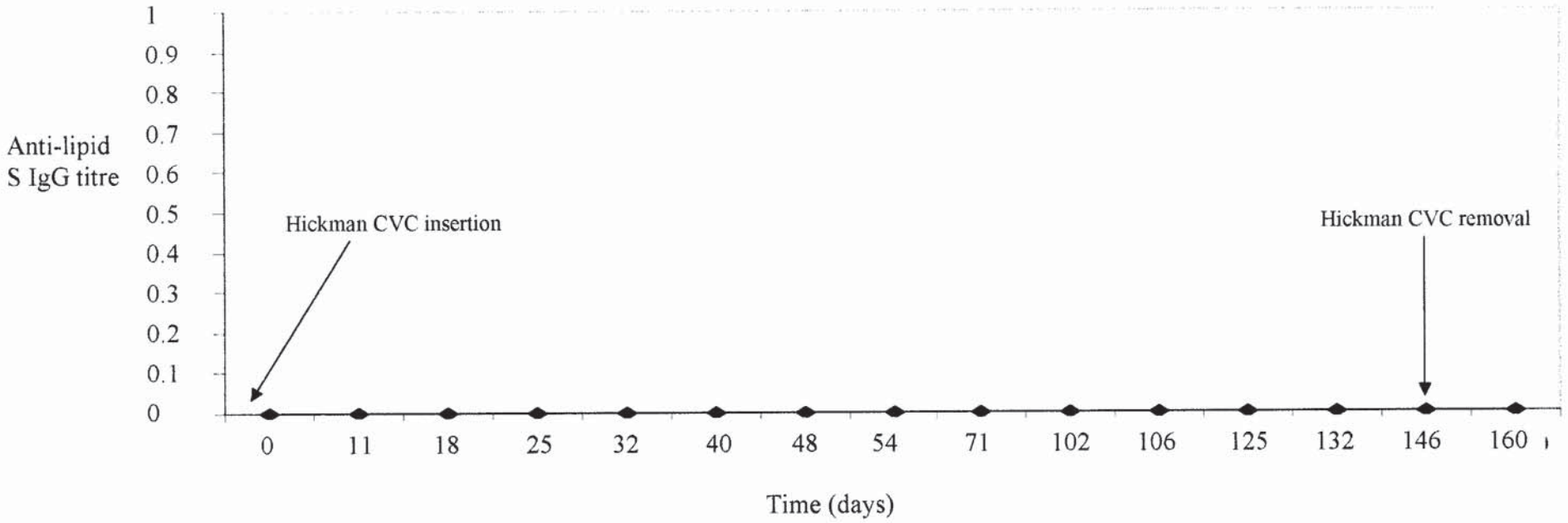
Figure 6-4 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 4



Day of blood collection	0	3	13	27	35	37	38	41	42	43	44	45	46	48	49	50	51	57	64	65
AOLC result	ND	-	+	+	+	ND	ND	+	ND	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	ND
WBC ($x10^9$/L)	5.4	2.5	9.5	6.3	4.3	5	4.4	4.1	4.3	4.1	0.1	0	0.2	0.5	1.1	4	4.7	5	4.5	7.8
Lymphocyte count ($x10^9$/L)	1.5	0.3	1.2	1.6	1.5	1.5	1.5	1.7	1.5	1.5	0.1	0	0.1	0.2	0.2	1.5	0.7	0.9	0.8	1.6

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

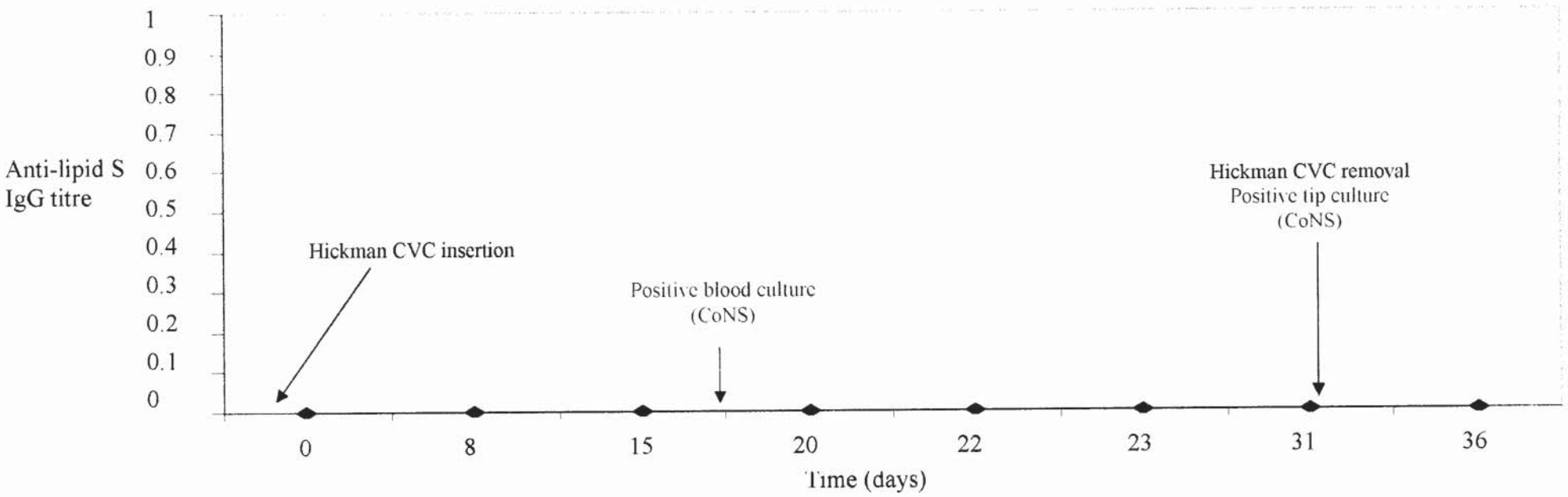
Figure 6-5 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 5



Day of blood collection	0	11	18	25	32	40	48	54	71	102	106	125	132	146	160
AOLC result	ND	-	-	+	+	-	+	+	+	ND	-	ND	ND	+	ND
WBC ($x10^9$/L)	4.5	4.5	1.6	0.2	6.4	11.9	17.3	19.5	16.4	7.8	9.8	10.1	7.1	7	4.1
Lymphocyte count ($x10^9$/L)	2.3	1.8	0.1	0.2	1.2	1	2.6	0.8	0.8	1	0.9	0.6	0.8	1.2	0.6

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count.
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

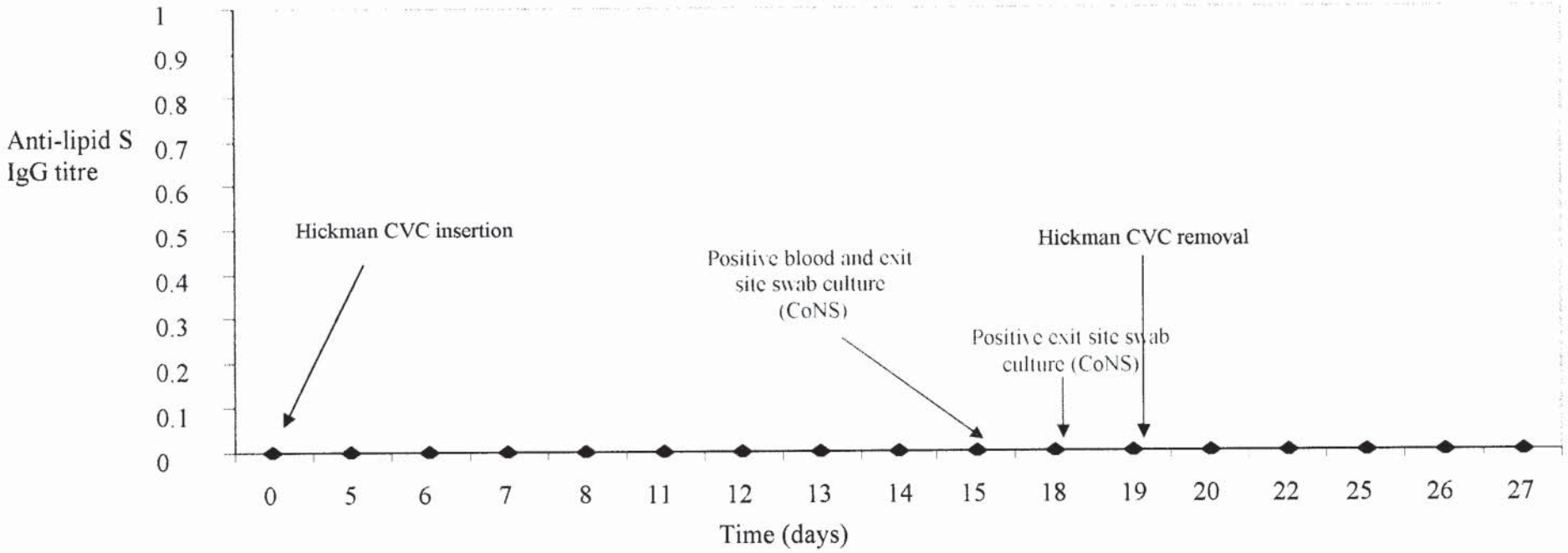
Figure 6-6 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 6



Day of blood collection	0	8	15	20	22	23	31	36
AOLC result	ND	+	+	ND	ND	+	+	ND
WBC ($x10^9$/L)	2.1	2.7	3.3	0.1	0	0	0.2	8.8
Lymphocyte count ($x10^9$/L)	0.5	0.5	0	0	0	0	0	0.2

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

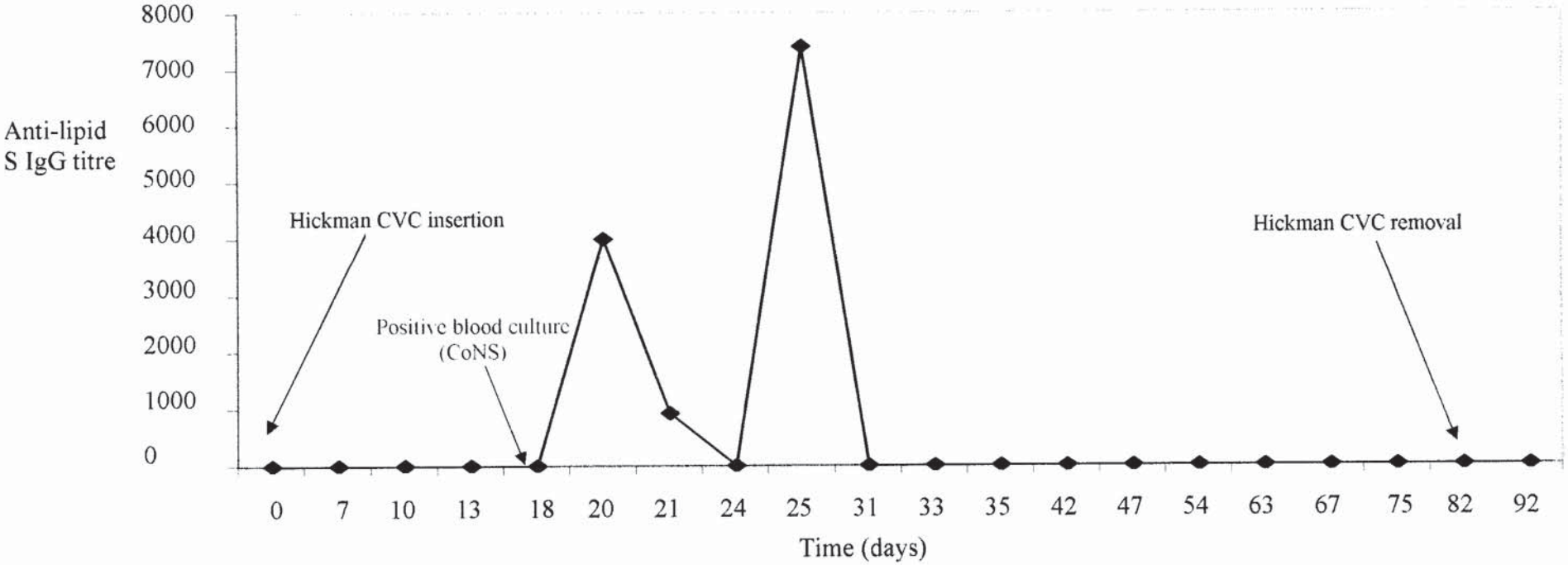
Figure 6-7 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 7



Day of blood collection	0	5	6	7	8	11	12	13	14	15	18	19	20	22	25	26	27
AOLC result	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
WBC ($x10^9$/L)	6.4	5.9	15.4	15	10.5	1.5	1.1	0.6	0.3	0.1	0.1	0.1	0.2	1.1	1.7	2.8	2.6
Lymphocyte count ($x10^9$/L)	1.6	1.4	0.3	0.3	0.2	0.1	0.2	0.2	0.1	0	0.1	0.1	0	0.1	0.1	0.2	0.2

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

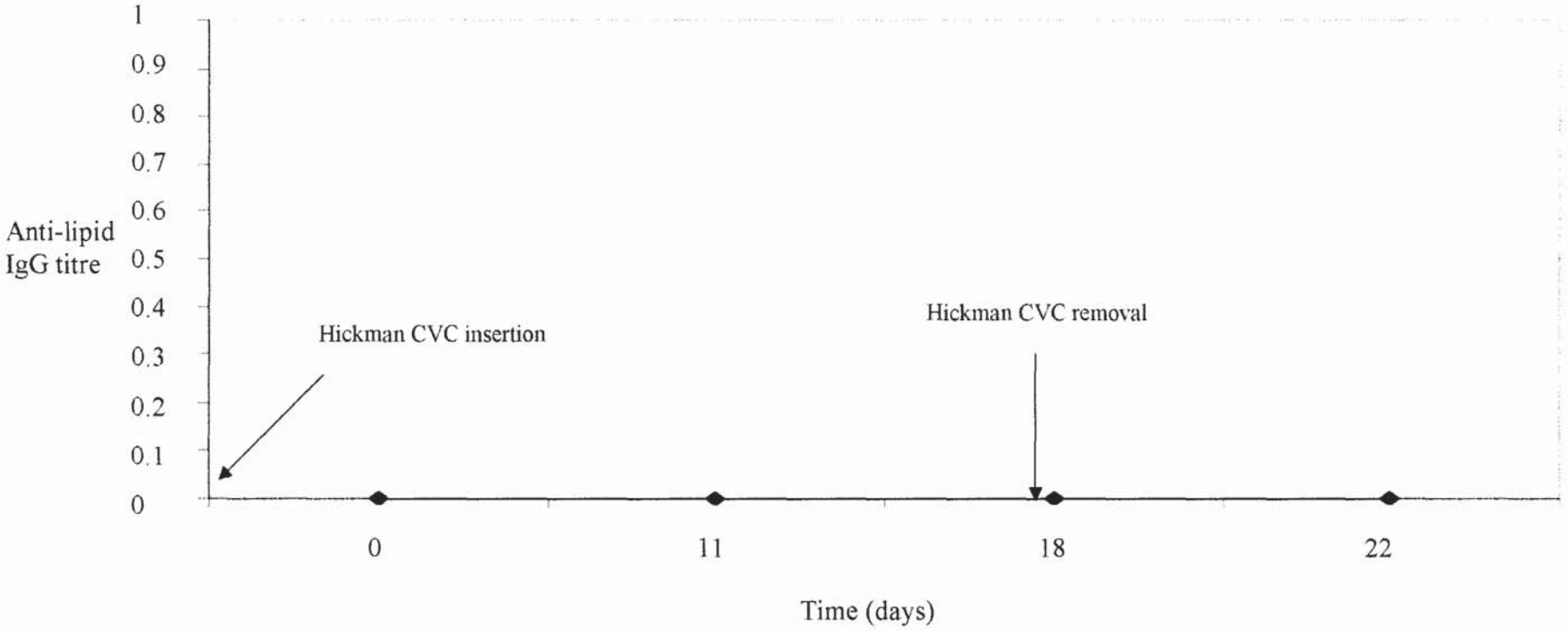
Figure 6-8 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 8



Day of blood collection	0	7	10	13	18	20	21	24	25	31	33	35	42	47	54	63	67	75	82	92
AOLC result	ND	ND	+	ND	+	ND	ND	+	ND	+	ND	ND	+	ND	ND	ND	ND	ND	ND	ND
WBC (x10^9/L)	25.1	14.1	8.1	1.1	0.1	4.1	0.2	0.8	6.2	10.3	10.5	7.8	10.7	12.7	13.8	6.3	18	11.7	14.5	4.6
Lymphocyte count (x10^9/L)	4.1	1.4	1.4	0.1	0.1	1.5	0.2	0.6	1.7	1.7	1.4	1.2	2	1.9	2.1	1.7	1.9	2.5	4	1.1

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

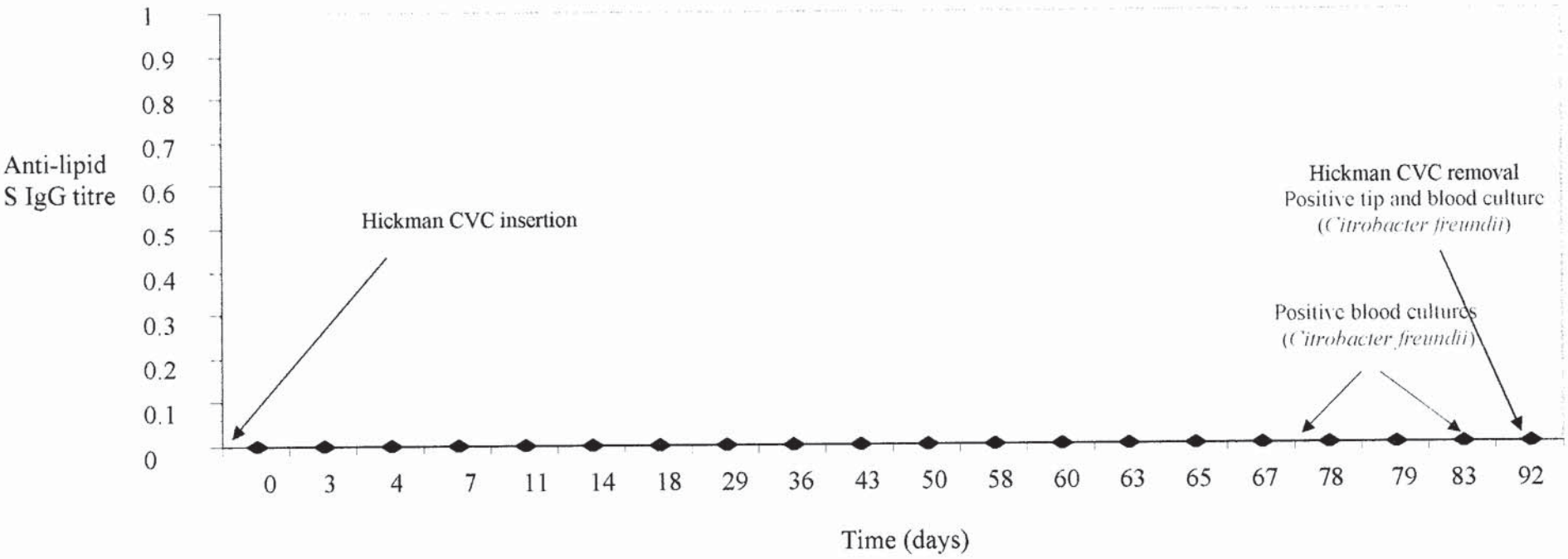
Figure 6-9 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 9



Day of blood collection	0	11	18	22
AOLC result	ND	+	ND	ND
WBC ($x10^9$/L)	6.3	5.2	0.1	1.6
Lymphocyte count ($x10^9$/L)	1.9	0.2	0.1	0.4

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

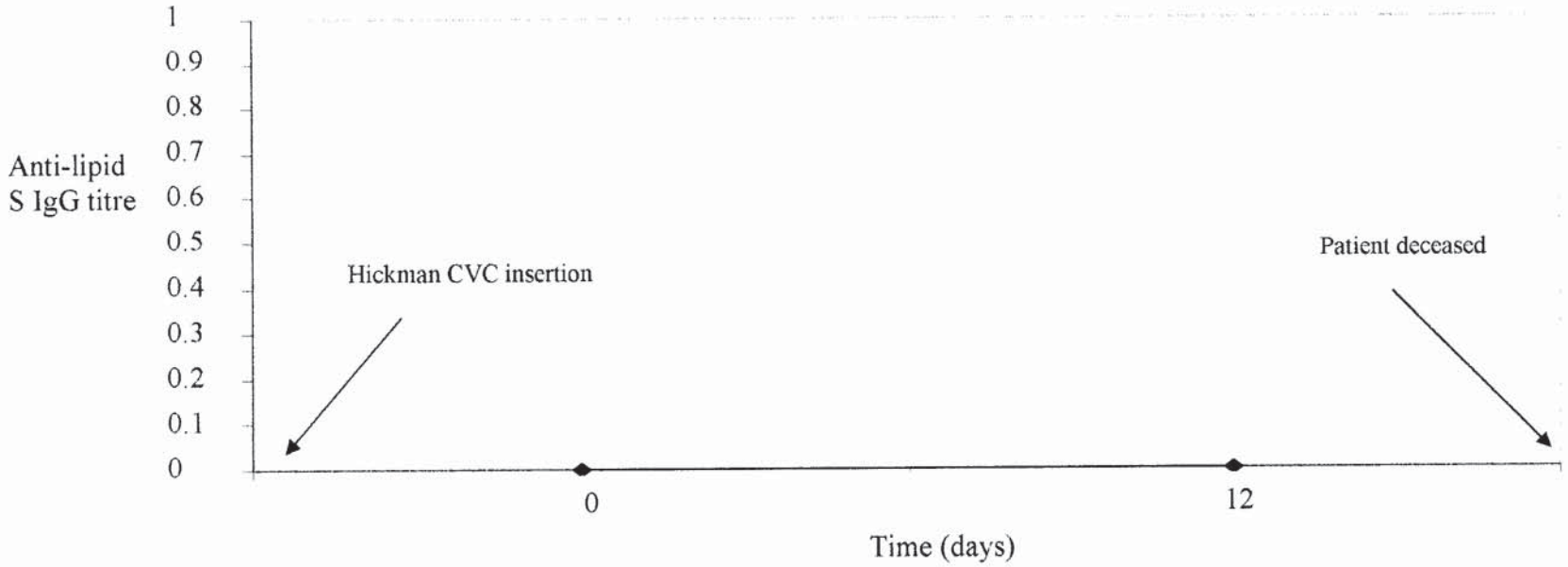
Figure 6-10 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 10



Day of blood collection	0	3	4	7	11	14	18	29	36	43	50	58	60	63	65	67	78	79	83	92
AOLC result	ND	+	ND	ND	ND	-	ND	ND	+	-	ND	ND	ND	-	ND	ND	ND	ND	ND	ND
WBC ($x10^9$/L)	3.5	4	4.6	0.4	0.2	18.5	13.2	3.1	5.5	5	5.6	4.5	8.9	3.4	4	3.4	6.6	4.4	6.9	7.1
Lymphocyte count ($x10^9$/L)	0.3	0.7	0.2	0.2	0.1	1.2	0.8	0.5	0.6	0.7	0.6	0.5	0.3	0.6	0.7	0.8	0.5	0.5	0.6	0.8

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

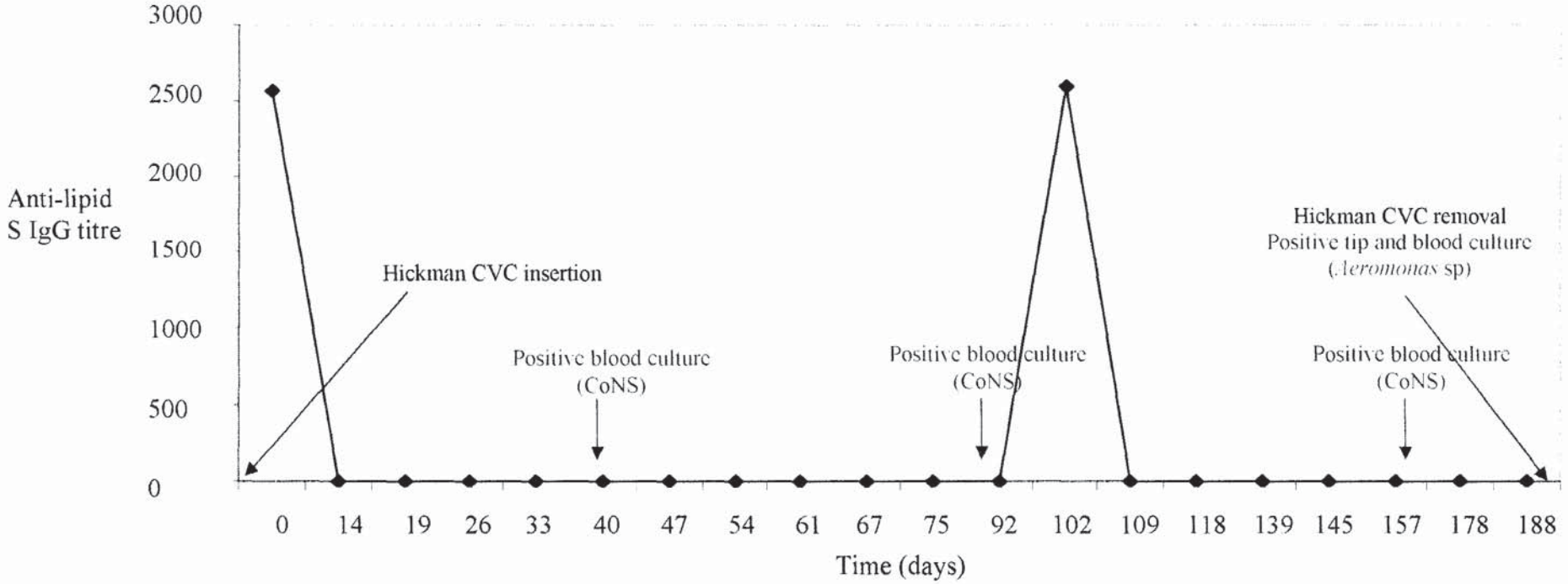
Figure 6-11 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 11



Day of blood collection	0	12
AOLC result	ND	+
WBC ($x10^9$/L)	0.2	8.6
Lymphocyte count ($x10^9$/L)	0.1	0.2

