HOSPITAL ACQUIRED INFECTION

Use of semi-quantitative and quantitative culture methods and typing for studying the epidemiology of central venous catheter-related infections in neonates on parenteral nutrition

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To study the epidemiology – especially the impact of contaminated stopcocks – on central venous catheter (CVC) infection and catheter-related sepsis (CRS), semi-quantitative (SQ) and quantitative (Q) culture methods and typing of coagulase-negative staphylococci (CNS) were employed in 49 neonates with clinical signs of sepsis while receiving parenteral nutrition in the paediatric intensive care unit. The patients were divided into two groups according to stopcock contamination: group A consisted of 18 patients (36%) with contaminated stopcocks and group B consisted of 31 patients (64%) with sterile stopcocks. Five specimens were obtained from each patient, in addition to that from the stopcock: a swab taken from the skin surrounding the catheter puncture site; the CVC tip; the intradermal segment (IDC); and samples of parenteral fluid and blood. A total of 294 specimens (392 sites) was cultured and micro-organisms were identified. All CNS isolated were typed by biotyping, antibiogram, plasmid analysis and pulsed-field gel electrophoresis (PFGE), and the discriminatory power of the typing methods was compared. The CVC tips were infected in 25 patients (51%); 15 (83%) in group A and 10 (32%) in group B. Sepsis was detected in 24 neonates (49%), 13 in group A and 11 in group B. This was catheter-related in 15 patients (63%), 12 in group A and 3 in group B. CNS were recovered from 13 (52%) of 25 infected CVCs, nine in group A and four in group B. Sixty-five CNS isolates were recovered from these patients and belonged to 14 biotypes, 22 antibiograms, 22 plasmid profiles and 26 PFGE types. Typing showed that in six of nine patients in group A, CNS of the same type were recovered from the catheter tip and the stopcock, in one patient the catheter tip and skin isolates were the same and in two others the catheter tip isolates were different from stopcock and skin isolates. In all four patients in group B, different CNS types were recovered from CVC tips and skin. Bacteraemia was caused by CNS in 14 patients (58%), six in group A and eight in group B. Typing confirmed that nine cases (six in group A and three in group B) were catheter-related but five were not. SQ and Q culture methods and typing, especially by PFGE, allowed the study to determine that bacteria from contaminated stopcocks were frequently the source of CVC infection and CRS.

Introduction

Vascular access *via* a central venous catheter (CVC) is an essential element of modern medical care in neonatal intensive care units. CVCs are used for parenteral nutrition, administration of medication,

blood pressure monitoring and for taking blood. However, despite many advances in the design and care of these devices, infections continue to be associated with their use. Vascular catheters interrupt the protective barrier of the skin and enable micro-organisms to gain access to the bloodstream. Micro-organisms can cause local and systemic complications, from CVC infection to catheter-related sepsis (CRS). Many micro-organisms are associated with CVC

infections and CRS, the most common being coagulase-negative staphylococci (CNS) [1–6]. Parenteral nutrition containing lipids seems to be a particular risk factor; in two studies 20–30% of newborns became infected [1, 2].

The pathogenesis of CVC infections and CRS has not been resolved and is the subject of ongoing controversy. Infections caused by CNS are especially controversial. There are several potential sources of CVC infections and bacteraemia: the stopcock, the administered parenteral fluid, the skin flora spreading from the outer surface of the line into the vein and other sources have all been implicated [7–13]. To detect infection, CVC tips are usually cultured by a semi-quantitative (SQ) roll-plate method, as suggested by Maki and co-workers [11]. However, this method detects bacteria solely on the outer catheter surface. Since it has been suggested that the catheter lumen may also be an important reservoir of micro-organisms, quantitative (Q) methods have been designed by Cleri and others to reveal bacteria on the internal CVC surface, in the hub and in the stopcock [12–15].

Within-species differentiation of CNS poses considerable difficulties. CNS are a genetically heterogeneous group of organisms found on the skin as part of the normal microbial flora, as well as in stopcocks, catheter tips and elsewhere. Therefore, when CNS are identified as the cause of CVC infection and CRS, it is difficult to establish the source and route of transmission to guide infection control interventions. Studies of the epidemiology of CRS are further complicated by the fact that organisms isolated may be contaminants rather than pathogens. An effective typing scheme is required to clarify the epidemiology of CNS infections. Several methods, including extended antibiotic susceptibility testing (antibiogram), biotyping, phage typing, plasmid analysis, ribotyping, SDS-PAGE and Western blotting of whole-cell proteins, have been explored previously [16–18].