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

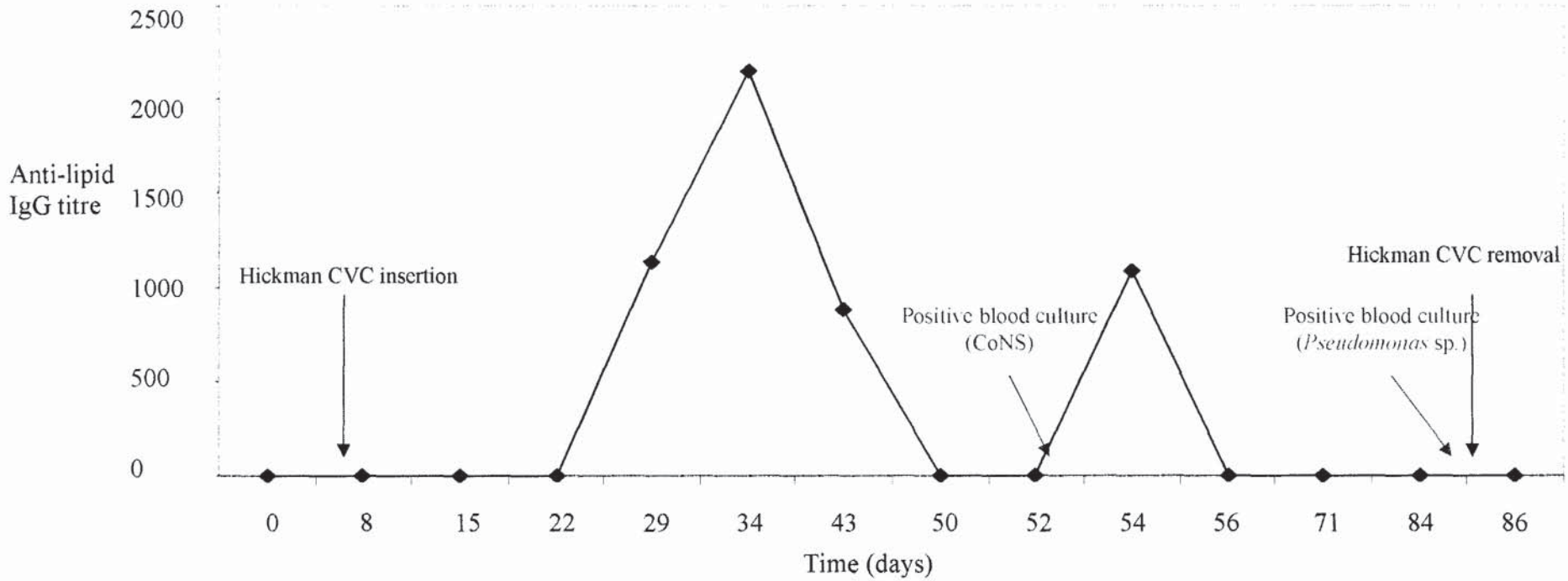
Figure 6-12 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 12



Day of blood collection	0	14	19	26	33	40	47	54	61	67	75	92	102	109	118	139	145	157	178	188
AOLC result	ND	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	ND
WBC ($x10^9$/L)	17.1	35	0.9	0	0	3	7.1	10.4	11.9	10.8	8.1	3.6	4.2	12	8.5	4.5	5.6	2.4	3.6	9.8
Lymphocyte count ($x10^9$/L)	3.5	1	0.1	0	0	0.1	0.7	0.7	0.5	0.5	0.5	0.6	1.6	0.7	0.4	1	2	0.8	1.1	2

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

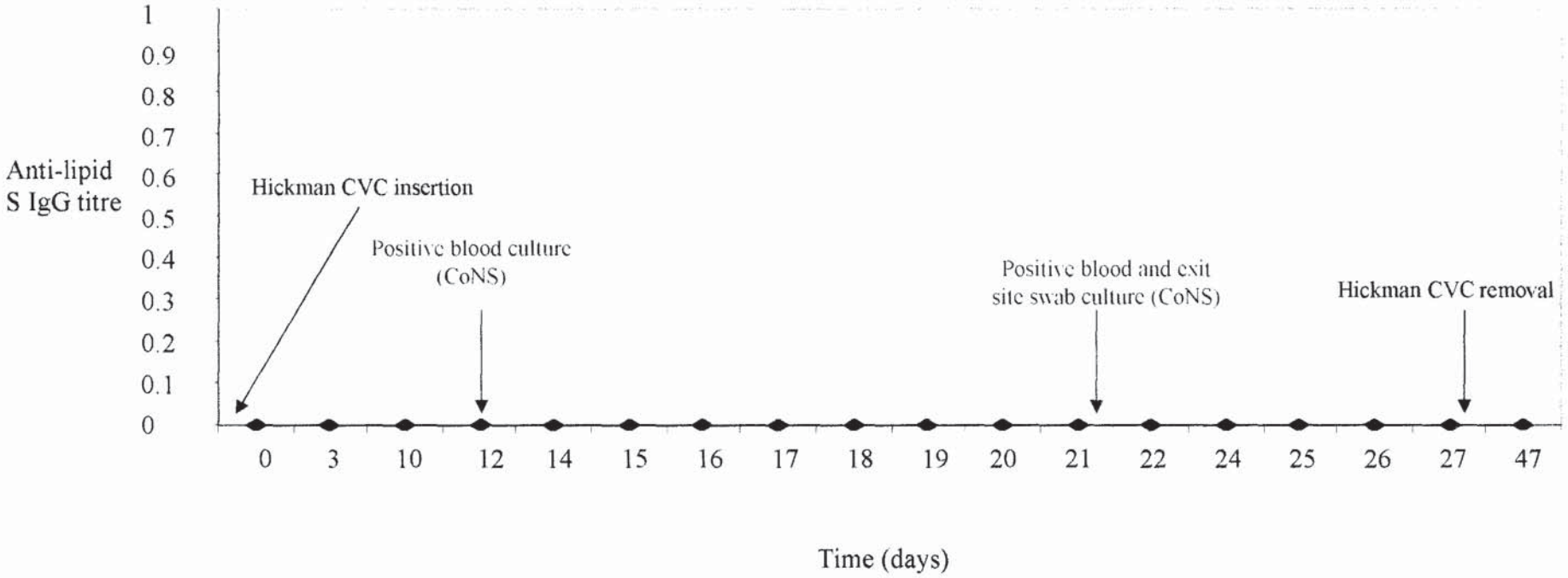
Figure 6-13 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 13



Day of blood collection	0	8	15	22	29	34	43	50	52	54	56	71	84	86
AOLC result	ND	-	-	-	-	-	-	-	-	-	-	-	-	-
WBC ($x10^9$/L)	4.4	5.1	1.1	0.9	7.9	4.8	4.2	0.8	1.1	6.8	21	5.6	0.1	2
Lymphocyte count ($x10^9$/L)	1.4	0.5	0.5	0.7	1.9	1.5	1.5	0.5	0.7	1.6	1.5	0.7	0	0.5

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

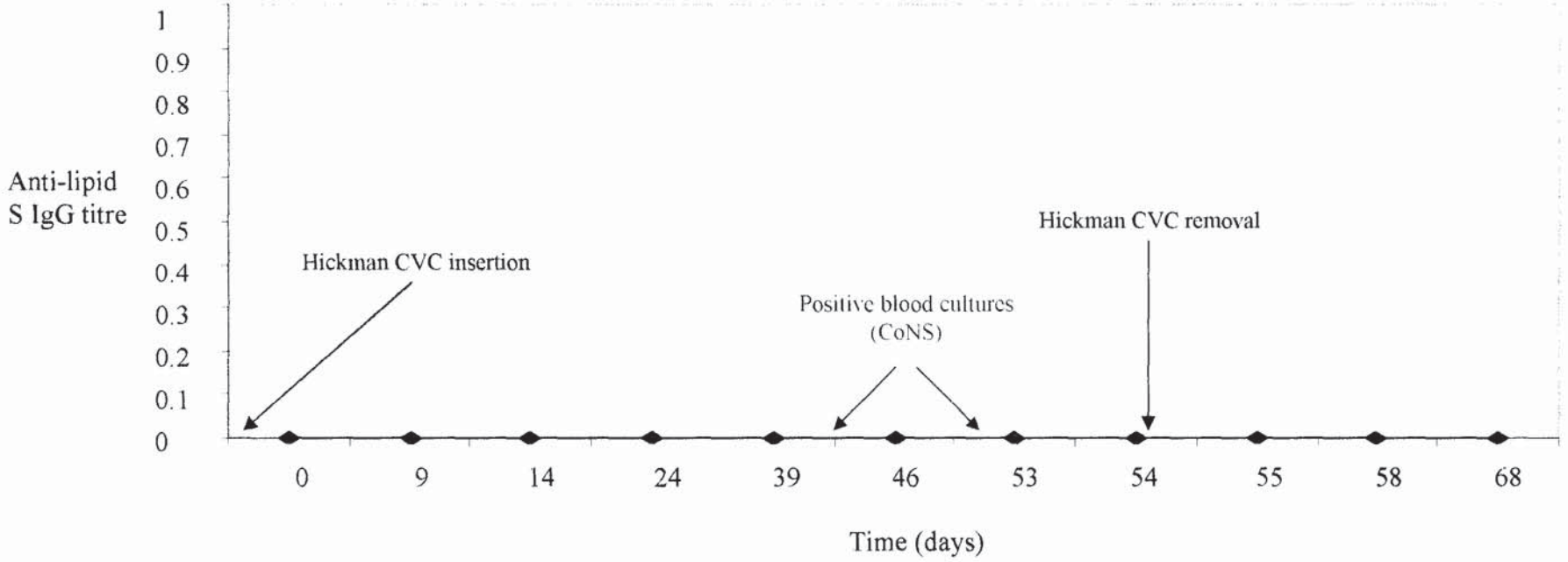
Figure 6-14 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 14



Day of blood collection	0	3	10	12	14	15	16	17	18	19	20	21	22	24	25	26	27	47
AOLC result	ND	-	-	ND	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	+	ND	ND	ND
WBC ($x10^9$/L)	11.7	10.2	9.6	3.9	3.8	3.9	3.7	1.6	0.4	0.1	0.1	0	0.1	0.4	0.8	2.3	3.9	3.5
Lymphocyte count ($x10^9$/L)	2	1.9	1.9	1.4	0.6	0.5	0.3	0.2	0.1	0	0.1	0	0.1	0.1	0.1	0.3	0.4	0.9

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

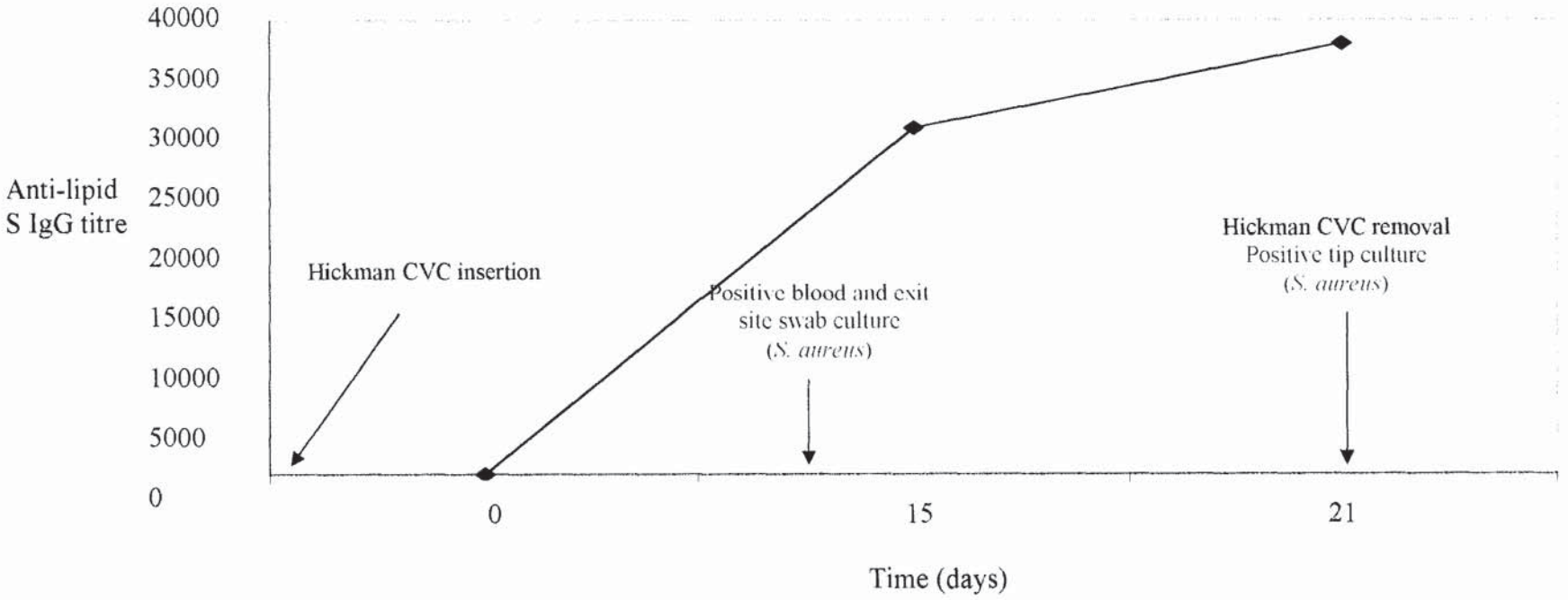
Figure 6-15 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 15



Day of blood collection	0	9	14	24	39	46	53	54	55	58	68
AOLC result	ND	-	-	-	+	+	ND	+	ND	ND	ND
WBC ($x10^9$/L)	6.9	4	0.2	19.3	3.9	3.4	0	0	0.1	1	5.4
Lymphocyte count ($x10^9$/L)	0.9	0.2	0.1	1.4	0.6	0.1	0	0	0	0.1	0.5

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

Figure 6-16 Anti-lipid S IgG titres, AOLC results and leucocyte counts for the duration of Hickman CVC placement in study patient 16



Day of blood collection	0	15	21
AOLC result	ND	+	ND
WBC ($x10^9$/L)	3.2	4	4.2
Lymphocyte count ($x10^9$/L)	1.4	1.6	3.5

ND = not done, + = positive AOLC result, - = negative AOLC result, WBC = white blood cell count
Leucocyte counts in blue represent those below 'normal' healthy adult ranges (Hoffbrand *et al.*, 2001).

6.3.3 Anti-lipid S IgG titres

Of the 12 patients presenting with CR-BSI due to staphylococci, 5 (42%) had elevated anti-lipid S IgG titres (figures 6-3, 6-4, 6-8, 6-13 and 6-16). All of these patients at the time of infection and lipid S IgG production had WBC and lymphocyte counts within the normal healthy adult range (Hoffbrand *et al.*, 2001). Conversely, 7 out of 12 (58%) patients with CR-BSI due to staphylococci did not produce anti-lipid S IgG (figures 6-1, 6-2, 6-6, 6-7, 6-12, 6-14 and 6-15). All of these patients at the time of infection had WBC and/or lymphocyte counts below the normal range stated for healthy adults. Positive lipid S titres were detected prior to clinical and routine microbiological diagnosis of CR-BSI in 4 out of 13 (31%) of infected patients (figures 6-3 to 6-4, 6-13 and 6-16).

Of the 3 patients presenting with CR-BSI due to Gram-negative microorganisms, none (0%) produced detectable levels of anti-lipid S IgG. One out of 3 (33.3%) of these patients had WBC and lymphocyte counts within the normal range (figure 6-12) whereas 2 (66.6%) had both reduced WBC and lymphocytes counts (figures 6-10 and 6-13).

6.3.4 Acridine orange leucocyte cytospin test results

Of the 13 patients presenting with CR-BSI, 12 (92%) had a positive AOLC result in 1 or more of the multiple blood samples during the placement of Hickman CVC (figures 6-1 to 6-4, 6-6 to 6-8, 6-10 to 6-12 and 6-14 to 6-16). Four out of 12 (33.3%) of these patients with CR-BSI and positive AOLC results also had negative AOLC results at some point during Hickman CVC placement (figures 6-2 to 6-3 and 6-10 to 6-12). Positive AOLC results were detected prior to clinical and routine microbiological diagnosis of CR-BSI in 8 out of 13 (62%) of infected patients (figures 6-1 to 6-2, 6-6 to 6-8, 6-10, 6-12 and 6-15). One patient who presented with CR-BSI did not yield any positive AOLC results during their time on the study (figure 6-13).

Of the 3 patients who presented with no clinical or routine microbiological evidence of CR-BSI, all (100%) demonstrated positive AOLC results in 1 or more of the multiple samples during Hickman CVC placement.

6.3.5 Comparison of lipid S ELISA and AOLC results

Of the 12 patients presenting with CR-BSI due to CoNS, 5 patients (42%) had both elevated lipid S IgG titres and positive AOLC results. One of these 12 patients (8%) had elevated lipid S IgG titres alone whereas 6 (50%) had positive AOLC results alone.

6.4 Discussion

The main objective of this study was to evaluate the use of the lipid S ELISA and the AOLC in the diagnosis of Hickman CR-BSI in haemato-oncology and stem cell transplant patients. These patients often require long-term CVC i.e. Hickman CVC as part of their clinical management. Since this group of patients are often immunocompromised, infection associated with CVC poses a serious problem. Indeed, intravascular catheter-associated infections are particularly prevalent in stem cell transplant patients within the first 60 days post transplantation (Hoffbrand *et al.*, 2001). Infection associated with long-term CVC also carries substantial financial costs. The average cost of infection associated with CVC designed for long-term use in haematology and oncology patients has been previously determined as £1781.81 (Moss *et al.*, 1997).

The results of this study suggest that the lipid S ELISA facilitates the diagnosis of CR-BSI in this patient group only during periods of immunocompetency. Indeed, less than half of the study patients with staphylococcal CR-BSI during their study period mounted an anti-lipid S IgG response. However, in 5 patients with CR-BSI, anti-lipid S IgG was detected prior to routine blood cultures becoming positive, thus demonstrating the potential of the lipid S ELISA in the rapid diagnosis of CR-BSI. All patients who produced anti-lipid S IgG did so during periods whereby their leucocyte counts were within the normal range. Those with non-detectable levels of anti-lipid S IgG, had leucocyte counts below this range, demonstrating the value of this serological test in immunocompetent patients. Indeed, the loss of humoral immunity to viral vaccination antigens in patients undergoing intensive chemotherapy or stem cell transplantation has been previously demonstrated (Avigan *et al.*, 2001; Nilsson *et al.*, 2002; Reinhardt *et al.*, 2003). The reduced sensitivity of the lipid S ELISA during periods of immunocompromisation is further illustrated in patients 4 and 8 (figures 6-4 and 6-8). Both patients mounted humoral responses to the lipid S antigen during CR-BSI caused by CoNS, however, anti-lipid S IgG titres declined during periods whereby leucocyte counts were markedly below normal ranges for healthy adults. The development of a direct ELISA, which detects the presence of specific antigen rather than antibodies may be more beneficial in immunocompromised patients. The sensitivity of the lipid S ELISA demonstrated in this patient group was lower than values achieved in a previous study which assessed the diagnostic potential of this test for CR-BSI (Elliott *et al.*, 2000). The assay sensitivity of 75% achieved during this study was obtained using haematology, oncology, general medical and general surgery patients, however,

sensitivity values for individual patient groups were not assessed and the immunological status of the subjects studied were not eluded to.

The excellent specificity of the lipid S ELISA for CR-BSI due to staphylococci was also demonstrated during this section of the study, concurring with results achieved in chapter 5. This is illustrated in patient 12 (figure 6-12) who failed to produce anti-lipid S IgG during a CR-BSI caused by *Aeromonas* sp. despite having normal leucocyte counts at the time of infection.

The AOLC assay is not dependant on the degree of immunocompetency of patients but relies on the microbiological detection of microorganisms in blood drawn through the CVC. Indeed, 92% of patients with CR-BSI had positive AOLC results at some point during Hickman CVC placement. The success of the AOLC in adult patients has also been reflected in previous studies (Kite *et al.*, 1999; Bong *et al.*, 2003). Furthermore, in 62% of infected patients AOLC results were positive prior to clinical and/or routine microbiological diagnosis of CR-BSI. This suggests that the AOLC test may detect catheter colonisation and may therefore have a clinical value as a predictive tool for CR-BSI. In a study by Von Baum and colleagues (1998), 20% of patients with colonised CVC but no signs of infection produced positive AOLC results. If catheter colonisation is the basis of the AOLC test, catheter brushing with an endoluminal brush may be required to yield sufficient microorganisms from the catheter lumen to be detected microscopically in the AOLC test (Tighe *et al.*, 1996). Indeed, 20 bacteria/ml of blood is required in order to achieve a positive AOLC result (Tighe *et al.*, 1996). However there are concerns in the clinical setting of the potential infectious hazards associated with catheter brushing, especially in immunocompromised patients (Blot *et al.*, 2000). The need for liberation of microorganisms from the internal CVC lumen surface may explain why infected patients showed intermittently negative AOLC results during this study. This observation demonstrates that in patients with CR-BSI, if a single sample is taken, the results may be negative, thus highlighting the need for multiple samples to be taken to support the diagnosis of CR-BSI by AOLC. Interestingly, 3 patients with no clinical or microbiological evidence of CR-BSI demonstrated positive AOLC results at some point during Hickman CVC placement again suggests that the test detects CVC colonisation and/or microbial contamination of hubs in addition to true bacteraemia. In a study by Tebbs and colleagues (1995) it was demonstrated that hub contamination rates can be as high as 22%. This highlights the need for adequate hub disinfection prior to collecting blood samples used to perform the AOLC test (Salzman *et al.*, 1993). From a clinical

perspective, positive AOLC results obtained from patients without CR-BSI may lead to the unnecessary prescription of antimicrobial therapy and/or removal of the CVC. This highlights the potential role of the AOLC as an adjunct to other conventional diagnostic methods rather than a method to be used in isolation.

Since the hub and subsequent intraluminal migration is the primary source of microorganisms responsible for CR-BSI in long-term CVC including Hickman devices (Linares *et al.*, 1985), the AOLC test may be most beneficial in patients with these devices *in situ*. The AOLC test has only been evaluated in patients with long-term intravascular catheters. Since the AOLC test detects catheter colonisation in addition to CR-BSI, it may only as valuable as routine catheter culture techniques. However, this method may reduce the costs associated with the unnecessary removal of CVC, since sampling is performed whilst the catheter is *in situ* (Bong *et al.*, 2003).

In summary, this study has demonstrated that the lipid S ELISA is a sensitive test for the diagnosis of staphylococcal CR-BSI in patients with leucocyte counts within the normal healthy adult range. However, the test is not of clinical value for immunocompromised patients. For patients with both normal and reduced leucocyte counts, the AOLC may provide a useful adjunct to other current diagnostic tools including the microbiological culture of blood. This test allows the *in situ* sampling of CVC in order to predict and diagnose CR-BSI, providing appropriate aseptic technique is employed to obtain blood samples. Both the lipid S ELISA and the AOLC test are simple, rapid and inexpensive to perform. These tests allow the diagnosis of CR-BSI whilst the catheter is *in situ,* avoiding the unnecessary removal of catheters. However, since the lipid S ELISA and the AOLC have only been fully evaluated by local research groups, further work is required to evaluate the assays in the hands of others.

7. Clinical evaluation of a novel computerised audit programme (Meditrend®, BD, UK) for central venous catheter-associated infection.

7.1 Introduction

Audit is an invaluable process in infection control. Indeed, surveillance activities are recognised as the first step in the prevention of nosocomial infection (Bouam *et al.*, 2003). Audit programmes allow the determination of infection rates, evaluation of current clinical practice and the review of infection control strategies. Clinical audit has been implemented to investigate infection in CVC, peripheral and CAPD catheters (Elliott *et al.*, 1995; Lawrance, 1994; Herbst, 1978; Gowardman *et al.*, 1998; Halstead, 2001; Webster *et al.*, 2003; Nelson *et al.*, 1996; Wilson *et al.*, 1994).

Clinical surveillance strategies may be either prospective or retrospective. Prospective clinical audit is generally favoured by most as it allows early identification of infection, allowing the rapid implementation of control measures, however this approach can be expensive and time consuming (Bouam *et al.*, 2003). Conversely, retrospective reviews of positive microbiological samples and medical records is less time-consuming but can be less accurate and may lead to a delay in the identification of problems (Bouam *et al.*, 2003).

Three infection surveillance methods were evaluated in high risk patients by Bouam and colleagues (2003). The techniques assessed were; laboratory-based ward surveillance (a retrospective review by a physician who evaluated positive bacteriology reports), a reference standard (assessment of clinical and microbiological data by infection control practitioners at the bedside) and automated surveillance (an intranet-based computer programme which translated test results from the microbiology laboratory into computerised medical records, amongst other things, allowing determination of whether an infection was hospital-acquired). This study demonstrated that the intranet automated system was more accurate and less time-consuming than the retrospective laboratory-based ward surveillance system.

This chapter evaluates a fully-integrated computer-based audit programme, (Meditrend® BD, UK) in the clinical setting. This tool aims to provide healthcare workers with a framework to monitor the clinical risk associated with CVC insertion and use. The

programme comprises a questionnaire containing 61 questions covering CVC; insertion, care and removal and CR-BSI; rates, profile and patient outcome (appendix 6). The audit programme allows data from all questionnaires to be consolidated and analysed as a whole in several ways. Analysis can take the form of standard reports which give an overview of best practice, localised and systemic infections, catheter blockages or performance against best practice. Alternatively, questionnaire responses can be presented graphically or analysed on a question by question basis depending on what information is required by the user. A correlation between 'cause and effect' can be measured by comparing clinical practice with localised and systemic infection rates. This, in turn allows the measurement of best practice.

The aims of this study were to;

a) Evaluate the Meditrend® audit tool in haemato-oncology and SCT patients.

b) Use the Meditrend® audit tool to determine CRI rates, causative microorganisms and patient outcome in haemato-oncology and SCT patients.

7.2 Materials and Methods

7.2.1 Patients

Patients from the haematology ward and BMT unit, University Hospital Birmingham NHS Trust who required a CVC as part of their normal clinical management were recruited both prospectively and retrospectively during a period of 2 years. Only patients with full, detailed and accurate medical records were entered into the audit.

7.2.2 Data input

For each CVC insertion, a Meditrend® questionnaire was completed by the study monitor and the responses entered into the Meditrend® computer database.

7.2.3 Data analysis

The 'analyse by question' option within the Meditrend® computer package was used to determine CVC infection rates, time of onset of infection, causative microorganisms, patient outcome and best practice for CVC insertion and management. This option allows a question to be analysed in addition to being able to select which respondents are included in the analysis. To determine overall CRI rates, only patients entered prospectively were analysed. Questions contained in the audit whereby all responses were identical were not analysed further.

7.2.4 Statistical analysis

Statistical analysis of data was performed using the Fishers Exact test.

7.3 Results

7.3.1 Patients recruited

Eighty-two patients from the haematology ward and BMT unit, University Hospital Birmingham NHS Trust were recruited into the audit programme. Patient demographics are given in table 7-1.

Table 7-1 Demographics of patients entered into the Meditrend® audit

Number of patients recruited	82
Male: female ratio	48:34
Mean age (range)	37 (18-69)

7.3.2 Meditrend® analysis

7.3.2.1 Questions not analysed

Many sections of the questionnaire were not analysed due to respondents all giving the same answer, for example, gloves were worn by all healthcare workers for CVC insertion.

7.3.2.2 Types of CVC inserted

The types of CVC inserted into audit patients both prospectively and retrospectively are summarised in table 7-2. The double-lumen Hickman CVC was the most frequently utilised intravascular device during the audit period.

7.3.2.3 Catheter-related infection profile

7.3.2.3.1 Overall CRI rates

Overall, 38 out of 52 (73%) prospectively audited CVC insertions resulted in an associated infection. Twenty out of the 52 (38%) CVC insertions resulted in both localised and systemic infection, 6 out of the 52 (12%) resulted in localised infection alone and 12 out of the 52 (23%) resulted in systemic infection alone. Fourteen out of the 52 (27%) CVC insertions were not associated with infection.

7.3.2.3.2 Clinical symptoms of systemic infection

Thirty-four out of 65 (52%) CVC insertions which resulted in systemic infection presented with low grade (<38.5°C) pyrexia. The remaining 31 (48%) presented with high grade (>38.5°C) pyrexia. Overall, 34 out of the 65 (52%) episodes of systemic infection were

characterised by pyrexia which was unresponsive to broad-spectrum antibiotics and 22 episodes (34%) where characterised by septic rigors on flushing of the catheter.

Table 7-2 Types of CVC inserted into study patients

CVC inserted	Total number of CVC inserted (%)	Number of CVC insertions prospectively audited (%)	Number of CVC insertions retrospectively audited (%)
Total	108	52	56
Single-lumen Hickman	8 (7)	6 (12)	2 (4)
Double-lumen Hickman	60 (56)	33 (63)	27 (48)
Triple-lumen short-term	16 (15)	5 (10)	11 (20)
Quad-lumen short-term	1 (1)	0 (0)	1 (2)
PICC	23 (21)	8 (15)	15 (27)

PICC = peripherally inserted central catheter

7.3.2.3.3 Time of onset of infection

The rates of times of onset post CVC insertion for localised and systemic infections are shown in tables 7-3 and 7-4. Localised infection most frequently developed >4 weeks post CVC insertion whereas systemic infection developed most frequently between 2 weeks and 6 months post CVC insertion.

Table 7-3 Times of onset of localised infection post CVC-insertion

Time of onset	Frequency (%)
0-3 days	0 (0)
4-7 days	9 (16)
1-2 weeks	14 (25)
2-4 weeks	13 (23)
> 4 weeks	20 (36)

Table 7-4 Times of onset of systemic infection post CVC-insertion

Time of onset	Frequency (%)
0-3 days	1 (2)
4-7 days	3 (7)
1-2 weeks	8 (19)
2-4 weeks	15 (35)
1-6 months	15 (35)
>6 months	1 (2)

7.3.2.3.4 Microorganisms associated with catheter-related infection

Coagulase-negative staphylococci were the most frequently isolated microorganisms from cultures of CVC tips, peripheral blood samples and blood samples drawn through the CVC in patients with systemic infection (Figures 7-1 to 7-3) and from exit site swabs in patients with localised infection (figure 7-4).

Figure 7-1 Pie chart demonstrating the microorganisms isolated from CVC distal tips in patients with systemic infection

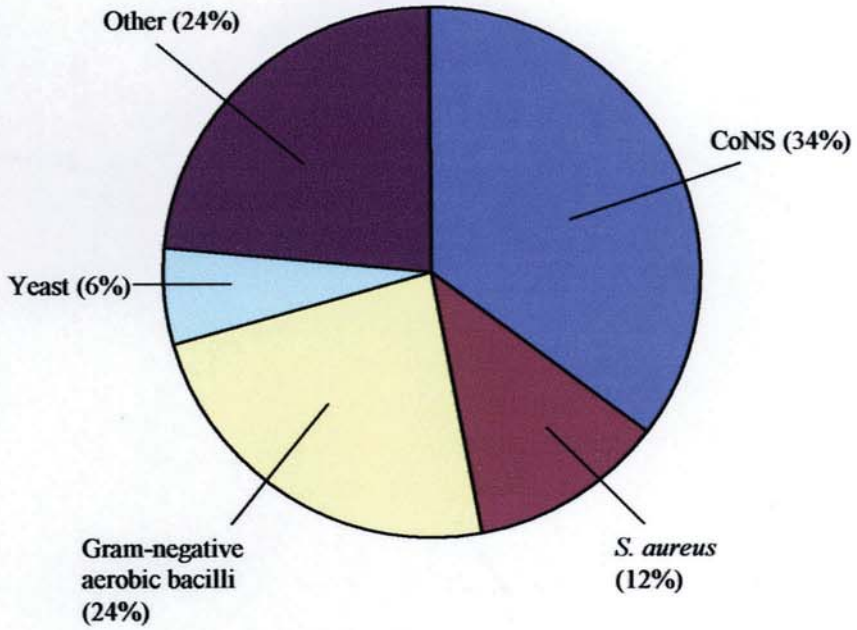


Figure 7-2 Pie chart demonstrating the microorganisms isolated from peripheral blood samples of patients with systemic infection

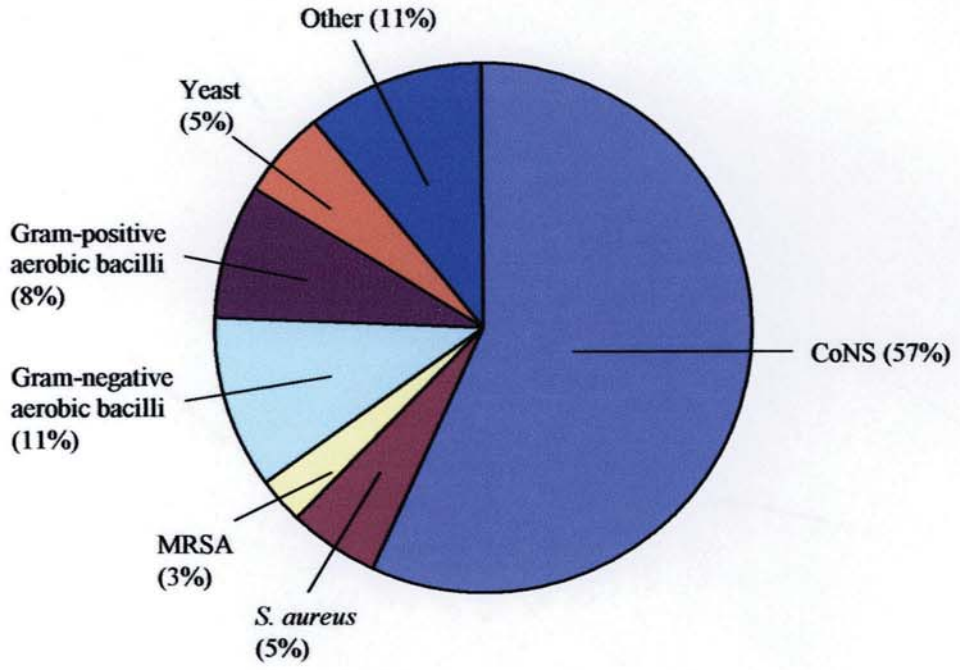


Figure 7-3 Pie chart demonstrating the microorganisms isolated from blood samples drawn through the CVC of patients with systemic infection

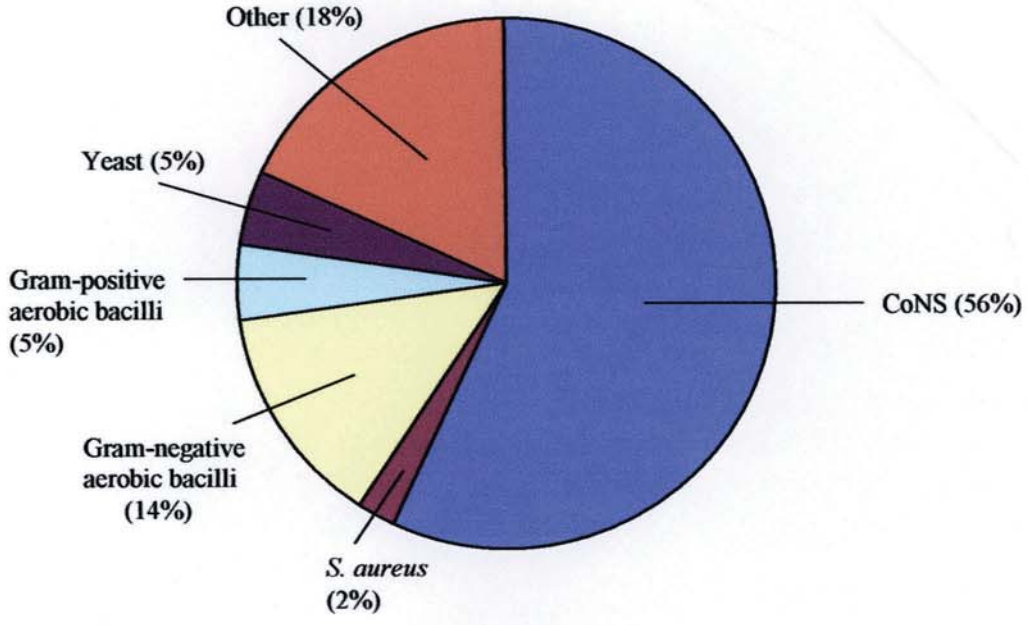
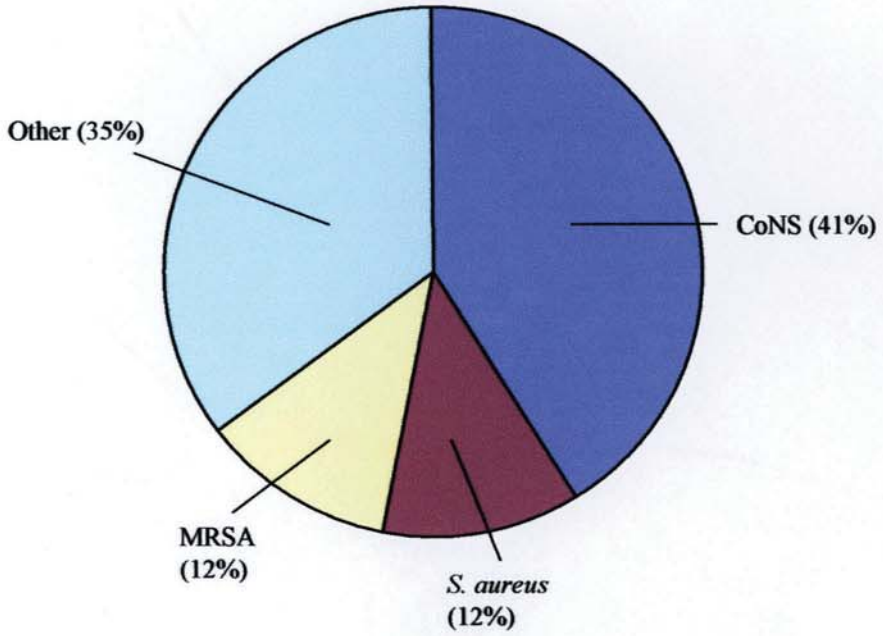


Figure 7-4 Pie chart demonstrating the microorganisms isolated from exit site swab cultures from patients with localised infection



7.3.2.4 CVC insertion

7.3.2.4.1 Healthcare workers inserting the CVC

As no CVC were inserted by intensivists during the audit period, this section of the questionnaire was used to identify when a CVC was inserted by a radiologist.

Table 7-5 outlines the number of localised and systemic infections which developed following insertion of CVC by 5 different types of healthcare workers. Insertion of CVC by registrars/house officers was associated with a significantly lower rate of localised and systemic infection than CVC inserted by surgeons ($p=0.001$ and $p=0.0004$, respectively) or anaesthetists ($p=0.02$ and $p=0.03$, respectively). A significantly lower rate of localised infections was also observed when the CVC was inserted by a registrar/house officer compared to insertion by a radiologists ($p=0.01$). Hickman CVC were inserted by surgeons, radiologists and anaesthetists only. Localised infection rates associated with Hickman insertion by surgeons, radiologists and anaesthetists were 66%, 71% and 59% respectively. Systemic infection associated with Hickman insertion by the above healthcare workers were 75%, 71% and 76% respectively.

Table 7-5 Localised and systemic infection rates associated with insertion of CVC by 5 groups of healthcare workers

Healthcare worker who inserted the CVC	Total number of CVC inserted by each type of healthcare worker	Number of CVC associated with localised infection (%)	Number of CVC associated with systemic infection (%)
Anaesthetist	9	7 (78)	7 (78)
Radiologist	18	10 (56)	13 (72)
Surgeon	44	29 (66)	33 (75)
Registrar/house officer	33	9 (27)	11 (33)
Nurse	4	1 (25)	1 (25)

7.3.2.4.2 Location of CVC insertion

As no CVC were inserted in an accident and emergency department during the audit period this 'tick box' on the questionnaire was used for CVC inserted in a radiology suite. Twelve out of 40 (30%) CVC insertions which took place on the ward resulted in localised infection compared to 34 out of 50 (68%) CVC insertions in an operating theatre and 10 out of 18 (56%) CVC insertions in a radiology suite. There was a significant difference in localised infection rates between those CVC inserted in the ward and operating theatre ($p=0.04$).

Fourteen out of 40 (35%) CVC insertions which took place on the ward resulted in systemic infection compared to 38 out of 50 (76%) CVC insertions in an operating theatre and 13 out of 18 (72%) CVC insertions in a radiology suite. Significant differences in systemic infection rates was found when comparing CVC inserted in the ward with the operating theatre ($p=0.0001$) and radiology suite ($p=0.01$).

7.3.2.4.3 CVC tunneling

Forty-four out of 68 (65%) patients with tunneled CVC developed localised infection compared to 12 out of 40 (30%) with non-tunneled CVC. Furthermore, 37 out of the 68 (54%) patients with tunneled lines developed a systemic infection compared to 6 out of 40 patients (15%) with non-tunneled CVC ($p<0.0001$).

7.3.2.4.4 CVC biomaterial

Of the 108 CVC studied, 82 were silicone and 26 were polyurethane. Silicone CVC were associated with a higher level of both localised infection (59% vs. 29%, p=0.02) and systemic infection (46% vs. 19%, p=0.02) compared with polyurethane CVC.

7.3.2.4.5 Infection rates associated with single and multi-lumen CVC and PICC

Table 7-6 summarises localised and systemic infection rates associated with single and multi-lumen CVC and PICC inserted during the audit period.

Table 7-6 Localised and systemic infection rates associated with single and multi-lumen CVC and PICC

CVC inserted	Total number inserted prospectively	Number associated with localised infection (%)	Number associated with systemic infection (%)
Single-lumen Hickman CVC	6	2 (33)	4 (67)
Double-lumen Hickman CVC	33	20 (61)	24 (73)
Triple-lumen short-term CVC	5	2 (40)	3 (60)
PICC	8	2 (25)	1 (13)

Double-lumen Hickman CVC were associated with the highest rate of localised and systemic infection and PICC with the lowest. However, significant differences between infection rates depending on CVC type were observed only when comparing systemic infection rates of single and double lumen Hickman CVC with PICC (p=0.01 and p=0.003, respectively).

7.3.2.4.6 CVC entry site

Table 7-7 summarises local and systemic infection rates associated with CVC insertion into 5 different entry sites. The external jugular was associated with the highest rates of localised and systemic infection.

Table 7-7 Localised and systemic infection rates associated with CVC insertion into 5 entry sites

CVC entry site	Number of CVC inserted into the entry site	Number of CVC associated with localised infection (%)	Number of CVC associated with systemic infection (%)
Subclavian vein	16	8 (50)	11 (69)
Internal jugular vein	27	15 (56)	19 (70)
External jugular vein	10	7 (70)	7 (70)
Peripherally inserted	47	24 (51)	25 (53)
Femoral vein	8	2 (25)	4 (50)

7.3.2.4.7 CVC insertion site enlargement

Twenty-four out of 44 (55%) patients for which a dilator was used to enlarge the CVC insertion site developed a localised infection compared to 40 out of 68 (59%) of patients for which a scalpel was used (p=0.7). Nineteen out of the 44 (43%) of patients for which a dilator was used to enlarge the CVC insertion site developed a systemic infection compared to 31 out of the 68 (46%) of patients for which a scalpel was used (p=0.8).

7.3.2.4.8 CVC insertion technique

Table 7-8 summarises the localised and systemic infection rates associated with CVC inserted by the Seldinger, cut-down and through introducer techniques.

Table 7-8 Localised and systemic infection associated with CVC inserted by the Seldinger, cut-down and through introducer techniques

CVC insertion technique	Number of CVC inserted	Number of insertions associated with localised infection (%)	Number of insertions associated with systemic infection (%)
Seldinger	44	24 (55)	19 (43)
Cut-down	41	25 (61)	20 (49)
Through introducer	23	6 (26)	3 (13)

Central venous catheters inserted by the cut-down technique were associated with the highest rate of both localised and systemic infection, however this did not result in significantly higher infection rates than CVC inserted via the Seldinger technique (p=0.7 for both localised and systemic infection). However, CVC inserted using the through introducer technique were associated with a significantly lower rate of localised infection than CVC inserted via the Seldinger and cut-down techniques (p=0.04 and p=0.01, respectively). Furthermore, CVC inserted using the through introducer technique were associated with a significantly lower rate of systemic infection than CVC inserted by the Seldinger or cut-down techniques (p=0.01, p=0.01).

7.3.2.4.9 Risk factors at the time of CVC insertion

Table 7-9 demonstrates localised and systemic infection rates associated with risk factors at the time of CVC insertion. Assisted ventilation, a concurrent CVC, colonisation by resistant microorganisms, neutropaenia and immunosuppression at the time of insertion were associated with the development of both localised and systemic infection. However, no risk factor was associated with a statistically significant higher rate of localised or systemic infection.

Table 7-9 Localised and systemic infection rates associated with risk factors at the time of CVC insertion

Risk factor	Total number of CVC insertions with risk factor present	Number of CVC insertions associated with localised infection (%)	Number of CVC insertions associated with systemic infection (%)
Assisted ventilation	33	20 (61)	17 (52)
Tracheostomy	1	0 (0)	0 (0)
Concurrent CVC	12	4 (33)	4 (33)
Anticoagulant	2	0 (0)	0 (0)
Colonised by resistant microorganisms	11	5 (45)	5 (45)
Neutropaenic	25	12 (48)	14 (56)
Immunocompromised	44	21 (48)	23 (52)

7.3.2.4.10 CVC insertion preparation

The use of a gown worn by the healthcare worker inserting the CVC was associated with significantly higher levels of localised and systemic infection than healthcare workers who did not utilise a surgical gown for CVC insertion (63% vs. 33%, p=0.003 and 73% vs. 38%, p=0.001, respectively).

During the audit period, either 10% (w/v) povidone-iodine or 0.5% (w/v) chlorhexidine gluconate in 70% (v/v) industrial methylated spirit was used for pre-CVC insertion skin preparation. A higher rate of both localised and systemic infection was associated with patients for whom chlorhexidine/alcohol was used compared to those who had their skin disinfected with iodine (p=0.01 and p=0.03, respectively). Thirty-four out of 52 (65%) patients for which chlorhexidine/alcohol was used developed a localised infection rate compared to 22 out of 56 (39%) for whom iodine was used. Furthermore, 37 out of 52 (71%) patients for which chlorhexidine/alcohol was used developed a localised infection rate compared to 28 out of 56 (50%) for whom iodine was used.

7.3.2.4.11 Dressing used following CVC insertion

Forty-five out of 90 (50%) patients for which a non-occlusive semi-permeable dressing was used immediately following insertion developed a localised infection compared to 11 out of 18 (61%) patients who received sterile gauze/dry dressings. Fifty-one out of 90 (56%) patients for which the non-occlusive semi-permeable dressings were used developed a systemic infection compared to 14 out of 18 (78%) patients who received sterile gauze/dry dressings. There was no significance in localised and systemic infection rates associated with the use of sterile gauze/dry or non-occlusive semi permeable dressings (p=0.4 and p=0.1, respectively).

7.3.2.5 CVC management/care

7.3.2.5.1 Blocked CVC lumens

Ten out of 108 (9%) of CVC became blocked *in situ*. Six out of the 10 (60%) blocked CVC were associated with systemic infection whereas 59 out of 98 (60%) CVC that did not block were associated with a systemic infection. There was no significant difference in systemic infection associated with blocked and patent CVC (p=1.0).

7.3.2.6 CVC removal

7.3.2.6.1 Indwell time of CVC

Table 7-10 summarises the indwell time of CVC associated with localised and systemic infection. Most CVC which were not associated with infection had an indwell time of >30 days whereas the most frequent indwell time for CVC associated with localised or systemic infection was 8-30 days.

Table 7-10 Indwell time of infected and non-infected CVC

CVC indwell time	Total number of CVC *in situ* (%)	Number of CVC *in situ* not associated with infection (%)	Number of CVC *in situ* associated with localised infection (%)	Number of CVC *in situ* associated with systemic infection (%)
<24 hr	0 (0)	0 (0)	0 (0)	0 (0)
24-72 hr	4 (4)	3 (10)	0 (0)	1 (2)
3-4 days	3 (3)	2 (7)	1 (2)	1 (2)
5-7 days	7 (6)	3 (10)	2 (4)	2 (3)
8-30 days	46 (43)	9 (31)	31 (56)	31 (48)
>30 days	48 (44)	12 (41)	22 (39)	30 (46)

7.3.2.6.2 Reason for CVC removal

The reasons for removal of CVC obtained during the audit period are summarised in table 7-11. Clinical suspicion of infection was the most frequently associated reason for CVC removal.

Table 7-11 Reasons for CVC removal and frequency

Reason for CVC removal	Number of CVC (%)
No longer required	25 (23)
Blocked	3 (3)
Suspected infection	69 (64)
Extended duration of catheterisation	4 (4)
Misplacement	3 (3)
Accidental removal	1 (1)
Other	3 (3)

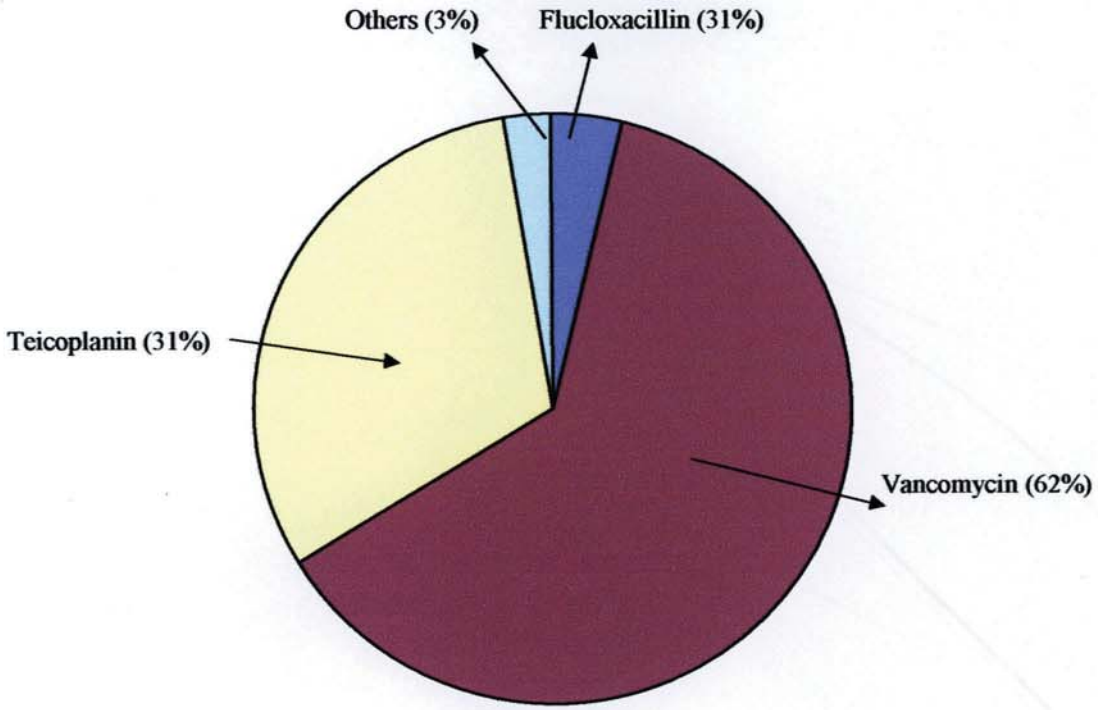
7.3.2.7 Microbiological investigation of specimens

Of the 65 systemic infections diagnosed, 9 (14%) CVC tips, 11 (17%) peripheral blood and 14 (22%) CVC blood specimens were not obtained for culture to confirm the diagnosis. Furthermore, of the 56 localised infections, 11(20%) exit site swabs were not obtained to confirm the diagnosis of localised infection at the CVC insertion site.

7.3.2.8 Treatment of catheter-related infection

The antibiotics used to treat CRI in patients during the audit period are presented in figure 7-5. Vancomycin was the most frequently administered antibiotic (62%).

Figure 7-5 Antibiotics administered to treat catheter-related infection



7.3.2.9 Patient outcome

7.3.2.9.1 Patient response to antibiotics

Fourteen out of 78 (18%) episodes of CRI were successfully treated with antibiotics compared with 64 out of 78 (82%) treatment failures ($p<0.0001$).

None of the 3 (0%) patients with CRI for which flucloxacillin was prescribed responded to treatment, compared to 11 out of 48 (23%) treated with vancomycin, 2 out of 24 (8%) with teicoplanin and 0 out of 2 (0%) with other antibiotics.