The aim of the present study was to investigate the epidemiology of CVC infections and CRS, and in particular to compare the discriminatory power of phenotypic (antibiogram and biotyping) and genotypic (plasmid analysis and pulsed-field gel electrophoresis) typing methods for CNS. To do this, stopcocks and other potential sites of bacterial access were cultured, patients were divided into two groups according to stopcock contamination and the impact of contaminated stopcocks on CVC tip infection and CRS was studied. Because many stopcocks were contaminated during 1992 and 1993, when 22 of the patients (11 each from group A and B) were studied, enhanced infection control measures (aseptic assembly of stopcocks by trained nurses, protection of stopcocks with semi-permeable dressings as used on the skin, changing of stopcocks every 3 days, and the use of irreversible valves to prevent retrograde flow of

parenteral fluid), were introduced, and the efficacy of these measures was evaluated in 27 patients (seven from group A and 20 from group B), treated during 1994 and 1995.

Patients and methods

Patients

The study was done in the paediatric neonatal intensive care unit (NICU) of the University Medical Centre, Ljubljana, Slovenia between Jan. 1992 and Jan. 1995. The unit has 15 beds located in three rooms. All neonates with clinical signs of sepsis whilst receiving parenteral nutrition *via* a CVC made of polyethylene, polyurethane or polyvinylchloride inserted percutaneously into a jugular, subclavian, femoral or umbilical vein were studied. Clinical evidence of sepsis comprised at least three of the following: fever $>38^{\circ}\text{C}$ or temperature instability, lethargy, irritability, bradycardia, apnoea, hypotension, acidosis, gastrointestinal disturbances, leukocytosis and elevated C-reactive protein (CRP) [1, 2]. A diagnosis of CRS was made if the patient had clinical evidence of sepsis, positive blood cultures and presence of local signs of catheter infection, or >15 cfu of bacteria of the same species and, in CNS, of the same genotype, either on the external or internal surface of the catheter tip with or without the above signs [1].

Forty-nine neonates were included in the study. They were divided into two groups: group A consisted of 18 neonates with contaminated stopcocks and group B included 31 neonates with sterile stopcocks. The subjects were matched by age, weight, surgery, receipt of lipids and duration of hospital stay. The mean age was 35 gestational weeks in both groups and the mean weight was 2.72 kg in group A and 2.49 kg in group B. Eleven patients (four in group A and seven in group B) underwent surgery. All patients were mechanically ventilated and received glucose, amino acids and catecholamines. Thirty-seven patients received lipids, seven did not and in five cases it was not known whether lipids were administered or not. Mean duration of hospital stay was similar for both groups: 37 days in group A and 28 days in group B. Parenteral fluid additives were prepared in an aseptic unit of the hospital pharmacy. The catheters were inserted aseptically by attending physicians. All catheters were triple-lumen four French (Arrow or Cook) with three-way stopcocks. One lumen was used for parenteral nutrition, the second for catecholamine administration and the third for taking blood. During catheterisation, the catheter sites were checked daily for evidence of infection in both groups. Every 48 h the catheter sites were cleaned with 0.05% aqueous chlorhexidine solution and disinfected with 0.5% chlorhexidine in 70% ethanol (tincture of chlorhexidine) and the dressings were changed. The infusion sets were changed every 24 h in neonates receiving lipids

and every 48 h in all others. The luer connectors were disinfected with tincture of chlorhexidine after each manipulation. Enhanced infection-control measures – aseptic assembly of stopcocks by trained nurses, protection of stopcocks with semi-permeable dressings, change of stopcocks every 3 days and the use of irreversible valves to prevent retrograde flow of parenteral fluid – were introduced during the second part of the study.

The groups differed in terms of the duration of catheterisation, catheter insertion site, number of consecutive catheters inserted and underlying illnesses. The catheters remained in place for a mean of 9.7 days in group A and 6.4 days in group B ($t = 3.15$, $p = 0.002$). Twenty-six catheters were inserted into jugular, subclavian or femoral veins (14 in group A and 12 in group B) and 23 into umbilical veins (four in group A and 19 in group B). Neonates in group A had a mean of 2.3 CVCs inserted compared with neonates in group B, who had a mean of 1.4 CVCs ($t = 3.96$, $p = 0.0003$). The underlying diseases included respiratory distress in 27 neonates (eight in group A and 19 in group B), congenital malformations in 17 (six in group A and 11 in group B), renal insufficiency in two in group A, haemorrhagia and ileus in one patient each in group A and duodenal perforation in one patient in group B.