7.3.2.9.2 CVC removal

Sixty-six out of 78 (85%) CVC insertions associated with CRI were removed as a consequence of infection compared to 12 out of 78 (15%) which were not explanted ($p<0.0001$).

7.4 Discussion

The main objective of this study was to evaluate the Meditrend® CVC audit tool to determine rates and risk factors for CRI in haemato-oncology and SCT patients. Clinical audit has previously been used to investigate infection associated with CVC (Gowardman *et al.*, 1998; Herbst, 1978; Lawrance, 1994).

During the current audit period, the intravascular device most commonly inserted for long-term venous access in this patient group was the double-lumen Hickman CVC. Hickman CVC are frequently utilised to achieve long-term venous access in patients whose upper extremity veins are rapidly exhausted by venepuncture and administration of irritating antibiotics and anti-neoplastic drugs (Press *et al.*, 1984). Overall, CRI rates with all CVC types during this study was relatively high. Indeed, 50% of patients developed a localised infection and 62% a systemic infection. However, these figures are discordant with those achieved in a meta-analysis of Hickman CVC infections carried out by Press and colleagues (1984) which were significantly lower. This may be due to the fact that the majority of patients entered into the Meditrend® audit programme received a CVC for stem cell transplantation and underwent intensive myeloablative therapy such as total body irradiation. In a previous CVC audit carried out on a paediatric BMT unit, 8 out of 15 (53%) patients had at least 1 episode of CR-BSI (Lawrance, 1994). Many patients (52%) with systemic infection presented with low-grade pyrexia during this study. This is of little surprise since CoNS was found to be responsible for the highest proportion of systemic infection during this audit and infection with these microorganisms often exhibit delayed, discrete symptoms (Archer and Armstrong, 1983). The development of localised and systemic infection in the later stages of the CVC indwell period (predominantly >1 month post CVC insertion) was concordant with findings by Mourau and colleagues (2002) who demonstrated that CR-BSI was reported most frequently more than 30 days after insertion of PICC. This suggests that prevention strategies may need to focus primarily towards CVC care rather than the insertion process. Indeed, it is believed that hub contamination followed by subsequent intraluminal migration is responsible for the highest proportion of CR-BSI in patients with long-term CVC (Linares *et al.*, 1985). Prevention strategies should therefore be aimed at reducing IV connection contamination.

Analysis of data entered into the Meditrend® audit demonstrated that insertion of CVC by registrars/house officers was associated with lower infection rates than CVC inserted by surgeons, anaesthetists and radiologists. These findings are surprising since

registrars/house officers are likely to be less experienced in inserting CVC. Physician inexperience has been previously cited as an important cause of CVC complications (Herbst, 1978). Furthermore, insertion of the CVC by registrars/house officers took place in the busy ward situation whereby maximum barrier precautions and aseptic technique may not have been fully utilised. It was indeed, observed during this audit that the rate of localised infection was significantly lower when CVC were inserted on the ward compared to those inserted in an operating theatre. Also, no significant difference between the rate of infectious complications was found between CVC inserted in the operating theatre and radiology suite which concurs with findings from a study by Nouwen and colleagues (1999). Other imposing risk factors including the type of CVC inserted and the technique employed may have more of an influence on infection rates associated with CVC than the type of healthcare worker inserting the device or the type of clinical area in which the procedure was performed. For example, CVC insertion techniques associated with a higher risk of infection are not normally performed in ward areas by junior or inexperienced members of staff. Peripherally-inserted central catheters were found to be associated with a significantly lower systemic infection rate than Hickman CVC. For this reason, the speciality of healthcare worker inserting the CVC was compared with using only the Hickman CVC. However, there was no significant difference between infection rates when either a surgeon, radiologist or anaesthetist inserted the device. Nevertheless current literature advocates the insertion of Hickman CVC by radiologists and anaesthetists rather than surgeons based on cost and time savings (Robertson *et al.*, 1989; Muhm *et al.*, 1997). The speciality of healthcare worker inserting the CVC was directly related to what type of CVC and insertion technique was employed. Hickman CVC were inserted by surgeons via the cut-down technique and by radiologists and anaesthetists using the Seldinger percutaneous approach. Multi-lumen short-term CVC were inserted by anaesthetists and registrars/house officers also using the Seldinger percutaneous approach, and finally, PICC were inserted by nurses, radiologists and registrars/house officers by the through introducer percutaneous technique. Overall, the cut-down technique was associated with the highest number of infectious complications. Despite not achieving statistical significance during this audit, the cut-down technique has been previously reported to be associated with higher rates of exit site, tunnel and bloodstream infections than CVC inserted via a percutaneous route (Ahmed and Mohyuddin, 1998). This is probably due to the fact that during CVC insertion using the cut-down technique an incision is made to access and cannulate the vein directly. The significantly reduced rates of infection associated with the use of the through introducer technique observed during

this audit may be due to the fact that the CVC is advanced through an introducer, thus preventing microorganisms from being advanced into the exit site.

Tunneling of the CVC during the audit period was associated with a significantly higher rate of localised and systemic infections. This questions the benefit of tunneling as a mechanism of reducing CRI. Previous studies have demonstrated that there is no clinical benefit to tunneling in reducing rates of CRI (Ahmed and Mohyuddin, 1998; Andrivet *et al.*, 1994). Silicone CVC were also associated with a significantly higher rate of infectious complications than polyurethane CVC which has also been demonstrated in a previous investigation (Sherertz *et al.*, 1995). However, the flexible, low thrombogenic nature of this polymer makes it ideal for its purpose in this patient group.

Two surprising observations regarding CVC insertion preparation were made during the audit. Firstly, the use of a gown worn by the healthcare worker inserting the CVC was associated with an increased level of infectious complications despite it being well-established that maximum sterile barrier precautions during the insertion of CVC can reduce the risk of associated infection (Raad *et al.*, 1994). Also, the use of 0.5% (w/v) chlorhexidine gluconate in 70% (v/v) industrial methylated spirit for pre-CVC insertion skin preparation was associated with significantly higher infection rates than patients who received 10% (w/v) povidone-iodine. However, it is well documented that chlorhexidine is superior to iodine for CVC insertion and care (Chaiyakunapruk *et al.*, 2003). Both of these observations, which are discordant with the current literature, may be due to: gowns were not utilised when inserting CVC with low infection risk (PICC); chlorhexidine in alcohol was used mainly in high-risk patients for example, those having Hickman CVC surgically inserted or simply that the number of subjects entered into the database was not adequate.

None of the risk factors present during CVC insertion in this audit were significantly associated with the development of infectious complications despite the well-documented impact of factors such as assisted ventilation/endotrachael intubation (Fridkin *et al.*, 1996; Polderman and Girkes, 2002) and neutropaenia/immunosuppression (Polderman and Girkes, 2002; Zitella, 2003; Nouwen *et al.*, 1999). This may be due to the number of respondents being too small to achieve statistical significance.

The lack of a statistically significant difference between infection rates associated with the use of sterile gauze/dry dressings and semi-permeable non-occlusive transparent dressings immediately following CVC insertion may again, indicate that the development of

infection is more dependant on the standard of CVC care rather than insertion. This audit also demonstrated no correlation between thrombus formation within the CVC and systemic infection, despite it being documented as a significant risk factor (Raad *et al.*, 1994; Press *et al.*, 1984). This may be due to the small number (10 out of 108) of blocked CVC observed during the audit.

The most frequent length of CVC indwell time was lower in patients presenting with localised and/or systemic infection than for non-infected patients. This is of little surprise since clinical suspicion of infection was the major reason for CVC removal (64%). Furthermore, 78% of infected patients did not respond to antibiotic therapy and 85% of CVC were removed as a result of infection. The audit also demonstrated that appropriate microbiological specimens were not always obtained to confirm the diagnosis of CRI. This in turn may have led to the use of inappropriate antimicrobial therapy.

The Meditrend® audit demonstrated variations in the CVC insertion process. Different types of CVC were inserted by different techniques, in different locations by different specialities of healthcare workers. The type of CVC inserted dictated the insertion technique, location, healthcare worker inserting the device and the preparation process. For example, PICC were primarily inserted using the through introducer technique, on the ward, by nurses or registrars/house officers, using iodine for pre-PICC insertion skin preparation. Therefore, it is difficult to determine which of these factors is responsible for associated infection rates. For this reason, the factor under investigation should be analysed in a select group of patients whereby all other variations in CVC insertion and care are absent. This would require large quantities of patients to be entered into the database in an environment whereby variations in clinical practice exist. Many of the results obtained during the current audit did not reach statistical significance or were discordant with trends in the current literature which is probably due to the number of respondents recruited being too small.

The value of audit tools such as Meditrend® depends on the ability to input accurate and reliable data. In order to achieve this, patients should be entered prospectively by personnel who play an integral part in patient care. All healthcare workers within the audit area could be trained so that patient capture is maximal and information is complete and accurate. To aid this process, the database could be available to all staff on a well-secured intranet web-site (Bouam *et al.*, 2003). The 'named nurse' approach (Turner, 1997) to

patient care adopted in many healthcare institutions would also aid the capture of accurate data.

The Meditrend® audit programme was simple to complete and contained full, comprehensive data regarding the insertion, care and removal of CVC. All of this information was self-contained within the computer database allowing rapid retrieval of specific analytical data as required by the user. However, the Meditrend® audit programme had several limitations. For example, analysis did not provide any statistical information on the raw data achieved in order to determine the significance of such results, for this, the user needed to analyse the raw data further by additional methods for example, statistical computer packages. It has also been demonstrated during this audit that it is necessary to modify specific questions in order to reflect local policies and practice. Also, flexibility within the audit tool would be beneficial for patients whereby multiple episodes of CRI occur without the removal of the CVC.

In conclusion, the results of this current audit demonstrate that the Meditrend® audit tool may be used to determine CRI rates, causative microorganisms and patient outcome. Furthermore, the results demonstrated that in order to reduce rates of CRI, a focus needs to be placed on the development of preventative strategies targeted at the care of CVC. In addition, in patients whereby Hickman CVC are not required, the use of non-tunneled CVC such as PICC inserted via the through introducer technique may provide long-term central venous access with a lower associated infection risk. Further audit of this patient group using the Meditrend® audit tool could be implemented to evaluate strategies for the prevention of CRI in structured trials.

8. Assessment of the potential infection risk associated with needleless connectors in the clinical setting

8.1 Introduction

Microorganisms, in particular CoNS, originating from the skin of healthcare workers or patients may gain access to intravascular catheters via contamination of hubs. Microorganisms migrate down the intraluminal surface to the catheter distal tip resulting in colonisation and potential subsequent infection. Several methods of preventing infection acquired via this route have been evaluated including the use of various disinfectants for decontamination of hubs (Salzman *et al.*, 1993) and attachment of needleless connectors (NC) as a protective barrier (Seymour *et al.*, 2000). In addition to preventing needlestick injuries, NC may facilitate aseptic technique, reduce the time spent manipulating IV connections and avoid ports being left open. However the infection risk associated with these devices remains unclear.

The PosiFlow® (BD, UK) and Clave® (ICU Medical Inc, USA) NC are one-piece bi-directional devices which uses standard luer-lock connections. The devices incorporate a silicone compression seal that opens the fluid path when a male luer is introduced and automatically seals on withdrawal. This closed system eliminates the need to remove and replace luer caps, which may leave the fluid path open and the entry port vulnerable to microbial contamination. Furthermore the PosiFlow® NC has the added feature of constant positive pressure, preventing the backflow of blood up the catheter during disconnection of the male luer and thereby maintaining catheter patency.

The aims of this study were to:

a) Compare the microbial contamination associated with the Clave® and the novel PosiFlow® NC in the clinical setting (figure 8-1).

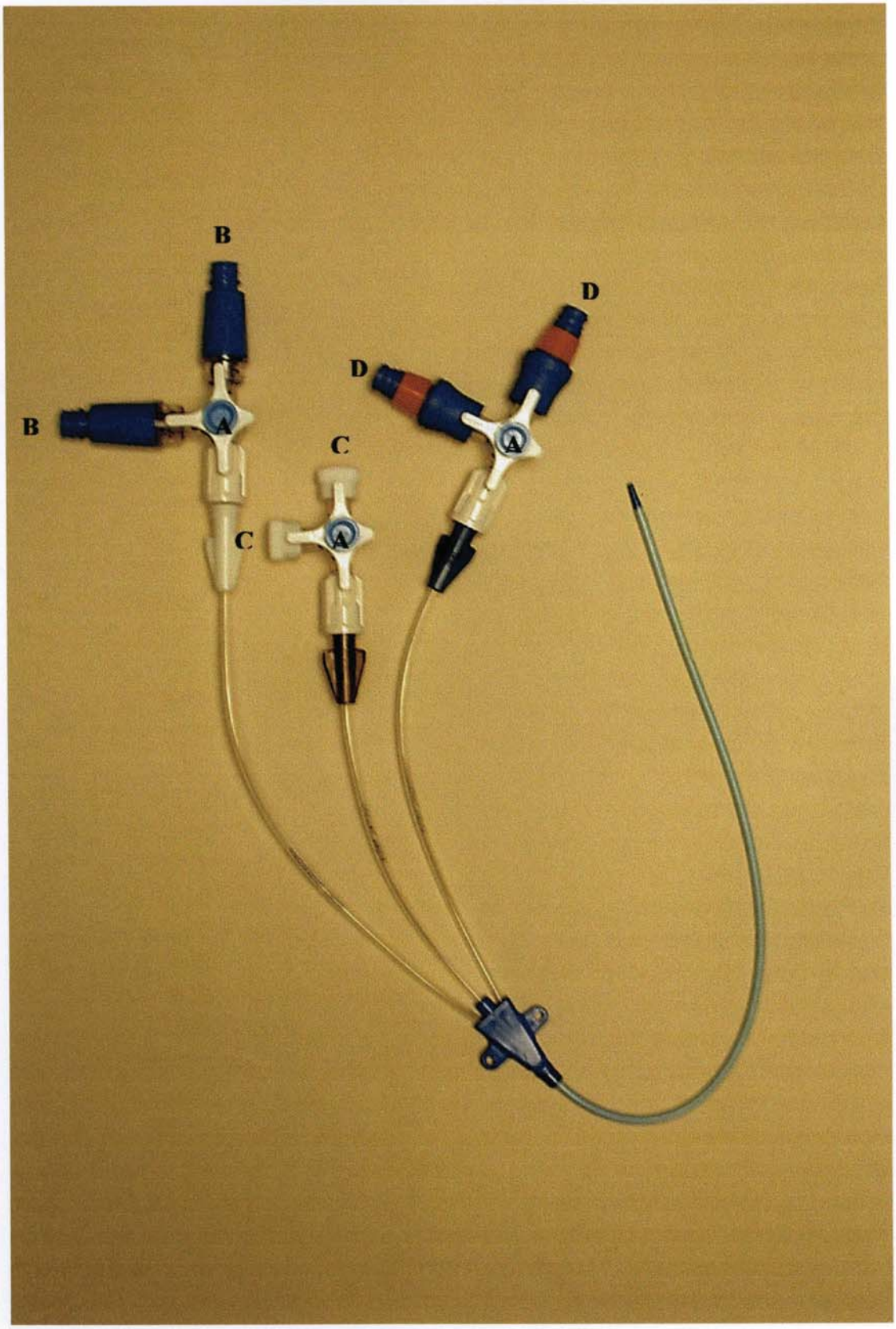
b) Compare stopcock entry port microbial contamination rates when either standard luer caps or PosiFlow® are attached.

c) Evaluate the efficacy of 70% (v/v) industrial methylated spirit (IMS) BP, 0.5% (w/v) chlorhexidine gluconate in 70% (v/v) IMS and 10% (w/v) aqueous povidone-iodine for decontamination of stopcock entry ports.

d) Determine if a correlation exists between the number of IV manipulations and the microbial contamination rate of NC/stopcock entry port contamination.

e) Establish if there is a correlation between the types of drugs administered through the NC/stopcock entry ports and microbial contamination.

Figure 8-1 Triple lumen central venous catheter complete with stopcocks and needleless connectors attached



A=Stopcock, B=PosiFlow®, C=standard luer caps, D=Clave®

8.2 Materials and Methods

8.2.1 Patient Selection

1) Local research ethical committee approval was sought for patients requiring a CVC as part of their clinical management to be recruited into the study (appendix 7). Informed consent was obtained from all patients recruited into the study (appendices 8 and 9).

Inclusion criteria:

2) Patients greater than 18 years of age.

3) Patients undergoing planned cardiac surgery with post operative admission to the cardiac intensive care unit, University Hospital Trust, Birmingham, U.K.

Exclusion criteria:

1) Patients with a psychiatric history or who are mentally handicapped.

2) Breast feeding or pregnant women.

3) Emergency admissions.

8.2.2 Phase 1 - Comparison of the microbial contamination rates of the PosiFlow® and Clave® following disinfection with 0.5% (w/v) chlorhexidine in 70% (v/v) IMS

Patients admitted onto the cardiac surgery ward who required a CVC as part of their clinical management were recruited into this part of the study.

Prior to CVC insertion the patient's skin was disinfected with chlorhexidine (0.5% (w/v) chlorhexidine gluconate in 70% (v/v) IMS BP spray, Hydrex® derma spray, Adams Healthcare, Leeds, U.K) and left to dry for 30 sec prior to cannulation.

A quad-lumen CVC was inserted into the internal jugular vein of each patient by the standard Seldinger technique. Stopcocks were then aseptically attached to each hub of the CVC. Each patient was randomly allocated to receive either the Clave® or PosiFlow® device and the designated NC was attached to each stopcock entry port. Each NC was numbered 1-8.

All NC were used as recommended by the manufacturer. Prior to manipulation, the silicone compression seals were disinfected with 0.5% (w/v) chlorhexidine gluconate in

70% (v/v) IMS and allowed to dry for 30 sec. Following all manipulations during the study period, the NC were disinfected again by the same method. The total number of activations and the types of administrations made through each device was recorded for the duration of the investigation (appendix 10). After 72 hr *in situ* all NC were aseptically removed aseptically from the stopcocks, placed into sterile specimen bags and sent to the laboratory for microbiological investigation. The entire CVC was removed from any patient exhibiting clinical signs of CRI and sent for routine microbiological examination. In patients with no signs of sepsis, the NC were replaced with standard luer caps. Patient demographics were recorded on a standard case report form (appendix 11).

8.2.3 Phase 2 - Comparison of stopcock entry port microbial contamination rates with either standard luer caps or PosiFlow® attached, following disinfection with 0.5% (w/v) chlorhexidine, 70% (v/v) IMS or 10% (w/v) povidone-iodine

Patients from the cardiac surgery ward who required a CVC as part of their clinical management were recruited onto the study. The cannulation site of each patient was swabbed prior to disinfection with a transport swab (Charcoal amies, Bibby Sterilin, Stone, U.K.) to determine the microbiological flora. Each patient was randomly assigned 70% IMS (Spiriclens®, Adams Healthcare, Leeds, U.K), chlorhexidine (0.5% w/v chlorhexidine gluconate in 70% v/v industrial methylated spirit BP spray, Hydrex® derma spray, Adams Healthcare, Leeds, U.K) or 10% aqueous povidone-iodine (Betadine®, Purdue Frederick, Norwalk, Connecticut, USA) for skin disinfection prior to CVC insertion. The disinfectant was allowed to dry for 30 sec, after which a quad-lumen CVC was inserted into an internal jugular vein by the standard Seldinger technique and stopcocks attached to each hub. The assigned disinfectant was subsequently used to clean NC and stopcock entry ports prior to and following all manipulations during the study. Each patient was randomly allocated either the PosiFlow® NC or standard luer caps, which were then attached to all ports of the stopcocks. Each port/PosiFlow® was numbered 1-8 and all administrations and/or aspirations made through the ports were recorded for the duration of the study (Appendices 10 and 12). Following 72 hr *in situ* the stopcocks complete with PosiFlow® or standard luer caps were aseptically removed, placed in sterile specimen bags and sent for microbiological analysis. Upon catheter removal, the distal tip was also sent for microbiological examination. The entire CVC was removed from any patient exhibiting signs of CRI and sent for routine microbiological culture. All information regarding patient demographics, NC and disinfectant allocation was recorded on a standard case report form (appendix 11).

8.2.4 Laboratory Methods

8.2.4.1 Culture media and incubation conditions

Needleless connectors, stopcocks, skin swabs and CVC tips received in the laboratory were cultured as described in chapter 2, section 2.2.2.1.

8.2.4.2 Sampling the stopcock entry ports for microbial contamination

The entry ports of each stopcock to which PosiFlow® or standard luer caps were attached to were sampled and cultured by the techniques outlined in chapter 2, section 2.2.2.2.

8.2.4.3 Sampling the external surface of the Clave® and PosiFlow® device

The silicone compression seal of each Clave® and PosiFlow® was sampled and cultured as described in chapter 2, section 2.2.2.3.

8.2.4.4 Sampling the internal surface of the Clave® and PosiFlow® device

The internal surface of the NC were sampled and cultured as described in chapter 2, section 2.2.2.4.

8.2.4.5 Sampling the external surface of the CVC distal tip (phase 2 only)

The external surface of the CVC distal tip was sampled and cultured as described in chapter 2. section 2.2.2.5.

8.2.4.6 Sampling the internal lumen of the CVC distal tip (phase 2 only)

The external surface of the CVC distal tip was sampled and cultured as described in chapter 2. section 2.2.2.6.

8.2.4.7 Skin swab cultures

Charcoal amies swabs from patients' skin were cultured as described in chapter 2, section 2.2.2.7.

8.2.4.8 Identification of microorganisms

Microorganisms were identified by standard microbiological tests including Gram-stain, catalase, oxidase and coagulase tests (chapter 2, section 2.2.2.8).

8.2.5 Statistical analysis

Statistical analysis of the data was undertaken using the Fishers Exact test, Spearman Rank test and Mann Whitney-U test.

8.3 Results

8.3.1 Phase 1 - Comparison of the microbial contamination rates of PosiFlow® and Clave® needleless connectors following disinfection with 0.5% (w/v) chlorhexidine in 70% (v/v) IMS

8.3.1.1 Patient demographics

Thirty-eight patients requiring cardiac surgery were recruited into the study - 13 female and 25 male with a mean age of 58 years (range = 20-85). The surgical procedures performed on study patients are given in table 8-1.

Table 8-1 Surgical procedures performed on study patients

Type of surgical procedure	Number of patients
Coronary artery bypass grafts (CABG)	22
Aortic valve replacement (AVR)	9
Mitral valve replacement (MVR)	3
CABG and AVR	3
Aortic root replacement and CABG	1

8.3.1.2 Incidence of CVC-related infections

None of the 38 patients who took part in the trial developed symptoms of a CVC-related infection during the study period.

8.3.1.3 The external and internal microbial contamination rates of the PosiFlow® and Clave® needleless connectors

The internal and external microbial contamination rates and numbers of microorganisms recovered from the PosiFlow® and Clave® NC are given in tables 8-2 and 8-3.

8.3.1.3.1 External microbial contamination rate of the PosiFlow® and Clave® needleless connectors

There was no significant difference between the rates of external microbial contamination of the silicone compression seal of the PosiFlow® and the Clave® (p=0.09, Fishers Exact Test). There was no significant difference between the number of patients with externally

contaminated PosiFlow® or Clave® NC (p=0.16, Fishers Exact Test). There was no significant difference between the numbers of cfu recovered from the external compression seal of the devices (p=0.8, Mann-Whitney Test). Colonies of CoNS recovered from the external compression seal of an NC following culture are shown in figure 8-2.

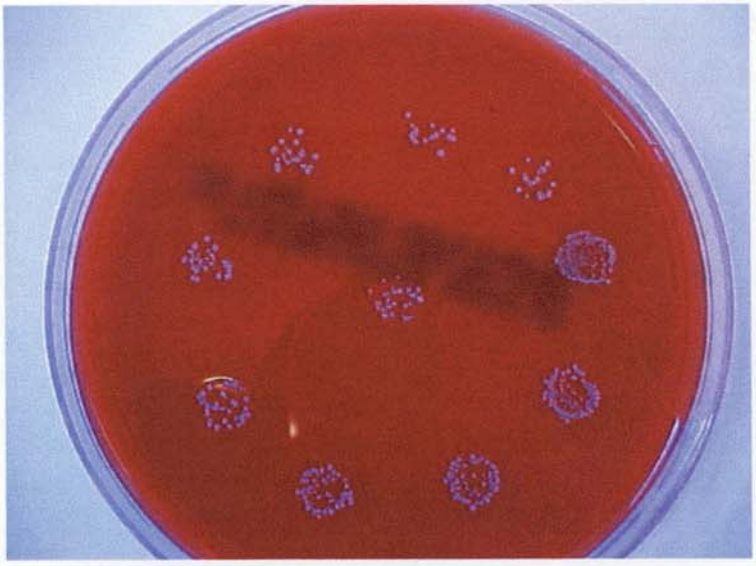
8.3.1.3.2 Internal microbial contamination rate

There was no significant difference between the internal contamination rates of the PosiFlow® and the Clave® (p=0.6, Fishers Exact Test). There was no significant difference between the number of patients with internally contaminated PosiFlow® or Clave® (p=1.0, Fishers Exact Test).

All Clave® NC with internal microbial contamination were also externally contaminated. Fifty-seven percent of PosiFlow® devices with internal microbial contamination were also externally contaminated. Microorganisms recovered from the internal and external surfaces of the devices were not identical.

There was no significant difference between the extent of internal microbial contamination between the devices; both internally contaminated Clave® and PosiFlow® NC yielded an average of 2 cfu per device (p=0.4, Mann-Whitney Test).

Figure 8-2. CoNS recovered from the external compression seal of a needleless connector



8.3.1.3.3 Microorganisms isolated

Skin and environmental microorganisms including CoNS, *S. aureus*, Gram-positive and negative aerobic bacilli and *Candida* spp were recovered from both types of NC.

Table 8-2. Internal and external microbial contamination rates of the PosiFlow® and Clave® NC

	PosiFlow®	Clave®
Number of patients investigated	20	18
Number of connectors investigated	127	128
% connectors externally contaminated (n)	28.3 (36)	39 (50)
% patients with externally contaminated connectors (n)	60 (12)	83 (15)
% connectors internally contaminated (n)	5.5 (7)	4 (5)
% patients with internally contaminated connectors (n)	20 (4)	22 (4)
% internally contaminated connectors which were also externally contaminated (n)	57.1 (4)	100 (5)

Table 8-3. Number of cfu recovered from the internal and external surfaces of the Clave® and PosiFlow® NC

	PosiFlow®	Clave®
External surface		
Mean	9	10
Range	1-66	1-78
Internal surface		
Mean	2	2
Range	1-9	1-4

8.3.1.4 Correlation between frequency of needleless connector manipulation and external and internal microbial contamination

The frequency of NC manipulation and associated external and internal microbial contamination rates are shown in tables 8-4 and 8-5. Each type of NC was manipulated an average of 1.6 times (ranges: PosiFlow®, 0-14 and Clave® 0-17). There was no correlation between the number of manipulations and the presence of internal microbial contamination in either the PosiFlow® or the Clave® ($p=0.2$ and $p=0.24$, respectively, Spearman Rank Test). The same observation was made when assessing external microbial contamination: Clave® ($p=0.3$) and PosiFlow® ($p=0.3$, Spearman Rank Test).

8.3.1.5. Relationship between frequency of manipulation and the extent of external and internal needleless connector contamination

There was no significant correlation between the number of manipulations and the extent of internal microbial contamination; for PosiFlow® or Clave® ($p=0.5$ and $p=0.8$ respectively, Spearman Rank Test). There was also no correlation between the number of manipulations and the number of cfu recovered from the external surface of each device (Clave® $p=0.5$ and PosiFlow® $p=0.2$, Spearman Rank Test). Table 8-6 contains the mean numbers of cfu recovered from both surfaces of both devices when manipulated on varying numbers of occasions.

Table 8-4. Frequency of Clave® manipulation and associated external and internal microbial contamination rates

Number of activations	Total number of Clave® investigated	Number of Clave® externally contaminated (%)	Number of Clave® internally contaminated (%)
0	20	8 (40)	1 (5)
1	73	23 (31.5)	4 (5.5)
2	19	11 (57.9)	0 (0)
3	6	1 (16.7)	0 (0)
4	4	2 (50)	0 (0)
5	1	1 (100)	0 (0)
6	3	1 (33.3)	0 (0)
7	-	-	-
8	-	-	-
9	-	-	-
10	1	1 (100)	0 (0)
11	-	-	-
12	1	0 (0)	0 (0)
13	-	-	-
14	-	-	-
15	-	-	-
16	-	-	-
17	1	1 (100)	0 (0)

Table 8-5. Frequency of PosiFlow® manipulation and associated external and internal microbial contamination rates

Number of activations	Total number of PosiFlow® investigated	Number of PosiFlow® externally contaminated (%)	Number of PosiFlow® internally contaminated (%)
0	30	10 (33.3)	1 (3.3)
1	59	15 (25.4)	4 (6.8)
2	16	5 (31.3)	1 (6.3)
3	8	1 (12.5)	0 (0)
4	6	2 (33.3)	1 (16.7)
5	2	0 (0)	0 (0)
6	-	-	-
7	2	1 (50)	0 (0)
8	-	-	-
9	-	-	-
10	1	1 (100)	0 (0)
11	-	-	-
12	-	-	-
13	-	-	-
14	1	0 (0)	0 (0)

Table 8-6. Cfu recovered from the external and internal surfaces of needleless connectors following manipulation

Number of manipulations	Clave®		PosiFlow®	
	Mean number of cfu from external surface (range)	Mean number of cfu from internal surface (range)	Mean number of cfu from external surface (range)	Mean number of cfu from internal surface (range)
0	5 (0-52)	0.2 (0-3)	4 (0-29)	0.03 (0-1)
1	5 (0-78)	0.1 (0-4)	2 (0-21)	0.1 (0-2)
2	4 (0-30)	0 (0)	7 (0-66)	1 (0-9)
3	0.2 (0-1)	0 (0)	1 (0-4)	0 (0)
4	1 (0-2)	0 (0)	2 (0-8)	0.2 (0-1)
5	3 (3)	0 (0)	0 (0)	0 (0)
6	4 (0-12)	0 (0)	-	-
7	-	-	1 (0-1)	0 (0)
8	-	-	-	-
9	-	-	-	-
10	1 (1)	0 (0)	2 (0-2)	0 (0)
11	-	-	-	-
12	0 (0)	0 (0)	-	-
13	-	-	-	-
14	-	-	0 (0)	0 (0)
15	-	-	-	-
16	-	-	-	-
17	1 (1)	0 (0)	-	-

8.3.1.6. Association between IV infusions and microbial contamination rates of needleless connectors

All administrations made through the NC were recorded during the study period. The frequency of contaminated devices following IV infusions is given in figure 8-3.

Each drug was administered through the same number of each device. The highest rate of external NC contamination was associated with; metoclopramide (100%), pethidine (100%) and sodium nitroprusside (100%). Each infusion was administered through one device only. Of the infusions administered through ten or more devices, the highest rate of external compression seal microbial contamination was associated with glycerol trinitrate (48.6%), 5% (w/v) dextrose (47.1%) and 0.9% (w/v) saline (40%).

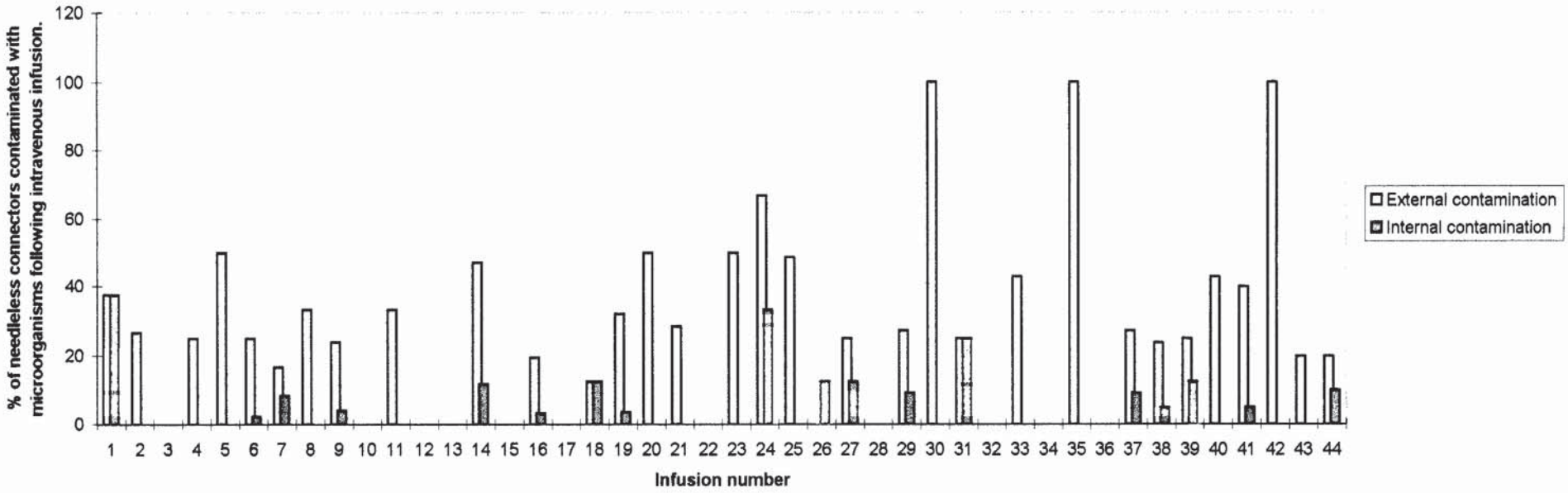
The infusions associated with the highest rate of internal microbial contamination were; adrenaline (37.5%), gelatin solution (33.3%) and morphine (25%). These infusions were administered through a total of eight devices each. Of the infusions administered through ten or more devices, the highest rate of internal needleless connector microbial contamination was associated with fentanyl (12.5%), heparin (12.5%) and 0.5% (w/v) dextrose (11.8%).

Needleless connectors through which several antibiotics were administered including; ceftazidime, cefuroxime, clarithromycin, flucloxacillin and vancomycin were also associated with microbial contamination both internally and externally.

8.3.1.7 Type of manipulations associated with contaminated needleless connectors

The type of manipulation most frequently associated with contamination of the external compression seal of the PosiFlow© was aspiration or administration of blood/blood products, however, for the same device, bolus injections were most frequently associated with internal contamination. For the Clave® bolus injections were most frequently associated with external microbial contamination whilst attachment of a central venous pressure (CVP) line accounted for the highest rate of internal microbial contamination (table 8-7).

Figure 8-3. Intravenous infusions associated with contaminated needleless connectors



Infusion Key and number of needleless connectors through which each infusion was administered (n).				
1) Adrenaline (8)	11) Clarithromycin (3)	21) Insulin (7)	31) Morphine (4)	41) 0.9% saline (40)
2) Alfentanil (15)	12) Cyclizine (1)	22) Isoprenaline (1)	32) Noradrenaline (5)	42) Sodium nitroprusside (1)
3) Amiodarone (1)	13) Cytarabine (1)	23) Ketamine (2)	33) Ondansetron (7)	43) Tramadol (5)
4) Aprotinin (4)	14) 5% Dextrose (17)	24) Gelofusine (3)	34) Pancuronium (1)	44) Vancomycin (10)
5) Atracurium (2)	15) Dobutamine (1)	25) Glyceryl trinitrate (35)	35) Pethidine (1)	
6) Blood/blood products (44)	16) Dopamine (31)	26) Glycopyrrolate (8)	36) Phenylephrine (3)	
7) Calcium chloride (12)	17) Ephedrine (1)	27) Heparin (16)	37) Potassium chloride (11)	
8) Ceftazidime (3)	18) Fentanyl (16)	28) Hydrocortisone (1)	38) Propofol (21)	
9) Cefuroxime (25)	19) Flucloxacillin (28)	29) Metaraminol (22)	39) Protamine (8)	
10) Ciprofloxacin (1)	20) Frusemide (6)	30) Metoclopramide (1)	40) Ranitidine (7)	

Table 8-7. Types of manipulation associated with external and internal microbial contamination of PosiFlow® and Clave® needleless connectors

Manipulation type	PosiFlow®			Clave®		
	Number of manipulations	% each manipulation type was associated with external microbial contamination	% each manipulation type was associated with internal microbial contamination	Number of manipulations	% each manipulation type was associated with external microbial contamination	% each manipulation type was associated with internal microbial contamination
Bolus injection	23	26.1 (n=6)	8.7 (n=2)	28	57.1 (n=16)	0 (n=0)
Continuous infusion	69	27.5 (n=19)	7.2 (n=5)	78	38.5 (n=30)	2.6 (n=2)
CVP line	13	7.7 (n=1)	0 (n=0)	18	22.2 (n=4)	11.1 (n=2)
Blood aspiration/ administration	24	33.3 (n=8)	4.2 (n=1)	14	21.4 (n=3)	0 (n=0)

CVP = Central venous pressure

8.3.2 Phase 2 - Comparison of stopcock entry port microbial contamination rates with luer caps or PosiFlow® attached following disinfection with either 0.5% (w/v) chlorhexidine, 70% (v/v) IMS or 10% (w/v) aqueous povidone-iodine

8.3.2.1. Patient demographics

Seventy-seven patients requiring were recruited into the study - 26 female and 49 male with a mean age of 63 years (range – 21-84). The surgical procedures performed on recruited patients are shown in table 8-8.

Table 8-8 Surgical procedures performed on study patients

Surgical procedure	Number of patients
Coronary artery bypass graft (CABG)	31
Aortic valve replacement (AVR)	13
Mitral valve replacement (MVR)	4
CABG + AVR	11
CABG + MVR	3
CABG + AVR + MVR	1
AVR + MVR	4
Aortic root replacement (ARR)	1
ARR + AVR	2
Pulmonary valve replacement (PVR)	2
Ventricular septal defect repair/closure (VSD)	2
Atrial septal defect repair/closure (ASD)	2
Excision of atrial myxoma	1

8.3.2.2 Incidence of CVC-related infections

None of the seventy-seven patients who took part in the trial exhibited any clinical signs of a CVC-related infection during the study period.

8.3.2.3 Comparison of total stopcock entry port microbial contamination rates with either luer caps or PosiFlow® attached

Two hundred and seventy-four stopcock entry ports to which PosiFlow® were attached and 306 stopcock entry ports to which standard luer caps were attached were assessed for

microbial contamination. The results irrespective of disinfection procedure are shown in table 8-9.

The stopcock entry ports to which the PosiFlow® were attached had a significantly lower microbial contamination rate compared to those with standard luer caps attached ($p=0.0002$, Fishers Exact Test).

Also, significantly more patients had CVC with contaminated entry ports when luer caps were attached when compared to those with the PosiFlow® ($p<0.0001$).

The level of entry port microbial contamination was higher when standard luer caps were attached compared to PosiFlow®, however, the difference was found to be statistically insignificant ($p=0.4$, Mann-Whitney Test).

Table 8-9 – Contamination rates and cfu recovered from stopcock entry ports with either standard luer caps or PosiFlow® attached

	Device attached to the stopcock entry ports	
	Luer caps	PosiFlow®
Number of patients investigated	39	38
Number of entry ports investigated	306	274
% entry ports contaminated (n)	18 (55)	6.6 (18)
% patients with contaminated entry ports (n)	66.7 (26)	34.2 (13)
Mean cfu recovered (range)	26 (1-810)	5 (1-22)

8.3.2.4 Positive CVC distal tip cultures

Rates of internal and external catheter tip microbial contamination are given in table 8-10. A higher proportion of CVC tips were externally contaminated with microorganisms compared to internally. There was no significant difference between the number of patients with externally or internally contaminated tips to which either PosiFlow® or caps were attached (p=0.3 and p=0.5 respectively – Fishers Exact Test).

Patients randomly allocated 70% IMS for skin and intravenous connection disinfection had significantly more CVC tips that were externally contaminated with microorganisms compared to 0.5% chlorhexidine/70% IMS and 10% povidone-iodine (p=0.01 and p=0.04 respectively). There was no statistical difference between external tip microbial contamination rates when comparing 10% povidone-iodine and 0.5% chlorhexidine/70% IMS (p=0.8).

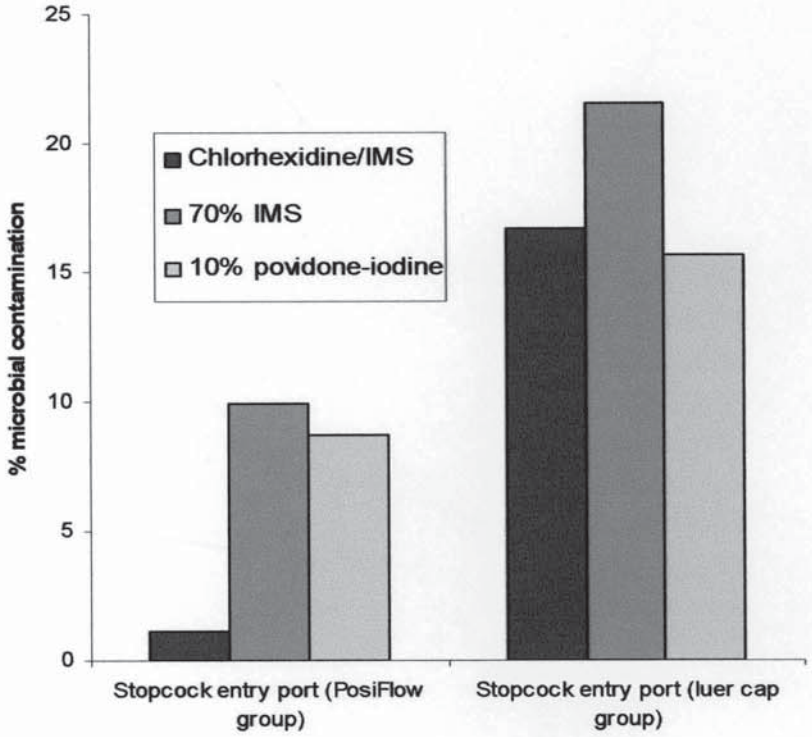
Table 8-10 Association between intravenous connection type, disinfection procedure and positive CVC distal tip cultures

	External tip culture	**Internal tip culture**
Number sampled	68 (32 PosiFlow +36 luer cap)	36 (16 PosiFlow +20 luer cap)
% Culture positive (n)	45.6 (31)	36.1 (13)
% PosiFlow® patients positive (n)	53.1 (17)	43.8 (7)
% Luer cap patients positive (n)	38.9 (14)	30 (6)
% Chlor/IMS patients positive (n)	28.6 (6/21)	42.9 (3/7)
% IMS patients positive (n)	69.6 (16/23)	42.9 (3/7)
% Pov-iodine patients positive (n)	37.5 (9/24)	31.8 (7/22)

8.3.2.5 Comparison of microbial contamination rates associated with stopcock entry ports following disinfection with 0.5% (w/v) chlorhexidine, 70% (v/v) IMS or 10% (w/v) povidone-iodine

The microbial contamination rates associated with stopcock entry ports following disinfection with 0.5% (w/v) chlorhexidine, 70% (v/v) IMS or 10% (w/v) povidone-iodine is shown in figure 8-4.

Figure 8-4 Microbial contamination rates of stopcock entry ports disinfected with 0.5% chlorhexidine/IMS, 70% IMS or10% povidone-iodine



Of those PosiFlow® disinfected with chlorhexidine, 1.1% of the entry ports to which the PosiFlow® were attached were contaminated with microorganisms compared to 9.9% disinfected with IMS (p=0.02) and 8.7% with povidone-iodine (p=0.03). There was no significant difference between the microbial contamination rates of the IV connections disinfected with either IMS or povidone-iodine (p=0.8).

Of the stopcock entry ports with standard luer cap attachments, 21.6% cleaned with IMS were contaminated compared to 16.7% disinfected with chlorhexidine (p=0.5) and 15.7% with povidone-iodine (p=0.4). There was no significant difference between the microbial contamination rates of stopcock entry ports disinfected with either chlorhexidine or povidone-iodine (p=1.0).

There was no significant difference between the numbers of microorganisms recovered from stopcock entry ports with either PosiFlow® or standard luer caps attached following disinfection with 0.5% chlorhexidine/IMS, 70% IMS or 10% povidone-iodine (table 8-11).

Table 8-11. Cfu recovered from stopcock entry ports following disinfection with 0.5% chlorhexidine/IMS, 70% IMS or 10% povidone-iodine

	Disinfectant type – Mean cfu number and (range)			Statistical significance (Mann-Whitney Test)
Intravenous connection sampled	**0.5% (w/v) chlorhexidine**	**70% (v/v) IMS**	**10% (w/v) povidine-iodine**	**a) chlorhexidine vs IMS b) chlorhexidine vs povidone-iodine c) IMS vs povidone-iodine**
Stopcock entry ports with PosiFlow® attached	5 (5)	3 (1-3)	7 (1-22)	a) p=1.0, b) p=1.0, c) p=0.2
Stopcock entry ports with luer caps attached	7 (1-103)	8 (1-67)	70 (1- 810)	a) p=0.9, b) p=0.2, c) p=0.2

8.3.2.6. Microorganisms recovered from intravenous connections, CVC and skin swabs

Microorganisms recovered from the NC, entry ports, skin swabs and CVC distal tips were skin and environmental microorganisms including CoNS, *S. aureus,* Gram-positive and negative aerobic bacilli, *Neisseria* spp. and *Candida* spp.

8.3.2.7. Frequency of PosiFlow®stopcock entry port manipulation and incidence of stopcock entry port contamination

The frequency of PosiFlow®/stopcock entry port manipulation and associated stopcock entry port microbial contamination rates are shown in table 8-12.

The PosiFlow® and stopcock entry ports were manipulated an average of 2.0 times (ranges: PosiFlow®, 0-20 and stopcock entry ports with luer caps 0-17). There was no significant correlation between the number of manipulations and stopcock entry port contamination either with PosiFlow® or luer caps attached (p=0.7 and p=0.5, Spearman Rank Test).

8.3.2.8. Correlation between frequency of manipulation and the extent of stopcock entry port contamination

There was no significant correlation between the number of manipulations and extent of stopcock entry port contamination in patients with either PosiFlow® or luer caps attached (p=0.8 and p=0.5, Spearman Rank Test) (table 8-13).

Table 8-12. Frequency of PosiFlow®/stopcock entry port manipulation and associated stopcock entry port microbial contamination rates

	Ports with PosiFlow® attached		Ports with standard caps attached	
Number of manipulations	Total number of PosiFlow® investigated	Number of ports contaminated (%)	Total number of stopcock entry ports investigated	Number of ports contaminated (%)
0	41	4 (9.8)	48	11 (22.9)
1	88	2 (2.3)	118	17 (14.4)
2	67	6 (9.0)	79	15 (19)
3	29	1 (3.4)	20	9 (45)
4	11	0 (0)	5	1 (20)
5	7	0 (0)	4	1 (25)
6	4	0 (0)	8	1 (12.5)
7	1	0 (0)	4	0 (0)
8	1	0 (0)	1	0 (0)
9	1	0 (0)	1	0 (0)
10	2	0 (0)	-	-
11	1	0 (0)	-	-
12	2	0 (0)	-	-
13	1	0 (0)	-	-
15	1	0 (0)	-	-
17	-	-	1	0 (0)
20	1	1 (100)	-	-

Table 8-13. Cfu recovered from stopcock entry ports with either luer caps or PosiFlow® attached

	Stopcock entry ports	
Number of manipulations	Mean number of cfu recovered from port with PosiFlow® attached (range)	Mean number of cfu recovered from port with luer caps attached (range)
0	0.2 (0-2)	4 (0-103)
1	0.3 (0-22)	10 (0-810)
2	1 (0-21)	1 (0-30)
3	0.03 (0-1)	1 (0-5)
4	0 (0)	0.2 (0-1)
5	0 (0)	1 (0-3)
6	0 (0)	0.1 (0-1)
7	0 (0)	0 (0)
8	0 (0)	0 (0)
9	0 (0)	0 (0)
10	0 (0)	0 (0)
20	1 (1)	-

8.3.2.9. Association between intravenous infusions and microbial contamination of stopcock entry ports

The frequency of contaminated stopcock entry ports and associated intravenous infusions is shown in figure 8-5.

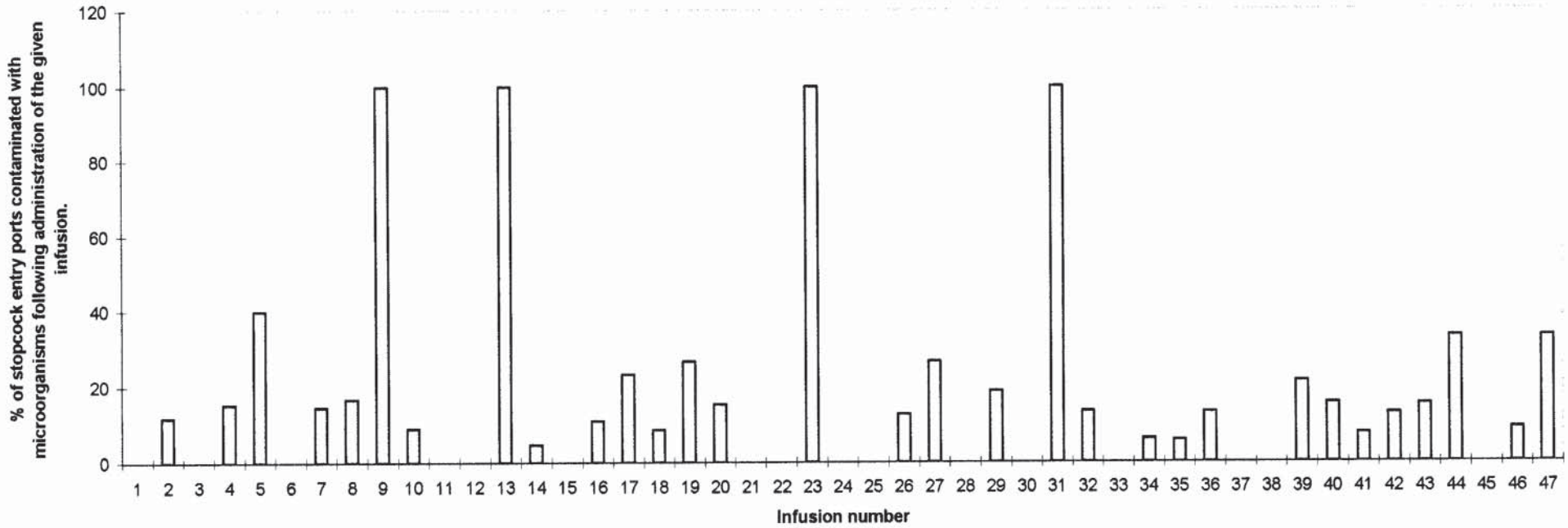
Infusions associated with the highest rate of stopcock entry port contamination were; ceftazidime (100%), dexamethasone (100%), lignocaine (100%) and magnesium sulphate (100%). Each infusion was administered through a maximum of 5 stopcock entry ports each. Of the infusions administered through 10 or more stopcock entry ports, the most commonly associated with stopcock entry port contamination were; frusemide (26.7%), fentanyl (23.3%), potassium chloride (21.4%), heparin (18.8%) and calcium chloride (16.7%).

Needleless connectors through which several antibiotics were administered (benzylpenicillin, ceftazidime, cefuroxime, flucloxacillin, gentamicin and vancomycin) were associated with contaminated stopcock entry ports.

8.3.2.10. Type of manipulations associated with contaminated stopcock entry ports

The manipulation most frequently associated with microbial contamination of stopcock entry ports to which both standard luer caps and PosiFlow® were attached was the attachment of a central venous pressure (CVP) line (table 8-14).

Figure 8-5. Frequency of contaminated stopcock entry ports following intravenous infusions



Drug Key and number of stopcock entry ports through which each infusion was administered (n).

1) Adrenaline (6)	11) Chlorphenamine (1)	21) Isoprenaline (1)	31) Magnesium sulphate (1)	41) Protamine (26)
2) Alfentanil (42)	12) Cyclizine (3)	22) Ketamine (4)	32) Metaraminol (52)	42) Ranitidine (31)
3) Amiodarone (1)	13) Dexamethasone (1)	23) Lignocaine (1)	33) Metoclopramide (4)	43) 0.9% saline (91)
4) Aprotinin (13)	14) 5% Dextrose solution (21)	24) Gelatin solution (2)	34) Morphine (16)	44) Sodium bicarbonate (3)
5) Atracurium (5)	15) Dobutamine (1)	25) Gentamicin (1)	35) Noradrenaline (17)	45) Sodium nitroprusside (1)
6) Benzylpenicillin (1)	16) Dopamine (73)	26) Glyceryl trinitrate (79)	36) Ondansetron (15)	46) Tramadol (11)
7) Blood/blood products (225)	17) Fentanyl (30)	27) Glycopyrrolate (15)	37) Pancuronium (1)	47) Vancomycin (3)
8) Calcium chloride (12)	18) Flucloxacillin (81)	28) Granisetron (2)	38) Phenylephrine (2)	
8) Ceftazidime (1)	19) Frusemide (15)	29) Heparin (48)	39) Potassium chloride (14)	
9) Cefuroxime (67)	20) Insulin (13)	30) Hydrocortisone (2)	40) Propofol (64)	

Table 8-14. Types of manipulations associated with microbial contamination of stopcock entry ports

	Stopcock entry ports with attached PosiFlow®		Stopcock entry ports with luer caps attached	
Manipulation type	Number of manipulations	% of contaminated stopcock entry ports (n)	Number of manipulations	% of contaminated stopcock entry ports (n)
Bolus injection	70	2.9 (2)	46	19.6 (9)
Continuous infusion	146	6.2 (9)	162	19.1 (31)
CVP line	33	12.1 (4)	32	21.9 (7)
Blood aspiration/administration	136	9.4 (10)	121	19 (23)

CVP = central venous pressure

8.4 Discussion

In phase one of this trial, the microbial contamination associated with the novel PosiFlow® and Clave® NC in the clinical setting was assessed. There was no significant difference between the external and internal microbial contamination rates or numbers of microorganisms recovered between the two devices. This suggests that the surface topography of the compression seals and the ability to effectively disinfect them are similar in both devices.

The rate of internal microbial contamination of both types of device was low compared to that of standard stopcock entry ports, which have reported to be as high as 22% (Tebbs *et al.*, 1996). This low microbial internal contamination rate may suggest that needleless connectors do not act as a microbial reservoir, potentially seeding the intravascular catheter with microorganisms as suggested by Donlan and colleagues (2001). However, Seymour *et al.* (2000) demonstrated internal Clave® microbial contamination rates as high as 16.5% following 72 hr use in the clinical setting.

During the clinical investigation, several nursing and medical staff reported that the PosiFlow® was more difficult to manipulate than the Clave® due to its positive pressure dynamics. This may account for the higher internal microbial contamination rate of the PosiFlow® as more time is spent manipulating the device.

One hundred percent of internally contaminated Clave® were also externally contaminated with microorganisms compared to only 57.1% of PosiFlow®. This suggests that the PosiFlow® are less likely to become internally contaminated if microorganisms are present on the external compression seal which may be due to the superior 'closed system' piercing element associated with the device. However as microorganisms were not characterised by molecular typing methods, no conclusions can be drawn regarding the source of the contamination. However, one hypothesis is that microorganisms recovered form the internal surface may have migrated from the external compression seal but the external disinfection process removed microorganisms prior to sampling. Conversely, internal microbial contamination may have been introduced via contaminated infusates or via seeding from the blood during aspiration.

There was no correlation between the rate of internal and external microbial contamination rates of both PosiFlow® and Clave® and the frequency of manipulation by healthcare workers. However, a single manipulation of a NC may be sufficient to result in internal microbial contamination. The direct effect of the frequency of manipulation on microbial contamination rates requires investigation by *in vitro* studies whereby all variables such as microbial exposure and bioload can be strictly controlled.

To summarise, phase 1 of this trial, there was no difference in microbial contamination rates between PosiFlow® and Clave® and the potential infection risk is not associated with increased activation of the devices. This preliminary work also highlights that the low microbial contamination rates associated with the use these devices may provide a strategy for the prevention of intravenous connection contamination and subsequently CRI compared to the widely used standard luer caps.

During phase 2 of the trial, microbial contamination associated with stopcock entry ports with either PosiFlow® or standard luer caps attached were assessed. In addition, as there is a lack of consensus regarding the most efficient disinfectant for cleaning these devices, 0.5% (w/v) chlorhexidine gluconate in 70% (v/v) IMS, 70% (v/v) IMS and 10% (w/v) povidone-iodine were investigated for efficacy.

Stopcock entry ports to which PosiFlow® were attached had a significantly lower microbial contamination rate compared to those with standard luer caps attached (6.6% vs 18%). The contamination rate of the stopcock entry ports with luer caps attached achieved in this study concurs with previous investigations (Tebbs *et al.*, 1996; Seymour *et al.*, 2000). Furthermore, in a similar clinical investigation, Bouza and colleagues (2003) demonstrated that the use of NC was associated with a lower hub microbial contamination rate. Four percent of hubs to which Clave® were attached were contaminated compared to 14% with standard connections. There are several hypotheses as to why the PosiFlow® needleless connector was associated with a reduced rate of stopcock entry port microbial contamination: the PosiFlow® is a closed system device compared to standard luer caps which are removed prior to each manipulation, exposing the entry port to the environment; the closed design of the PosiFlow® may also reduce handling time during manipulation; extended handling of the luer caps may provide a significant source of microorganisms in stopcock entry ports increasing the microbial contamination rate. Indeed, the microbial

load recovered from the stopcock entry ports was higher when standard luer caps were employed compared to PosiFlow®.

None of the patients in this trial presented with infection related to the catheter. However catheters removed for culture from both patient groups yielded tips that were colonised or contaminated with microorganisms. The tips may have become contaminated or colonised via a number of routes including: contamination by the patients and healthcare workers skin flora during removal of the CVC; by haematogenous seeding; extraluminal migration from the exit site or by impaction of the microbes during insertion (Elliott, 1995). However, further epidemiological studies to characterise microorganisms by molecular typing methods is required to support this hypothesis.

In this current study, the efficacy of 0.5% (w/v) chlorhexidine/70% (v/v) IMS, 70% (v/v) IMS and 10% (w/v) povidone-iodine was also compared in their ability to decontaminate stopcock entry ports. Chlorhexidine and povidone-iodine were associated with significantly lower rates of microbial contamination than 70% IMS for stopcock entry ports to which PosiFlow® were attached, which concurs with previous work. Indeed, chlorhexidine has been reported to be more effective than 70% isopropyl alcohol for general disinfection purposes (Maki *et al.*,1991). Furthermore, a study conveyed by Choudhuri *et al.* (1990) reported that povidone-iodine was more effective than alcohol for skin sterilisation prior to venepuncture which reflects the results described in this study. A higher proportion of patients who had positive tip cultures had their skin disinfected with 70% IMS. This suggests that 70% IMS is less effective at reducing the microbial load on the skin prior to catheterisation compared to tincture of chlorhexidine or povidone-iodine. This suggests that the use of tincture of chlorhexidine or povidone-iodine may reduce the potential: impaction of microorganisms on to the CVC; extraluminal migration of microorganisms from the exit site and contamination during removal of the catheter. Alcohol based disinfectants have a rapid mode of action which involves the denaturation of microbial cellular proteins (Larson, 1987). They have a broad spectrum of activity and minimal side effects, however there is no residual antimicrobial action after evapouration of the alcohol (Kobayashi, 1993). Chlorhexidine and povidone-iodine also have a broad spectrum of antimicrobial activity which is by means of disruption of the electrostatic charge of cell walls and by oxidation/substitution by free iodine respectively (Larson, 1987). However, both disinfectants require between 3 and 5 min for microbial killing; a waiting time which is frequently ignored in the clinical setting (Widmer, 1997). In this current study, there was no statistically significant difference in the efficacy of

chlorhexidine and povidone-iodine to disinfect the IV connections, reflecting the results of previous work which investigated skin antisepsis prior to epidural catheterisation and for exit site antisepsis (Kasuda *et al.*, 2002; Humar *et al.*, 2000). However, several previous studies have also found that 0.5% tincture of chlorhexidine is superior to 10% povidone-iodine in a number of clinical situations including skin preparation prior to venepuncture for phlebotomy and peripheral and epidural cannulation (Mimoz *et al.*, 1999; Garland *et al.*, 1995; Kinirons *et al.*, 2001). These observations could be due to the combination of alcohol and chlorhexidine which increased the antimicrobial spectrum (the remanent effect) (Widmer, 1997). Indeed, it was shown by Kjolen and Andersen (1992), that alcoholic chlorhexidine was more effective than 70% ethanol and 40% isopropanol in the disinfection of skin heavily contaminated with different species of bacteria. As in phase 1 of the study, there was no significant correlation between the number of PosiFlow®/stopcock entry port manipulations and stopcock entry port contamination.

Drugs and infusions administered through the needleless connectors and stopcock entry ports during both phases of the study were recorded and analysed to determine if any correlation exists between microbial contamination and type of administration. The administrations most commonly associated with microbial contamination in this study were; fentanyl, dextrose solution, heparin, glycerol trinitrate, frusemide, potassium chloride and calcium chloride. However, there is no published data on the ability of; glycerol trinitrate, frusemide, potassium chloride and calcium chloride to support or inhibit microbial growth therefore the association in this trial may be incidental. Previous work has demonstrated that fentanyl has no bacterial growth inhibition properties which compares well with our findings (Feldman *et al.*, 1994; Rosenberg and Renkonen, 1985). Five percent dextrose solution was also associated with microbial contamination of the IV connections in this study. The solution is a readily available source of carbohydrate providing a source of nutrients to support microbial growth. The antimicrobial effects of heparin remain unclear. Catheters which are coated with heparin are less likely to become colonised and infected. However a study examining the sterility of recombinant factor VIII inoculated with a variety of bacteria followed by the introduction of heparin showed that the heparin had no effect on bacterial growth and numbers were not reduced which would concur with the results from this study (Belgaumi *et al.*, 1999). Microbial contamination of devices used for antibiotic administration during this study is likely to be incidental as there is no published data to suggest that antibiotics encourage microbial growth. Contamination may have occurred after the antibiotic was infused through

manipulation of the device by patients and staff, however further work is required to test these hypotheses.

There was no specific type of manipulation that was associated with the highest incidence of IV connection contamination. However attachment of a CVP line was associated with the internal microbial contamination of the Clave® and stopcock entry ports to which either with PosiFlow® or standard luer caps were attached. A CVP line is attached to the distal lumen of the CVC and a transducer, and allows continuous CVP monitoring in the cannulated central vein. A CVP port is present approximately midway down the line which allows administration of drugs and fluids. The CVP line continually opens the fluid path of the needleless connector/stopcock entry port which is often neither closed nor disinfected between use, creating a portal of entry for microorganisms to access the CVC. The repeated administration of bolus injections through the CVP port potentially creates a renewed source of contamination each time.

Microbial contamination of intravenous connections may be reduced by ensuring all devices are flushed with sterile saline following administration. Furthermore the use of syringes pre-filled with the appropriate infusion may aid in reducing contamination (Worthington *et al.*, 2001). Pre-filled syringes avoid the need for healthcare workers to prepare the infusion thus reducing manipulation time and possible microbial contamination.

In summary, the cardiac surgery critical care unit of the University Hospital Birmingham NHS Trust currently uses stopcocks with standard luer caps on CVC which are disinfected with 70% IMS. In this current study, this resulted in 21.6% of stopcock entry ports being contaminated with microorganisms after 72 hr *in situ*. The results of this clinical trial suggest that microbial contamination of stopcock entry ports may be reduced by incorporating NC into clinical practice for use with intravenous connections. Indeed, the PosiFlow® disinfected with 0.5% chlorhexidine in 70% IMS before and after each manipulation reduced stopcock entry port contamination to 1.1%. These strategies may aid in reducing catheter-related bloodstream infection acquired via the intraluminal route and therefore reduce costs associated with such infections.

9. Final Discussion

The first infections associated with the use of IV catheters were reported shortly after the introduction of these devices into clinical practice (Neuhof and Seley, 1947). Coagulase-negative staphylococci are currently the most common aetiological agents implicated in bloodstream infections associated with CVC (Coello *et al.*, 2003). This is of little surprise since the CoNS are a major part of the cutaneous ecosystem (Kloos and Bannerman, 1994). The CoNS comprise a large proportion of commensal microorganisms on the skin's surface, however, if the skin is damaged by implantation of a foreign body for example, an IV catheter, these may gain entry into the host and potentially adopt a pathogenic role (Kloos and Bannerman, 1994). Indeed, the skin is recognised as a major source of microorganisms responsible for IV catheter contamination, colonisation and infection (Sherertz, 1997). Research undertaken in this study has supported this by demonstrating that strains of CoNS originating from patients' skin can be recovered from several locations on colonised CVC including the internal and external CVC tip surfaces and IV connections (chapter 2). These CoNS may have translocated to these areas via a number of routes such as intraluminal migration from contaminated hubs (Sitges-Serra *et al.*, 1984) or extraluminal migration from the skin at the exit site (Elliott, 1988).

The incidence of infection caused by CoNS has increased with advances in modern medical practice for example, the increased use of indwelling medical devices and the increased number of immunocompromised patients. This rise in infections due to CoNS may also be associated with the increased acceptance of this group of microorganisms as opportunistic pathogens. Indeed, prior to the 1970s, CoNS were generally disregarded as specimen contaminants with *S. aureus* being the only staphylococcal species widely accepted as being pathogenic (Kloos and Bannerman, 1994). The association of CoNS with disease and their species diversity has been further enhanced by the development of sophisticated methods for the identification and characterisation of staphylococci (Geary *et al.*, 1997; Sloos *et al.*, 2000; De Paulis *et al.*, 2003; Garcia *et al.*, 2004).

Many microbial typing techniques have been evaluated in their ability to characterise staphylococci. Genotypic methods are particularly suited to this purpose as analysis of genetic material is not affected by environmental factors unlike phenotypic techniques whereby results are dependant on gene expression. During this study, several microbial typing techniques (biotyping, antibiograms, PFGE and RAPD) were compared based on their ability to characterise CoNS and *S. aureus* implicated in infection. In agreement with

previous studies, PFGE was the most discriminatory typing technique for the characterisation of both groups of microorganisms (Saulnier *et al.*, 1993; Schmitz *et al.*, 1998; Worthington *et al.*, 2000; Raimundo *et al.*, 2002). Indeed, PFGE of macrorestricted DNA is suited to the characterisation of microorganisms as the whole genome is investigated and a unique fingerprint of the microbial DNA is obtained (Weller, 2000). However, PFGE is a time-consuming technique and results achieved are often retrospective (Matushek *et al.*, 1996). Although RAPD only samples randomly selected regions of the microbial genome, it was demonstrated during this study to be highly discriminatory in the characterisation of both CoNS and *S. aureus*. Since this genotypic method is extremely rapid to perform, it may provide information during epidemiological investigations in a more timely manner. Indeed, further work to optimise this technique utilising multiple primers to further increase discriminatory power is warranted. No single common strain of *S. aureus* or CoNS was found to be associated with either infection during this research, however, the heterogeneic nature of CoNS isolates implicated in CR-BSI was demonstrated which concurred with findings by Lang and colleagues (1999).

The importance of genetic characterisation of heterogeneic microbial populations such as CoNS was further demonstrated in chapter 4. This section of the study was undertaken to determine if phenotypic characterisation of CoNS by routine microbiological methods is adequately discriminative to diagnose CR-BSI. The microbiological diagnosis of CR-BSI is based on the recovery of identical microorganisms (species and antibiogram) from pure cultures obtained from the catheter tip and blood culture (O'Grady *et al.*, 2002). However, it was demonstrated that multiple genotypes of CoNS were found within young, phenotypically similar CoNS populations, indicating that cases of CR-BSI may be mis-diagnosed in the routine microbiology laboratory. Indeed, recent reports suggest that cultures of colonially identical CoNS may contain multiple genotypes (Viedma *et al.*, 2000; Kloos and Bannerman, 1994). Furthermore, only single colonies of CoNS from colonially identical cultures are selected for phenotypic characterisation including antibiotic sensitivity testing, thus potentially facilitating the mis-diagnosis of CR-BSI. It would be useful to determine the exact extent of heterogeneity among colonies within morphologically pure, young cultures of CoNS. This investigation has demonstrated that to aid the diagnosis of CR-BSI, multiple colonies of CoNS should be selected for genotypic characterisation from tip and blood cultures which have been subjected to an extended incubation period (Viedma *et al.*, 2000; Kloos and Bannerman, 1994). Extended incubation periods for CoNS would, however, delay the clinical diagnosis and treatment of

patients. Thus further highlighting the need for simple, rapid genotypic characterisation techniques such as RAPD to be incorporated into routine laboratory practice.

The diagnosis of CRI is complicated. Clinical signs of CRI are often non-specific and indicative symptoms are often absent (Michalopoulos and Geroulanos, 1996). This difficulty in making the clinical diagnosis often leads to the unnecessary removal of IV catheters and highlights the vital role of laboratory investigations to confirm the diagnosis (Eggiman and Pittet, 2002). Standard microbiological methods used in the diagnosis of CRI include the culture of blood, catheter tips and exit site swabs (Pearson, 1996; Douard *et al.*, 1991; Blot *et al.*, 1999; Maki *et al.*, 1977; Cleri *et al.*, 1980; Guidet *et al.*, 1994). Other methods such as the use of an endoluminal brush to sample the internal lumen of catheters are being subjected to clinical evaluation (Kite *et al.*, 1999). Interpretation of microbiological culture results for the diagnosis of infections such as those associated with IV devices is often complex as positive cultures of CoNS may be representative of contamination or colonisation rather than true infection.

Serological diagnosis of infection provides a useful adjunct to microbiological culture as levels of specific antibodies or antigens may be estimated to confirm diagnosis and aid the interpretation of positive microbiological cultures. Indeed, many serological tests have been developed for the diagnosis of staphylococcal infection but have been of limited use (chapter 1, section 1.5.2.2.6). A rapid, indirect ELISA, incorporating lipid S - a novel exocellular lipoteichoic antigen produced by staphylococci was developed by Worthington and colleagues (2002). In this current investigation the potential of this assay to facilitate the diagnosis of Gram-positive infection was assessed (chapter 5). The assay was evaluated for the diagnosis of CR-BSI in haematology patients and SSI in cardiothoracic surgery patients. In addition, the correlation between elevated serum antibody titres with CVC colonisation was assessed. The lipid S ELISA had low sensitivity for the diagnosis of Gram-positive CR-BSI in haematology/SCT patients. Indeed, sensitivity values of only 33% for CoNS, 33% for *S. aureus* and 25% for *Enterococcus* species were achieved. This, however may be explained by the immunosuppressed state of this patient population. Indeed, many haematology patients had reduced leucocyte counts at the time of infection due to the receipt of myeloablative therapy such as chemotherapy. The potential of the lipid S ELISA as a rapid serological test to facilitate the diagnosis of staphylococcal infection was demonstrated in an immunocompetent patient group. For cardiothoracic surgery patients with SSI following median sternotomy, the assay had sensitivities of 69% for *S. aureus* infection, 100% for CoNS and 64% for cultures routinely disregarded as

'skin' microorganisms. These sensitivities compared well to results from previous studies which assessed the lipid S ELISA for serodiagnosis of CR-BSI (Worthington *et al.*, 2002), endocarditis (Connaughton *et al.*, 2001) and prosthetic joint infection (Rafiq *et al.*, 2000). Interestingly, elevated serum levels of anti-lipid S IgG were detected in 64% of patients whose cultures yielded CoNS but were regarded as non-significant suggesting that the assay may provide a useful adjunct when interpreting cultures yielding CoNS. In addition, the assay achieved a higher sensitivity for patients with deep sternal SSI due to *S. aureus* compared to those with superficial SSI due to *S. aureus* (54% vs 79%). Indeed, the sensitivity of the lipid S assay has been previously demonstrated to be higher in chronic infections (Connaughton *et al.*, 2001; Rafiq *et al.*, 2000). Anti lipid S IgG titres were not detected in non-infected cardiothoracic surgery patients whose CVC tips were colonised with CoNS, further demonstrating the use of this assay as an adjunct to facilitate the interpretation of microbiological cultures.

The reduced sensitivity of the lipid S ELISA for facilitating the diagnosis of CR-BSI in immunocompromised patients was further demonstrated in chapter 6. The lipid S ELISA and AOLC were performed on a regular basis during Hickman CVC placement in haematology and SCT patients in order to assess the ability of these tests to diagnose CR-BSI. However, in several immunocompetent patients with CR-BSI due to CoNS, a humoral anti-lipid S response was detected prior to routine blood cultures testing positive, demonstrating the potential of the lipid S ELISA in the rapid diagnosis of CR-BSI in immunocompetent patients. Evaluation of this rapid assay should be applied to further types of infection with different Gram-positive aetiological agents. In addition, the lipid S ELISA should be evaluated by different research teams to investigate inter-laboratory reproducibility of results.

The AOLC involves the microscopic examination of blood drawn through a CVC. This test has previously been demonstrated to be sensitive in the diagnosis of CR-BSI in both adults and neonates (Kite *et al.*, 1999; Bong *et al.*, 2003; Rushforth *et al.*, 1993; Tighe *et al.*, 1995). In agreement with reports of it's success in adults, 92% of adult patients with CR-BSI had positive AOLC results during Hickman CVC placement during this study. Furthermore, in 62% of infected patients, AOLC results were positive prior to clinical and routine microbiological diagnosis of CR-BSI, suggesting that the AOLC test may detect catheter colonisation in addition to bacteraemia. Indeed, in a study by Von Baum and colleagues (1998), positive AOLC results were obtained in patients with colonised CVC but no signs of infection. During this current study, a limited number of patients with no

clinical or routine microbiological symptoms of CR-BSI also had positive AOLC results, this again, may be due to the detection of colonisation or hub contamination, highlighting the need for adequate hub disinfection prior to collecting blood samples for the AOLC test. This demonstrates that the AOLC test should be used as an adjunct to conventional tests rather than in isolation and requires further evaluation in various patient groups with different types of CVC. Since both the lipid S ELISA and the AOLC can be performed whilst the catheter is *in situ*, the unnecessary removal of CVC may be avoided.

Since CR-BSI is the main cause of hospital acquired bacteraemia and imposes serious financial implications on healthcare systems worldwide, the development of preventative strategies are crucial (Correa and Pittet, 2000; Mermel, 2000). However, in order to obtain infection rate statistics and assess whether preventative strategies are effective, methods of surveillance are required. Previous audit programmes have highlighted areas in CVC care where preventative strategies should be targeted (Herbst, 1978; Lawrance, 1994). During this study, the Meditrend® audit programme was successfully used to identify current CR-BSI rates in haematology and SCT patients (chapter 7). In addition, it was demonstrated using this tool that where possible, PICC should be used in place of Hickman CVC since they carry a lower infection risk. However, the results should be interpreted with caution as it is difficult to assess the efficacy of one preventative measure if numerous variables are present. Indeed, during this audit, it was difficult to determine risk factors associated with CRI as many variables were present during the CVC insertion process, mostly dependant on the type of CVC employed. This highlights the need for further evaluation and development of the Meditrend® tool in order for it to successfully assess local clinical practice guidelines associated with the insertion and care of CVC.

Many preventative strategies have been evaluated for reducing CR-BSI (chapter 1, section 1.7). There has been growing evidence to support the theory that microbial contamination of the catheter hub and subsequent intraluminal migration is an important portal of entry (Salzman *et al.*, 1993; Segura *et al.*, 1996; Salzman and Rubin, 1997). Several measures for the prevention of CR-BSI acquired via the intraluminal route have been assessed (Salzman *et al.*, 1993; Segura *et al.*, 1996).

Needleless connectors have been primarily introduced into clinical practice to reduce the rate of needlestick injuries to healthcare workers (Russo *et al.*, 1999). However, it is thought that these devices may also facilitate aseptic technique and reduce the time spent manipulating IV connections, thereby minimising the risk of intraluminal contamination

(Brown *et al.*, 1997). Clinical trials that have evaluated the potential infection risk associated with NC have however generated conflicting results (Russo *et al.*, 1999; Brown *et al.*, 1997; Seymour *et al.*, 2000; Adams *et al.*, 1993; Lucet *et al.*, 2000; Kellerman *et al.*, 1996). At present there has also been limited research to evaluate the most appropriate disinfectant for cleaning intravenous connections (Salzman *et al.*, 1993). In this current study, the microbial contamination of stopcock entry ports of CVC was assessed when either NC or standard luer caps were attached. In addition, the efficacy of 0.5% (w/v) chlorhexidine gluconate in 70% (v/v) industrial methylated spirit (IMS), 70% (v/v) IMS and 10% (w/v) aqueous povidone-iodine for the disinfection of intravenous connections was determined (chapter 8). It was demonstrated during this study that the rate of stopcock entry port contamination was significantly lower with NC attached following 72 hrs *in situ*. Furthermore, disinfection of NC with 0.5% (w/v) chlorhexidine gluconate in 70% (v/v) IMS or 10% (w/v) aqueous povidone-iodine significantly reduced microbial contamination associated with the external compression seal. Both these strategies may reduce the risk of CR-BSI acquired via the intraluminal route. However, microbial contamination of hubs and subsequent intraluminal migration is the most common route of entry for microorganisms associated with CR-BSI in patients with long-term CVC (Sherertz, 1997). Indeed, further clinical trials assessing the direct impact of NC use on CR-BSI rates is warranted in these patients.

10. References

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Appendix 1 – Local Research Ethical committee approval for lipid S ELISA testing using sera sent to the routine pathology laboratories (chapter 5)

Birmingham NHS
Health Authority

South Birmingham Local Research Ethics Committee
27 Highfield Road, Edgbaston, Birmingham B15 2TH
Tel: 0121 245 2534
Fax: 0121 245 2535

Committee 2

Chairman: *Professor C Clifford*
Administrator: *Mrs A P McCullough*

Our ref: CC/APM/DD
Please *quote:*
14 January 2002

Professor T S J Elliott
Consultant Microbiologist
Clinical Laboratory Services Directorate
Queen Elizabeth Hospital

Dear Professor Elliott

Serological Markers of Gram Positive and Gram Negative Sepsis

Thank you for your letter and I apologise for the delay responding due to an office move.

The Vice Chairman reviewed what you wish to do and is happy to give approval for you to go ahead. In addition it will not be necessary for you to consent patients prior to further testing of samples.

Yours sincerely

Mrs A P McCullough
LREC Administrator

Appendix 2 – Local Research Ethical Committee approval for a comparative study of the lipid S ELISA and AOLC test (chapter 6)

4th Floor, Nuffield House, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH
Tel: 0121 627 8734 [internal 3811] - **Fax:** 0121 627 8575 [internal 3188]
Committee 1

Chairman: Mrs P K Moseley Mrs A P
Administrator: McCullough

Our ref: PKM/mlbt//01
Please quote: **5578**

Professor T S J Elliott
Consultant Microbiologist Department
of Clinical Microbiology Queen
Elizabeth Hospital Birmingham

Dear Professor Elliott

LREC reference number 5578
A Comparative Study of the Lipid S ELISA, the acridine orange leucocyte cytospin test and the endoluminal brush for the diagnosis of catheter-related sepsis due to staphylococci

South Birmingham Local Research Ethics Committee are happy to Approve your Study subject to the following:

- Satisfactory Indemnity arrangements being in place.
- Clearance from your Trust or relevant employer.
- That you produce an annual review in line with the Good Clinical Practice Guidelines.
- Active Approval is required until the Study has been completed.
- The Committee would wish to be kept informed of Serious Adverse Events, Amendments and any modifications to Patient Information Leaflets and Consent Forms.

Approval is valid for three years, however, if it is intended to continue the study after THREE YEARS from the date of this letter South Birmingham Local Research Ethics Commiftee would wish to re- examine it.

Would you please communicate this approval immediately to all members of the investigating team and where appropriate the sponsoring commercial company. Please also advise your Research and Development Office of this approval.

Yours sincerely

Mrs P K Moseley
Chairman
Local Research Ethics Committee

cc Appropriate Trust,
MREC if applicable

Appendix 3 – Patient information sheet for the comparative study of the lipid S ELISA and AOLC test (chapter 6)

PATIENT INFORMATION SHEET

STUDY: A COMPARATIVE STUDY OF THE SEROLOGICAL RESPONSE TO LIPID S AND THE ACRIDINE ORANGE LEUCOCYTE CYTOSPIN TEST FOR THE DIAGNOSIS OF CATHETER- RELATED SEPSIS DUE TO STAPHYLOCOCCI.

We wish to compare currently available tests to improve the diagnosis of infection and therefore improve further care of our patients. We therefore want to study the immune response in patients who are having a catheter inserted as part of their clinical treatment.

For the purpose of the study we need to collect small samples of blood at the time of catheter insertion and at intervals while your catheter remains in place. A sample of blood will be collected before you have your catheter inserted, another at the time your catheter is inserted and one each week of your hospital stay. The blood sample will be taken from your arm or through the catheter. All the blood samples will be taken to the laboratory for analysis and will not be used for anything other than the purpose of this study. Some clinical information will be recorded from your medical notes and again will only be used for the purpose of the study.

If you do decide to take part in the study you may at any time decide to withdraw without giving reason. This will not affect the care you receive in this hospital. If you do not want to take part in the study this will in no way affect the care you receive in this hospital. Please feel free to ask any questions you may have at any time.

Enquiries to:

Professor T. S. J. Elliott (ext. 2366).

Appendix 4 – Patient consent form for the comparative study of the lipid S ELISA and AOLC test (chapter 6)

TO BE RETAINED IN THE PATIENT NOTES **PATIENT ID LABEL**

CONSENT FORM

STUDY: A COMPARATIVE STUDY OF THE SEROLOGICAL RESPONSE TO LIPID S AND THE ACRIDINE ORANGE LEUCOCYTE CYTOSPIN TEST FOR THE DIAGNOSIS OF CATHETER- RELATED SEPSIS DUE TO STAPHYLOCOCCI.

For the purpose of the study we need to collect some small samples of your blood. A sample of blood will be taken from a vein in your arm or from the catheter, which you will have in place for the purpose of your treatment. All the blood samples will be taken to the laboratory for analysis and will not be used for anything other than the purpose of this study.

To be completed by the Patient

I have read and understood the patient information sheet about this study and have been given the chance to ask any questions I may have. I understand and accept the answers I have been given.

I confirm that I freely agree to take part in the study. I know that I am free to ask for more information and that I may withdraw, without giving reason, from the study at any time without this affecting my care or treatment in any way.

Patient's signature.....

Date of consent.....

Signature of Clinician/Nurse.....

Date of consent.....

Principle investigator: Prof TSJ Elliott, Dept of Clinical Microbiology, Queen Elizabeth Hospital. Ext. 2366

Appendix 5 – Standard case report form for the comparative study of the lipid S ELISA and AOLC test (chapter 6)

CENTRAL VENOUS CATHETER INFECTION STUDY

CASE REPORT FORM

- PATIENT STUDY NUMBER:
- DATE OF RECRUITMENT:
- PATIENT DETAILS:

  REGISTRATION NUMBER:

  NAME:

  AGE:

  WARD:

  CLINICAL DIAGNOSIS:

  CONSULTANT:

- CATHETER TYPE:
- DATE OF INSERTION:
- COMMENTS (AVAILABILITY FOR BLOOD SAMPLING):

SHOULD A PATIENT HAVE A CATHETER INFECTION PLEASE COMPLETE THE CLINICAL DIAGNOSIS SECTION BELOW.

Clinical Diagnosis

Date the infection was first suspected .....
Episode number (with the present catheter).....

Systemic infection.

- ☐ hypothermia <37°C
- ☐ pyrexia >38'C
- ☐ pyrexia unresponsive to broad spectrum antibiotics
- ☐ rigors following flushing of catheter
- ☐ no obvious focus of infection
- ☐ hypotension

Localised infection.

- ☐ cellulitis☐
- ☐ purulent exudates/pus from insertion site
- ☐ site induration (greater than 2cm)
- ☐ oedema/swelling
- ☐ pain
- ☐ erythema
- ☐ tenderness
- ☐ no other focus of infection

treatment for the line infection .....
outcome: infection successfully treated? Date .....
line removed? Date .....

Laboratory Diagnosis

- ☐ A recognised pathogen isolated from one or more blood cultures not related to an infection at another site.
- ☐ More than 15cfus cultured from the catheter tip.
- ☐ Same microorganism isolated from the catheter tip as the blood cultures (species and antibiogram) Same microorganism isolated from the catheter blood as the peripheral blood (species and antibiogram)
- ☐ Simultaneous quantitative blood cultures with a >5:1_ratio (CVC vs. Peripheral).
- ☐ Differential time period of CVC culture vs. Peripheral blood culture of 2 hours.

Appendix 6 – Meditend® audit questionnaire

BD CVC Audit Programme

1. Patient Details

1.1. Audit Number/Ward Number

1.2. Date of Birth

1.3. Hospital Registration Number

1.4. Was the patient?

a. ☐ Surgical **b.** ☐ Medical

1.5. What was the location of the patient after catheter insertion?

a. ☐ Ward **c.** ☐ High Dependenty Unit
b. ☐ ITU **d.** ☐ Other

2. Catheter Insertion Details

2.1. What month was the catheter inserted?

a. ☐ January **g.** ☐ July
b. ☐ February **h.** ☐ August
c. ☐ March **i.** ☐ September
d. ☐ April **j.** ☐ October
e. ☐ May **k.** ☐ November
f. ☐ June **l.** ☐ December

2.2. What year was the catheter inserted?

a. ☐ 2000 **d.** ☐ 2003
b. ☐ 2001 **e.** ☐ 2004
c. ☐ 2002 **f.** ☐ 2005

2.3. Who inserted the catheter?

a. ☐ Anaesthetist **d.** ☐ Resident/House Officer
b. ☐ Intensivist **e.** ☐ Nurse
c. ☐ Surgeon

2.4. Where did insertion take place?

a. ☐ Ward - please enter the ward number in the space below **d.** ☐ High Dependency Unit
b. ☐ Operating Room - please enter the operating room number in the space below **e.** ☐ Accident and Emergency
c. ☐ Intensive Care Unit

Ward/ Operating Room number

2.5. Was it tunnelled?

a. ☐ Yes **b.** ☐ No

BD CVC Audit Programme

2.6. Was the catheter valved (Groshong TM catheter)?

a. ☐ Yes
b. ☐ No

2.7. What was the biomaterial used to make the catheter?

a. ☐ Polyurethane
b. ☐ Polyurethane + Hydrophilic coating
c. ☐ Polyurethane + Antiseptic
d. ☐ Polyurethane + Antibiotic
e. ☐ Silicone
f. ☐ PTFE
g. ☐ Other

2.8. What type of catheter was used?

a. ☐ Single central line (Go to section 4)
b. ☐ Double central line (Go to section 4)
c. ☐ Triple central line (Go to section 4)
d. ☐ Quad central line (Go to section 4)
e. ☐ Quin central line (Go to section 4)
f. ☐ PICC (Go to section 3)
g. ☐ Sheath introducer (Go to section 4)
h. ☐ Midline (Go to section 3)
i. ☐ Other (Go to section 4)
j. ☐ Vascular port (Go to section 4)
k. ☐ CVC/Sheath introducer combination (Go to section 4)
l. ☐ PA/Sheath introducer combination (Go to section 4)

3. Parenteral Nutrition

3.1. What is the Patients Body Mass Index?

a. ☐ Low (less than 19)
b. ☐ Normal (20-30)
c. ☐ High (more than 31)

3.2. How long was the PICC inserted before PN commenced?

a. ☐ One day
b. ☐ Two days
c. ☐ Three days
d. ☐ Four days
e. ☐ Five days
f. ☐ More than 5 days

3.3. Was lipid infused?

a. ☐ Yes
b. ☐ No

4. Catheter and Insertion Details

4.1. Where was the catheter entry site?

a. ☐ Left
b. ☐ Right

4.2. Was the catheter entry site?

a. ☐ Subclavian
b. ☐ Internal jugular
c. ☐ External jugular
d. ☐ Peripheral
e. ☐ Femoral

5.1. Was a gloved finger used to enlarge the insertion site?

a. ☐ Yes (Go to 7)
b. ☐ No (Go to 6)

BD CVC Audit Programme

6. Was the insertion site enlarged with:

		Yes	No
6.1.	A dilator?	☐	☐
6.2.	A scalpel?	☐	☐

7. Were any of the following connectors fitted to the CVC hubs?

		Yes	No
7.1.	Stopcock	☐	☐
7.2.	Needleless I.V. access valve	☐	☐
7.3.	Injection port	☐	☐
7.4.	I.V. Line	☐	☐
7.5.	Luer lock plug	☐	☐

8.1. What catheter insertion technique was used?

a. ☐ Seldinger
b. ☐ Cut down
c. ☐ Through introducer
d. ☐ Guidewire exchange
e. ☐ Tunnelled

8.2. Was the same vessel used for any other cannulation?

a. ☐ Yes **b.** ☐ No

9. What were the patient risk factors at the time of insertion?

		Yes	No
9.1.	Assisted ventilation	☐	☐
9.2.	Tracheostomy	☐	☐
9.3.	Concurrent central line	☐	☐
9.4.	PN Infusion	☐	☐
9.5.	Anticoagulant	☐	☐
9.6.	Colonised by resistant organisms	☐	☐
9.7.	Neutropaenic	☐	☐
9.8.	Immunocompromised	☐	☐
9.9.	Other	☐	☐

10.1. Prior to insertion, were hands washed?

a. ☐ Yes **b.** ☐ No

10.2. Was hand disinfectant used?

a. ☐ Yes **b.** ☐ No

BD CVC Audit Programme

11. Were the following used in the preparation procedure?

	Yes	No
11.1. Gloves	☐	☐
11.2. Gown	☐	☐
11.3. Drapes	☐	☐

12. Insertion Site Preparation and Dressing

12.1. What type of skin disinfectant was used on the insertion site?

- **a.** ☐ Isopropyl Alcohol
- **b.** ☐ Chlorhexidine
- **c.** ☐ Chlorhexidine/Alcohol
- **d.** ☐ Iodine
- **e.** ☐ Other
- **f.** ☐ None

12.2. How long prior to CVC placement was disinfectant used?

- **a.** ☐ Less than two minutes
- **b.** ☐ More than two minutes

12.3. What type of dressing was used immediately following insertion?

- **a.** ☐ Non-occlusive semi-permeable
- **b.** ☐ Sterile gauze/dry dressings
- **c.** ☐ Occlusive
- **d.** ☐ None

12.4. Did the dressing contain an antimicrobial?

- **a.** ☐ Yes
- **b.** ☐ No

13. Insertion Site Management

13.1. What type of dressing was used in the ward?

- **a.** ☐ Non-occlusive semi-permeable
- **b.** ☐ Sterile gauze/dry dressing
- **c.** ☐ Occlusive
- **d.** ☐ None
- **e.** ☐ Non-sterile gauze/dry dressing

13.2. Did the dressing contain an antimicrobial?

- **a.** ☐ Yes
- **b.** ☐ No

13.3. If the dressing appeared soiled was it changed?

- **a.** ☐ Yes
- **b.** ☐ No

13.4. How often were dressings changed?

- **a.** ☐ 1 day intervals
- **b.** ☐ 2-3 day intervals
- **c.** ☐ 4-7 day intervals
- **d.** ☐ Less than once a week

13.5. What was used to clean the insertion site at dressing change?

- **a.** ☐ Normal Saline
- **b.** ☐ Iodine
- **c.** ☐ Isopropyl/Alcohol
- **d.** ☐ Iodine/Alcohol
- **e.** ☐ Chlorhexidine/Alcohol
- **f.** ☐ Not cleaned

13.6. How frequently is the insertion site and catheter junction boot cleaned?

- **a.** ☐ Every 24 hours
- **b.** ☐ 24 hours-72 hours
- **c.** ☐ More than 72 hours
- **d.** ☐ Not cleaned

BD CVC Audit Programme

14. I.V. Line Management

14.1. Was PN being infused?
- a. ☐ Yes
- b. ☐ No

14.2. Was a dedicated lumen used to infuse PN?
- a. ☐ Yes
- b. ☐ No

14.3. Was the catheter flushed after catheter usage?
- a. ☐ Yes
- b. ☐ No

14.4. Did any of the lumens become blocked?
- a. ☐ Yes
- b. ☐ No

14.5. Post insertion, did catheter blockage occur after:
- a. ☐ 0-3 days
- b. ☐ 4-7 days
- c. ☐ 1-2 weeks
- d. ☐ 2-4 weeks
- e. ☐ 1-6 months
- f. ☐ More than 6 months

14.6. Which flush was used to unblock the catheter?
- a. ☐ Urokinase
- b. ☐ Heparinised saline
- c. ☐ Saline
- d. ☐ Heparin
- e. ☐ Other
- f. ☐ None

15. Line Removal

15.1. How long was the catheter in-situ?
- a. ☐ Up to 24 hours
- b. ☐ 24-72 hours
- c. ☐ 3-4 days
- d. ☐ 5-7 days
- e. ☐ 8-30 days
- f. ☐ More than 30 days

15.2. Why was the catheter removed?
- a. ☐ No longer required
- b. ☐ Blocked
- c. ☐ Suspected infection
- d. ☐ Extended duration of catheterisation
- e. ☐ Misplacement
- f. ☐ Accidental removal
- g. ☐ Other

15.3. Was it replaced by guidewire exchange?
- a. ☐ Yes
- b. ☐ No

16. Localised Infection at the Insertion Site

16.1. Did the patient have a catheter-related infection at the insertion site?
- a. ☐ Yes (Go to 16.2)
- b. ☐ No (Go to section 17)

16.2. How many days post insertion did infection develop?
- a. ☐ 0-3 days
- b. ☐ 4-7 days
- c. ☐ 1-2 weeks
- d. ☐ 2-4 weeks
- e. ☐ Greater than 1 month

BD CVC Audit Programme

17. Catheter Related Sepsis

17.1. Did the patient have a systemic infection associated with the catheter?

a. ☐ Yes (Go to 17.2) **b.** ☐ No (Go to section 18)

17.2. How many days was the catheter in-situ before systemic infection was diagnosed?

a. ☐ 0-3 days
b. ☐ 4-7 days
c. ☐ 1-2 weeks
d. ☐ 2-4 weeks
e. ☐ 6 months
f. ☐ More than 6 months

18. If the patient did have a systemic infection, what was the infection profile?

	Yes	No
18.1. Pyrexia (low grade <38.5C)	☐	☐
18.2. Pyrexia (high grade >38.5C)	☐	☐
18.3. Pyrexial and unresponsive to broad spectrum antibiotics	☐	☐
18.4. Septic rigors on flushing	☐	☐

19. Microbiology

19.1. If catheter related sepsis was suspected, was the distal tip of the catheter cultured?

Yes (Go to 20)	No (Go to 21)
☐	☐

20. Which of the following micro-organisms were isolated from the distal tip?

	Yes	No
20.1. Co-agulase negative staphylococcus	☐	☐
20.2. Staphylococcus aureus	☐	☐
20.3. Staphylococcus aureus (MRSA)	☐	☐
20.4. Gram-negative aerobic bacilli	☐	☐
20.5. Gram-positive aerobic bacilli	☐	☐
20.6. Yeast	☐	☐
20.7. Other	☐	☐

BD CVC Audit Programme

21.1. If catheter related sepsis was suspected, was a peripheral blood sample analysed?

Yes (Go to 22)	No (Go to 23)
☐	☐

22. Which of the following were isolated from the peripheral blood cultures?

	Yes	No
22.1. Co-agulase negative staphylococcus	☐	☐
22.2. Staphylococcus aureus	☐	☐
22.3. Staphylococcus aureus (MRSA)	☐	☐
22.4. Gram-negative aerobic bacilli	☐	☐
22.5. Gram-positive aerobic bacilli	☐	☐
22.6. Yeast	☐	☐
22.7. Other	☐	☐

23.1. If catheter related sepsis was suspected, was a blood culture taken via the catheter?

Yes (Go to 24)	No (Go to 25)
☐	☐

24. Which of the following were isolated from blood cultures taken via the catheter?

	Yes	No
24.1. Co-agulase negative staphylococcus	☐	☐
24.2. Staphylococcus aureus	☐	☐
24.3. Staphylococcus aureus (MRSA)	☐	☐
24.4. Gram-negative aerobic bacilli	☐	☐
24.5. Gram-positive aerobic bacilli	☐	☐
24.6. Yeast	☐	☐
24.7. Other	☐	☐

25.1. If a localised infection was suspected, was a swab from the exit site analysed?

Yes (Go to 26)	No (Go to 27)
☐	☐

26. Which of the following were isolated from the exit swab site?

	Yes	No
26.1. Co-agulase negative staphylococcus	☐	☐
26.2. Staphylococcus aureus	☐	☐
26.3. Staphylococcus aureus (MRSA)	☐	☐
26.4. Gram-negative aerobic bacilli	☐	☐
26.5. Gram-positive aerobic bacilli	☐	☐
26.6. Yeast	☐	☐
26.7. Other	☐	☐

BD CVC Audit Programme

27. Patient Outcome

27.1. Were antibiotics given to treat the infection?
- **a.** ☐ Yes
- **b.** ☐ No

27.2. If yes, which antibiotics were given for related sepsis?
- **a.** ☐ Flucloxacillin
- **b.** ☐ Vancomycin
- **c.** ☐ Teicoplanin
- **d.** ☐ Other

27.3. Did the patient respond to antibiotics?
- **a.** ☐ Yes
- **b.** ☐ No

27.4. Was the catheter removed as a consequence of the infection?
- **a.** ☐ Yes
- **b.** ☐ No

27.5. Did the patient die as a result of catheter related sepsis?
- **a.** ☐ Yes
- **b.** ☐ No

Comments
If you have any further comments, please list them in the space provided.

Comments

Appendix 7 – Local Research Ethical Committee approval for the NC study (chapter 8)

Birmingham

HEALTH AUTHORITY

South Birmingham Local Research Ethics Committee
4th Floor, Nuffield House, Queen Elizabeth Hospital Edgbaston, Birmingham B15 2TH
Tel: 0121 627 8733 - internal 3404 Fax: 0121 627 8575 - internal 3188
Committee 2

Chairman Professor C Clifford Mrs
Administrator: A P McCullough

Our ref: CC/APM/DD/C2/01/07
Please Quote: 0446

Professor T S J Elliott
Consultant Microbiologist
Department of Clinical Microbiology
Queen Elizabeth Hospital
Birmingham

Dear Professor Elliott

LREC reference number 0446
A clinical study to compare the microbial contamination of NC systems and conventional luers using isopropanol, iodine and chlorhexidine disinfection

South Birmingham Local Research Ethics Committee are happy to Approve your Study subject to the following:

- Satisfactory Indemnity arrangements being in place.
- Clearance from your Trust or relevant employer.
- That you produce an annual review in line with the Good Clinical Practice Guidelines.
- Active Approval is required until the Study has been completed.
- The Committee would wish to be kept informed of Serious Adverse Events, Amendments and any modifications to Patient Information Leaflets and Consent Forms.

Approval is valid for three years, however, if it is intended to continue the project after THREE YEARS from the date of this letter South Birmingham Local Research Ethics Committee would wish to re-examine it.

Would you please communicate this approval immediately to all members of the investigating team and where appropriate the sponsoring commercial company. Please also advise your Research and Development Office of this approval.

Yours sincerely

Professor C Clifford
Chairman
Local Research Ethics Committee

CC Appropriate Trust

Appendix 8 – Patient information sheet for the NC study (chapter 8)

PATIENT INFORMATION SHEET

A clinical study to compare the contamination of NC systems and conventional systems using isopropanol, iodine and chlorhexidine disinfection

You have been asked to choose whether or not you would like to participate in our study whereby we wish to compare different types of connectors that are attached to the end of your catheter, which is a tube connected to your bloodstream. Different disinfectants will be used to clean them. The purpose of the study is to determine which connector and disinfectant reduces the contamination of your catheter. You should not encounter any additional risks whichever connector or disinfectant is used.

If you do decide to take part in the study, you will be one of approximately 400 patients and will receive one of three types of different connector attached to your catheter. The connector attached to your catheter will then be disinfected with one of three types of disinfectant whilst the catheter remains in place. A research assistant will monitor you on a daily basis and record any clinical information from your notes until the catheter is removed

You are free to ask any questions at any stage of the study. You may also withdraw from the study at any point without giving any reason and this will in no way affect the treatment and care you receive in this hospital.

Principle investigator: Prof TSJ Elliott, Dept of Clinical Microbiology, Queen Elizabeth Hospital. Ext 2366

Appendix 9 – Patient consent form for the NC study (chapter 8)

TO BE RETAINED IN THE PATIENT NOTES **PATIENT ID LABEL**

CONSENT FORM

A clinical study to compare the contamination of NC systems and conventional systems using, isopropanol, iodine and chlorhexidine disinfection

As part of your care, blood samples are taken and drugs are given through a connector on your catheter, which is a tube connected to your bloodstream. We are undertaking a study to compare the usual connector with an alternative that does not require the use of a needle. We also wish to investigate the cleaning process of your connector, therefore whichever connector is attached to your catheter, it will be cleaned with one of three recognised disinfectants. Clinical information from your notes will be recorded and used only for the purpose of this study.

To be completed by the Patient

I have read and understood the patient information sheet about this study and have been given the chance to ask any questions I may have. I understand and accept the answers I have been given.

I confirm that I freely agree to take part in the study. I know that I am free to ask for more information and that I may withdraw, without giving reason, from the study at any time without this affecting my care or treatment in any way.

Patient's signature.....

Date of consent.....

Signature of Clinician/Nurse.....

Date of consent .....

Appendix 10 – NC study administration sheets (phase 1 and 2) (chapter 8)

Affix patient label here.

CONNECTOR, THREE WAY TAP AND DISINFECTANT STUDY

- Please complete the attached sheet for each manipulation of the connectors. Connectors are numbered 1-8.
- Each connector should be disinfected with.....spray (provided) and allowed to dry for 30 seconds prior to use. After use the connector should then be disinfected again using.....spray.
- When the connectors have been in situ for **72 hours**, the three way taps complete with the connectors should be removed and sent to microbiology labs (specimen bags and a form are provided). Please place each tap in a separate bag labelled with the patients name. You may then replace the taps with new ones used on your ward and the patient will have completed their contribution to the study.
- **If the line is removed before the 72 hour deadline please send the three way taps with the connectors attached to the lab in the manner detailed above.**
- If the patient is suspected of having a line infection and the catheter is removed, please send the complete catheter with taps and connectors attached to the microbiology lab.
- If the patient is dependent on any drugs being administered IV through a entry port on a three way tap that has reached the 72-hour removal point, then the tap should be removed during the change over of infusions or another appropriate time.
- Thank you for taking the time to assist us with this study your efforts are appreciated. If you are unsure of anything, or have any questions please contact Anna 3451 or Tony on 3229/8693.

Appendix 10 - continued

CENTRAL LINE CONNECTOR, THREE WAY TAP AND DISINFECTANT STUDY STUDY: PATIENT NAME.....**DATE**.....

Please complete the table below by using a tick to identify:- 1) Disinfection of each connector with..... spray before and after administration of drugs.
2) Name of drug administered through each connector.
3) The number of times each connector is utilised. (Attachment of infusions and CVP line count as one use.)
4) All drugs given via the CVP port if attached to a connector on the central line. (Please indicate the number of the connector that the CVP is attached

Drugs given	Pre clean	Post clean	Connector 1	Connector 2	Connector 3	Connector 4	Connector 5	Connector 6	Connector 7	Connector 8
INFUSIONS										
Glyceryl Trinitrate										
Dopamine										
Noradrenaline										
Propofol										
Alfentanil										
Ketamine										
5% Dextrose										
0.9% Saline										
Vancomycin										
Blood/blood products										
BOLUS INJECTION										
Fentanyl										
Pancuronium										
Phenylepherine										
Metaraminol										
Glycopyrrolate										
Heparin										
Protamine										
Calcium chloride										
Calcium gluconate										
Flucloxacillin										
Cefuroxime										
Flush										
BLOOD WITHDRAWN										

PLEASE REMOVE ALL THREE WAY TAPS WITH CONNECTORS ATTACHED ON:.....**AND SEND TO MICROBIOLOGY LABS (BAGS AND FORM PROVIDED)**

CONNECTOR STUDY

CASE REPORT FORM

- DATE OF RECRUITMENT:
- PATIENT DETAILS

REGISTRATION NUMBER:

NAME:

AGE:

WARD:

CLINICAL DIAGNOSIS:

- CONNECTOR / DISINFECTANT

(STUDY DESIGNATION)

TYPE OF CATHETER: SINGLE / DOUBLE / TRIPLE / QUAD / QUIN
DISINFECTION PROCEDURE: ISOPROPANOL / CHLORHEXIDINE / IODINE
TYPE OF CONNECTOR: POSIFLOW / CONNECTA CLAVE / LUER

- **CONNECTOR MANIPULATION**

PLEASE COMPLETE THE CONNECTOR INFORMATION SHEETS FOLLOWING ANY CONNECTOR MANIPULATION

CVC ASSOCIATED SEPSIS

NOT INFECTED ☐

SIGNS OF LOCALISED INFECTION

OEDEMA ☐

ERYTHEMA ☐

PURULENT EXUDATE ☐

SIGNS OF SYSTEMIC INFECTION

PYREXIA (>38.5°C) ☐

PYREXIA (<38.5°C) ☐

RIGORS ON FLUSHING ☐

PYREXIA UNRESPONSIVE

TO BROAD SPECTRUM ANTIBIOTICS ☐

NO OTHER FOCUS OF INFECTION ☐

MICROBIOLOGY

COMPLETE CVC SENT TO LAB ☐

BLOOD CULTURES SENT TO LAB:

VIA CVC ☐

VIA PERIPHERAL LINE ☐

Appendix 12 – NC study administration sheets (phase 2 only) (chapter 8)

Affix patient label here.

THREE WAY TAP AND DISINFECTANT STUDY

- Please complete the attached sheet for each manipulation of the three way tap entry ports. The ports are numbered 1-8.
- Each port should be disinfected with.....spray (provided) and allowed to dry for 30 seconds prior to use. After use the port should then be disinfected again using.....spray.
- When the three way taps have been in situ for **72 hours**, they should be removed and sent to microbiology labs **complete with caps** (specimen bags and a form are provided). Please place each tap in a separate bag labelled with the patients name. You may then replace the taps with new ones used on your ward and the patient will have completed their contribution to the study.
- **If the line is removed before the 72 hour deadline please send the three way taps to the lab in the manner detailed above.**
- If the patient is suspected of having a line infection and the catheter is removed, please send the complete catheter with taps attached to the microbiology lab.
- If the patient is dependent on any drugs being administered IV through a entry port on a three way tap that has reached the 72-hour removal point, then the tap should be removed during the change over of infusions or another appropriate time.
- Thank you for taking the time to assist us with this study your efforts are appreciated. If you are unsure of anything, or have any questions please contact Anna 3451 or Tony on 3229/8693.

Appendix 12 – continued

CENTRAL LINE THREE WAY TAP AND DISINFECTANT STUDY STUDY: PATIENT NAME.....**DATE**.....

Please complete the table below by using a tick to identify:- 1) Disinfection of each port with..... spray before and after administration of drugs.
2) Name of drug administered through each port.
3) The number of times each port is utilised. (Attachment of infusions and CVP line count as one use.)
4) All drugs given via the CVP port if attached to a port on the central line. (Please indicate the number of the port that the CVP is attached to).

Drugs given	Pre clean	Post clean	Port 1	Port 2	Port 3	Port 4	Port 5	Port 6	Port 7	Port 8
INFUSIONS										
Glyceryl Trinitrate										
Dopamine										
Noradrenaline										
Propofol										
Alfentanil										
Ketamine										
5% Dextrose										
0.9% Saline										
Vancomycin										
Blood/blood products										
BOLUS INJECTION										
Fentanyl										
Pancuronium										
Phenylepherine										
Metaraminol										
Glycopyrrolate										
Heparin										
Protamine										
Calcium chloride										
Calcium gluconate										
Flucloxacillin										
Cefuroxime										
Flush										
BLOOD WITHDRAWN										

PLEASE REMOVE ALL THREE WAY TAPS ON:.....**AND SEND TO MICROBIOLOGY LABS (BAGS AND FORM PROVIDED).**

Conferences Attended

Federation of Infection Societies: 8th conference. Manchester, 28th – 30th November 2001.

Systemic fungal infections: new drugs and treatment strategies. Birmingham, 21st February 2002.

Development of a national hepatitis C strategy. NHS conference. Birmingham, 12th March 2002.

R+D team conference, UHB NHS Trust conference. Birmingham, 10th September 2002.

International Conference of the Hospital Infection Society: 5th conference. Edinburgh, 15th – 18th September 2002.

Society for General Microbiology: 152nd conference. Edinburgh, 7th – 11th April 2003.

European Congress of Clinical Microbiology and Infectious Disease: 13th conference. Glasgow, 10th - 13th May 2003.

3M Healthcare Advanced IV Study Day. Manchester, 8th September 2003.

Society for General Microbiology: 153rd conference. Manchester, 8th – 11th September 2003.

Federation of Infection Societies: 10th conference. Cardiff 19th – 21st November 2003.

Society for Applied Microbiology: January meeting. 'Microbial Interactions with Medical Devices: a matter of life and death', Newcastle, 7th – 8th January 2003.

List of Publications

Casey AL, Worthington T, Lambert PA, Faroqui MH, Elliott TSJ. NCs – the way forward in the prevention of catheter-related sepsis? *Journal of Hospital Infection* 2002;**50**:77-81.

Casey AL, Worthington T, Lambert PA, Quinn D, Faroqui MH, Elliott TSJ. A randomized, prospective clinical trial to assess the potential infection risk associated with the PosiFlow® NC. *Journal of Hospital Infection* 2003;**54**: 288-293.

Casey AL, Worthington T, Lambert PA, Bonser RSB, Elliott TSJ. Lipid S serodiagnosis of staphylococcal surgical site infection following median sternotomy. Submitted for publication.

List of Poster Presentations

Casey A, Worthington T, Lambert PA, Faroqui MH, Elliott TSJ. NCs – the way forward in the prevention of catheter-related sepsis. Eighth conference of the Federation of Infection Societies, Manchester 28-30th November 2001.

Casey A, Worthington T, Lambert P, Faroqui M, Elliott T. Making the connection: Preventative measures in reducing catheter-related sepsis. Fifth International Conference of the Hospital Infection Society. Edinburgh 15-18th September 2002.

Casey A, Worthington T, Lambert P, Elliott T. Does charcterisation of coagulase-negative staphylococci by routine microbiological techniques mislead the diagnosis of catheter-related bloodstream infection? Tenth conference of the Federation of Infection Societies, Cardiff 19th-21st November 2003.

NEEDLELESS CONNECTORS

THE WAY FORWARD IN THE PREVENTION OF CATHETER-RELATED INFECTIONS?

A. Casey[1], T. Worthington[1], P. Lambert[2], M. Faroqui[1], T, Elliott[1].
[1]University Hospital Birmingham NHS Trust, Birmingham, U.K and [2]Aston University, Birmingham, U.K.

INTRODUCTION

- Intravascular catheters are now an integral part of the clinical management of patients in intensive care.[1]
- Catheter-related bloodstream infections are the main cause of hospital acquired bacteraemias.[2]
- Microorganisms may gain access to intravascular catheters by five main routes: extraluminal, intraluminal, contaminated infusates, haematogenous seeding and impaction during insertion (Figure 1).[3]
- In a recent study, 22% of stopcock entry ports were contaminated with microorganisms after 72 hours *in situ*.[4]
- To reduce hub colonisation, several methods have been investigated including disinfection with ethanol or chlorhexidine[5] and the use of a new hub design incorporating a iodinated alcohol chamber.[6]
- Needleless connectors (NCs) have recently been introduced into clinical practice to reduce the risk of needlestick injuries.[7] Furthermore, NCs may facilitate aseptic technique and reduce the time spent on manipulation of intravenous connections.
- The potential infection risk associated with NCs is currently unclear.

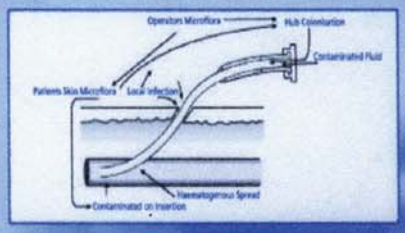

Figure 1

Microbial Sources of Intravascular Line Associated Infections

AIMS

- To assess the infection risk associated with the use of a NC (PosiFlow®, Becton Dickinson, Utah, USA).
- To compare the microbial contamination rates of stopcock entry ports with either standard luer caps or PosiFlow® attached (Figure 2).
- To evaluate the efficacy of two disinfectants currently used in the U.K. - 70% isopropyl alcohol (Spiriclens®, Adams Healthcare, Leeds, U.K.) and 0.5% chlorhexidine gluconate in 70% isopropyl alcohol (Hydrex DS®, Adams Healthcare, Leeds, U.K.) in the decontamination of NCs.

METHODS

Patients and Connectors.

- Thirty-one patients undergoing cardiac surgery who required a central venous catheter (CVC) as part of their clinical management were recruited onto the study.
- Each patient was randomly assigned 70% isopropyl alcohol or 0.5% chlorhexidine/alcohol for skin disinfection prior to CVC insertion and also decontamination of the PosiFlow® NCs and stopcock entry ports.
- A quad-lumen CVC was inserted into the internal jugular vein of each patient and stopcocks were attached to each hub. Each patient was randomly allocated either the PosiFlow® NC or standard luer caps to be attached to all ports of the stopcocks.
- After 72 hours in situ the stopcocks complete with PosiFlow® NC or standard luer caps were aseptically removed and sent for microbiological assessment.

Microbiological Assessment.

- The silicone compression seal of each PosiFlow® NC was imprinted ten times onto the surface of a 7% blood agar plate (Oxoid, Basingstoke, U.K.).
- Stopcock entry ports were sampled by inserting a small sterile swab and rotating through 360° ten times.
- Microorganisms were identified by standard laboratory techniques.
- All samples were inoculated onto 7% blood agar and incubated at 37°C for 48 hours in air.

Figure 2

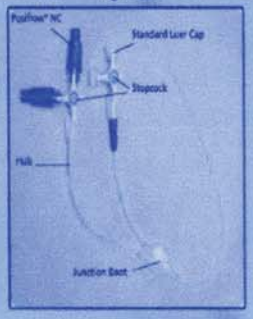

Figure 2. Triple lumen central venous catheter complete with stopcocks. From left to right; PosiFlow® NCs and standard luer caps attached to the entry ports.

Designed and produced by Medical Illustration Queen Elizabeth Hospital, Birmingham (0121) 627 2428

RESULTS

- None of the 31 patients who took part in the study had clinical or microbiological evidence of a CVC-related infection during the study period.
- Of 118 stopcock entry ports with standard caps, 20% were contaminated with microorganisms. In comparison 8 out of 122 (6%) luers with PosiFlow® NCs attached were contaminated (p=0.002, Fishers Exact Test) (Figure 3).
- The number and types of microorganisms recovered from stopcock entry ports with either PosiFlow® NCs or standard luer caps attached is given in Table1.
- PosiFlow® NCs cleaned with 0.5% chlorhexidine/alcohol had a lower external contamination rate (42%) compared to those cleaned with 70% isopropyl alcohol (64%)(p=0.002, Fishers Exact Test) (Figure 4).
- The number and types of microorganisms recovered from the external compression seal of the PosiFlow® NC following disinfcetion with either 0.5% chlorhexidine/alcohol or 70% isopropyl alcohol is given in Table 2.

Figure 3

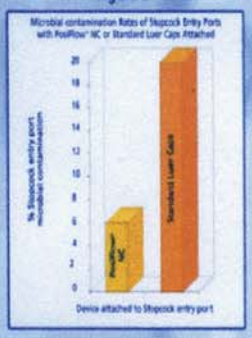

Figure 4

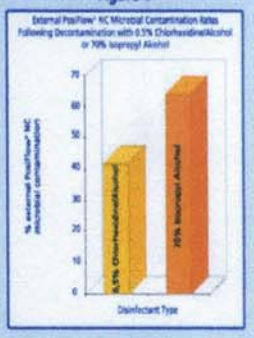

Table 1

Microorganisms recovered from Stopcock entry ports with either PosiFlow® NCs or standard Luer Caps attached.

	PosiFlow® NC	Standard luer caps
Mean cfu (range)	2 (1-3)	10 (1-60)
Microorganisms isolated.	CNS, Gram-negative bacilli, Neisseria spp.	CNS, Gram-negative and positive bacilli, S. aureus and Neisseria spp.

p=0.4 (Mann Whitney Test)
cfu = colony-forming unit
CNS = Coagulase negative staphylococci

Table 2

Microorganisms recovered from the external compression seal of the PosiFlow® NC following disinfection with 0.5% chlorhexidine/alcohol or 70% isopropyl alcohol

	0.5% Chlorhexidine/ alcohol	70% Isopropyl alcohol
Mean cfu (range)	1 (1-10)	14 (1-101)
Microorganisms isolated.	CNS and Neisseria spp.	CNS, Gram-negative bacilli and Neisseria spp.

p=0.2 (Mann Whitney Test)
cfu = colony-forming unit
CNS = Coagulase negative staphylococci

DISCUSSION

- The use of NCs may reduce hub and stopcock entry port contamination rates associated with intravascular catheters.
- The results also suggest that 0.5% chlorhexidine/alcohol is more effective than 70% isopropyl alcohol in the disinfection of NCs.
- The use of NCs may reduce the rate of catheter-related bloodstream infections.
- Disinfection with 0.5% chlorhexidine/alcohol combined with the use of pre-filled syringes may further reduce catheter-related infections acquired via the intraluminal route[3]. Further studies are needed.

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1. **Elliott T.S.J.** Catheter-associated infections: new developments in prevention. In: Burchardi, H (Ed). *Current topics in intensive care*. London: WB Saunders Co Ltd. 1997; 182-205.
2. **Correa L., Pittet D.** Problems and solutions in hospital-acquired bacteraemia. *Journal of Hospital Infection*. 2000; 46:89-95.
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4. **Tebbs S.E., Ghose A., Elliott T.S.J.** Microbial contamination of intravenous and arterial catheters. *Intensive Care Medicine*. 1996; 22:272-273.
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7. **Orenstein R., Reynolds L., Harabaic M. et al.** Do protective devices prevent needlestick injuries among health care workers? *American Journal of Infection Control*. 1995; 23:344-351.
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This work was supported by BD.

Making the Connection

Preventative measures in reducing catheter-related sepsis

A. Casey[1], T. Worthington[1], P. Lambert[2], M. Faroqui[1], T, Elliott[1].

[1]University Hospital Birmingham NHS Trust, Birmingham, UK and
[2]Aston University, Birmingham, UK

1 Introduction

- Intravascular catheters are now an integral part of the clinical management of patients in intensive care.[1] Indeed, 190,000 short-term central venous catheters (CVCs) are inserted annually in the UK.[2]
- Catheter-related infection may present locally at the exit site (Figure 1) or as a catheter-related bacteraemia.
- Catheter-related bloodstream infections are the main cause of hospital acquired bacteraemias.[3]
- The estimated annual cost of bacteraemia associated with short-term CVC use in the UK is £5–7 million.[4]
- Microorganisms may gain access to intravascular catheters by five main routes: extraluminal, intraluminal, contaminated infusates, haematogenous seeding and insertion during insertion (Figure 2).[5]
- In a recent study, 22% of stopcock entry ports were contaminated with microorganisms after 72 hours *in situ*.[6]
- Needleless connectors (NCs) have recently been introduced into clinical practice to reduce the risk of needlestick injuries.[7] Furthermore, NCs may facilitate aseptic technique and reduce the time spent on manipulation of intravenous connections.
- In a recent investigation, the microbial contamination rate associated with the PosiFlow® and Clave® NCs (Becton Dickinson, Utah, USA and ICU Medical Inc., California, USA respectively) was assessed.
- The internal and external contamination rate of PosiFlow® was 5.5% and 28.3% respectively whereas Clave® was 4% and 39%.
- The potential infection risk associated with NCs is currently unclear.

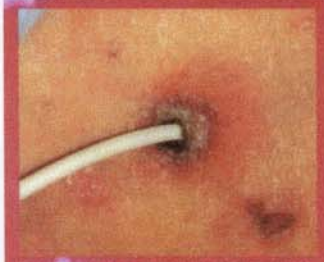


Fig 1 A localised catheter-related infection exhibiting erythema & exudate at the exit site.

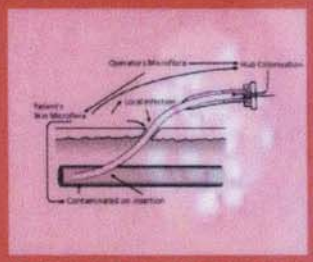


Fig 2 Microbial Sources of intravascular line associated infections.

2 Objectives

- To assess the infection risk associated with the use of a NC (PosiFlow®, Becton Dickinson, Utah, USA).
- To compare the microbial contamination rates of stopcock entry ports with either standard luer caps or PosiFlow® attached (Figure 7).
- To assess the efficacy of three disinfectants in the decontamination of NCs —
  70% isopropyl alcohol (Spirichlor®, Adams Healthcare, Leeds, UK),
  0.5% chlorhexidine gluconate in 70% isopropanol (Hydrex DS®, Adams Healthcare, Leeds, UK) &
  10% aqueous povidone-iodine (Betadine®, Purdue Frederick, Norwalk, Connecticut, USA).

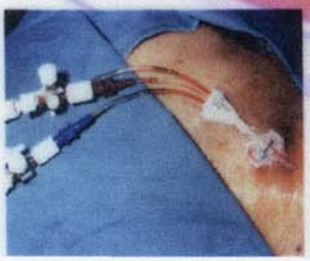
3 Methods

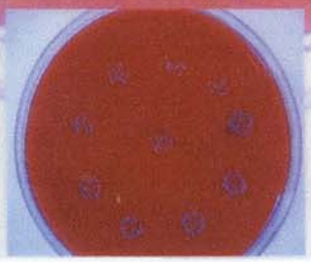
Patients and Connectors

- Seventy-eight patients undergoing cardiac surgery who required a central venous catheter (CVC) as part of their clinical management were recruited onto the study.
- Each patient was randomly assigned 70% isopropyl alcohol, 0.5% chlorhexidine / alcohol or 10% povidone-iodine for skin disinfection prior to CVC insertion and also decontamination of the PosiFlow® NCs and stopcock entry ports.
- A quad-lumen CVC was inserted into the internal jugular vein of each patient (Figure 3) and stopcocks were attached to each hub. Patients were randomly allocated either the PosiFlow® NC or standard luer caps to be attached to all ports of the stopcocks.
- After 72 hours *in situ* the stopcocks complete with PosiFlow® NC or standard luer caps were aseptically removed and sent for microbiological assessment.

Microbiological Assessment

- The external silicone compression seal of each PosiFlow® NC was imprinted ten times onto the surface of a 7% blood agar plate (Oxoid, Basingstoke, UK) (Figure 4).
- The seals were then disinfected with 70% isopropyl alcohol (Steretz®, Seton Prebble Ltd, Merseyside, UK). The internal microbial contamination rate of the NCs was assessed by flushing with 100 microlitres of brain heart infusion broth (Oxoid, Basingstoke, UK), which was subsequently cultured onto blood agar.
- Stopcock entry ports were sampled by inserting a small sterile swab and rotating through 360° ten times.
- Microorganisms were identified by standard laboratory techniques.
- All samples were inoculated onto 7% blood agar and incubated at 37°C for 48 hours in air.





4 Results

- None of the 77 patients who took part in the study had clinical or microbiological evidence of a CVC-related infection during the study period.
- Of 306 stopcock entry ports with standard caps, 18% were contaminated with microorganisms, in comparison 18 out of 274 (6.6%) lues with PosiFlow® NCs attached were contaminated (p=0.0001, Fisher's Exact Test) (Figure 5).
- The number and types of microorganisms recovered from stopcock entry ports with either PosiFlow® or standard luer caps attached is given in Table 1.
- The microbial contamination rates associated with the PosiFlow® NC following disinfection with one of the three disinfectants is given in Figure 6.
- Of those PosiFlow® NCs disinfected with isopropyl alcohol, 59.2% were externally contaminated with microorganisms compared to 30.8% disinfected with chlorhexidine/alcohol (p=0.0011) and 25% with povidone-iodine (p=0.0011).
- The number and types of microorganisms recovered from the external compression seal and internal surface of the PosiFlow® NC following disinfection with either 70% isopropyl alcohol, 0.5% chlorhexidine / alcohol or 10% povidone-iodine are given in Table 2.

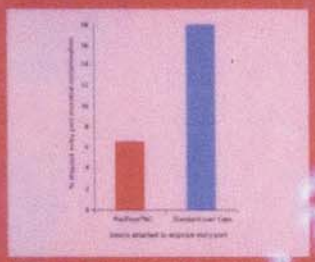


Fig 5 Microbial contamination rates of stopcock entry ports with PosiFlow® NC or standard Luer Caps attached.

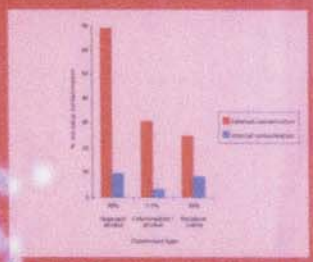


Fig 6 PosiFlow® NC microbial contamination rates following decontamination with 70% isopropyl alcohol, 0.5% chlorhexidine or 10% aqueous Povidone-iodine.

Table 1

	PosiFlow®	Standard Luer Caps
Mean cfu (range)	5 (1–22)	26 (1–810)
Micro-organisms isolated	CNS, Gram-negative and postive bacilli Neisseria spp.	CNS, Gram-negative and postive bacilli, S. Aureus, Neisseria spp and yeasts

cfu= colony-forming unit
CNS = Coagulase negative staphylococci

Table 1 Microorganisms recovered from stopcock entry ports with either PosiFlow® NCs or Standard Luer Caps.

Table 2 Microorganisms recovered from the PosiFlow® NC following disinfection with 0.5% chlorhexidine / alcohol, 70% isopropyl alcohol or 10% povidone-iodine.

Table 2

	70% Isopropyl Alcohol	0.5% chlorhexidine alcohol	10% povidone-iodine
External contamination Mean cfu (range)	18 (1–138)	9 (1–98)	12 (1–121)
Internal contamination Mean cfu (range)	3 (1–13)	1 (1–2)	4 (1–16)
Micro-organisms isolated	CNS, Gram-negative & postive bacilli Neisseria spp and S. aureus	CNS, Neisseria spp and yeasts	CNS, Gram-negative & postive bacilli Neisseria spp and S. aureus

5 Conclusions

- The use of NCs may reduce the rate of catheter-related bloodstream infections acquired via the intra-luminal route.
- NCs may reduce hub and stopcock entry port contamination rates associated with intravascular catheters.
- Disinfection of NCs with 0.5% chlorhexidine / alcohol or 10% aqueous povidone-iodine is more effective than 70% isopropyl alcohol.

6 References

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7. **Casey AL, Spare MK, Worthington T *et al.*** Needleless connectors – the way forward in the prevention of catheter-related infections? *Journal of Hospital Infection*. 2002; 50: 77–79.

This work was supported by BD

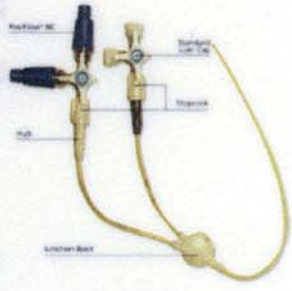

Fig 7 Triple lumen central venous catheter complete with stopcocks. Left to Right: PosiFlow® NCs and Standard Luer Caps attached to the entry ports.

Does characterisation of coagulase-negative staphylococci by routine microbiological techniques mislead the diagnosis of catheter-related bloodstream infection?

Aston University

University Hospital Birmingham NHS Trust

A Casey[1], T Worthington[1], P Lambert[2] and T Elliott[1]
Department of Clinical Microbiology and Infection Control[1], University Hospital Birmingham NHS Trust, UK and School of Life and Health Sciences[2], Aston University, UK

Introduction

- Intravascular catheters are an integral part of the management of patients in modern clinical practice
- The primary cause of hospital-acquired bacteraemia is intravascular catheter-related blood stream infections (CR-BSI)[1]
- Coagulase-negative *staphylococci* (CoNS) are the most frequent cause of central venous CR-BSI, accounting for more than one third of such infections[2]
- Diagnosis of CR-BSI is complicated, since CoNS recovered from specimens may represent contamination from the skin surface
- Confirmation of the identity of CoNS is commonly based on biochemical profiles and antibiogram
- Microbiological diagnosis of CR-BSI is based on the recovery of identical microorganisms (species and antibiogram) from the catheter tip and blood culture[3]
- Recent reports suggest that multiple strains of CoNS are associated with CR-BSI[4,5]

Aims

- To characterise strains of CoNS recovered from catheter tips and blood cultures by biotyping, antibiograms and pulsed-field gel electrophoresis (PFGE)
- To determine the discriminatory power of phenotypic and genotypic methods for characterisation of CoNS
- To determine if multiple strains of CoNS are associated with CR-BSI

Methods

A) Routine identification of CoNS

- Eight bone marrow transplant patients with a clinical diagnosis of CR-BSI were recruited onto the study
- A single colony from the blood culture and catheter tip of each patient was identified as CoNS by routine microbiological methods
- Identical antibiograms obtained from patient isolates confirmed the clinical diagnosis of CR-BSI

B) Further assessment of CoNS

- For each patient, 8 representative colonial types of CoNS (3 from the blood culture and 5 from the catheter tip) were individually subcultured and incubated at 37°C for 24 hours to obtain pure cultures
- Eight isolates from each patient were characterised by antibiogram using the BSAC standardised disc diffusion method. The panel of antibiotics employed were: flucloxacillin, erythromycin, trimethoprim, fusidin, mupirocin (low and high concentration), tetracycline, gentamicin and clindamycin
- Isolates were biotyped using the API ID 32 STAPH (Biomerieux, France)
- Isolates were characterised genotypically by *SmaI* chromosome macrorestriction profiling using PFGE

Results

- Analysis of all isolates studied demonstrated that PFGE distinguished more strains of CoNS compared to biotyping or antibiograms (20, 10 and 10 respectively)
- Phenotypic and genotypic characteristics of all 8 isolates of CoNS correlated in only 4 out of 8 patients (tables and figures 1–4)
- Three out of 4 of these patients had identical microorganisms from both blood culture and catheter tip thus supporting the diagnosis of CR-BSI (tables and figures 1–3)
- Genotypic and phenotypic characteristics of CoNS recovered from the remaining 4 patients did not correlate (tables and figures 5–8)
- Characterisation of multiple colonies of CoNS recovered from blood and catheter tip cultures revealed that strains were heterogeneous in 5 out of 8 patients which did not support the clinical diagnosis of CR-BSI (tables and figures 4–8)

Tables & Figures

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Conclusions

- Characterisation of CoNS based on biotype and antibiogram lacks discriminatory power
- Characterisation by PFGE is highly discriminative
- Current routine methods of identifying and typing a single colony of CoNS may generate misleading results as multiple strains may be present in culture due to contamination or polyclonal infection[4,5]
- Genotypic typing techniques are essential in order to accurately compare strains of CoNS implicated in infection
- Multiple colonies of CoNS should be selected for characterisation from patients with CR-BSI

References