Catheter and blood cultures

Central venous catheters were removed after skin cleansing with chlorhexidine 0.05% solution and disinfection with chlorhexidine 0.5% in ethanol 70% for 2 min and divided into a 1-cm catheter tip and a 1-cm intradermal segment (IDC) in both groups. Six specimens were prepared from each patient: the stopcock, a swab taken from the skin surrounding the catheter puncture, the CVC tip and intradermal segment (IDC), a 10-ml sample of parenteral fluid and a 1-ml blood sample, which was taken from a peripheral vein site *via* a closed system for taking blood. The skin swab was taken before the skin was cleaned and disinfected, and was immersed into brain heart broth containing Tween 80 (Difco) 3%, lecithin (Merck) 0.3%, histidine (Difco) 0.1% and thiosulphate 0.5% to inactivate the disinfectant remaining on the skin. Parenteral fluid consisting of glucose, amino acids and lipids was aspirated from the parenteral fluid bag after disinfection of the bag. A total of 294 specimens (392 sites) was obtained and cultured by SQ and Q methods. The stopcocks were flushed with 1 ml of Trypticase Soy Broth (Gibco) 3% according to Cleri's Q method and agitated vigorously to dislodge most of the adherent micro-organisms. The broth was diluted 1 in 10 and 1 in 100 with fresh broth and seeded on to a blood agar plate (Difco) [12]. Parenteral fluid samples and skin swabs were cultured similarly. The catheter tips and IDCs were cultured according to Maki's SQ method, each segment being rolled back and forth across the

surface of a chocolate blood agar (Oxoid) plate [11]. Subsequently the catheter tips and IDCs were flushed. The plates were incubated at 37°C for 48 h in an aerobic atmosphere. Blood was sampled from the peripheral vein after the skin had been cleaned and disinfected twice with tincture of chlorhexidine and was cultured by a standard procedure [13]. A stopcock was considered to be contaminated if ≥ 15 cfu were found in it. A parenteral fluid was considered to be contaminated if any growth was detected [14]. A catheter was considered to be colonised if ≥ 15 cfu were recovered from the outer surface of its tip [11]. Because catheter segments were very short, 15 cfu and not 1000 cfu, as suggested by Cleri *et al.*, were taken as the cut-off for catheter lumens considered to be colonised [12, 13]. Morphologically distinct colonies from all investigated sites were identified. Coagulase-negative staphylococci were subsequently typed.

Bacterial typing

Four typing methods were used and their discriminatory power was compared. Two methods were phenotypic (extended antibiotic susceptibility testing and biotyping) and two were genotypic (plasmid analysis and pulsed-field gel electrophoresis).

Extended antibiotic susceptibility testing. Extended antibiotic susceptibility testing of CNS isolates was performed on nutrient agar (Oxoid) by a comparative disk-diffusion method [18] with the following antibiotics: chloramphenicol 10 µg, lincomycin 2 µg, erythromycin 10 µg, tetracycline 10 µg, streptomycin 10 µg, penicillin G 1 IU, gentamicin 10 µg, kanamycin 30 µg, neomycin 10 µg, novobiocin 5 µg, fusidic acid 10 µg, rifampicin 5 µg, vancomycin 30 µg, ciprofloxacin 5 µg, mupirocin 5 µg and methicillin 10 µg. Sensitivity plates were read after incubation for 24 h at 35°C. Isolates were recorded as either sensitive or resistant and the results were recorded with the initials of those antibiotics to which bacteria were resistant (antibiogram). Bacteria were considered to be different if there was a difference in susceptibility to one or more antibiotics [18].

Biotyping. All CNS isolates were biotyped with the API ID 32 Staph system (bioMérieux, France) as described previously. They were considered to be different if there was at least one biochemical difference [19].

Plasmid analysis. Bacterial plasmids were isolated as described previously [20] and the gels were stained with ethidium bromide 1 mg/L for 30 min before viewing. Bacterial isolates were typed by plasmid number and mol. wt. Isolates were considered to be indistinguishable (had the same profile) if they possessed plasmids of identical mobility [20].

Pulsed-field gel electrophoresis (PFGE). Organisms

were grown on nutrient agar plates and resuspended in SE buffer (75 mM NaCl, 25 mM EDTA, pH 7.5). Chromosomal DNA was isolated, digested with *Sma*I and analysed by PFGE with a contour-clamped homogeneous electric field (CHEF) II apparatus (Bio-Rad Lab, Hercules, CA, USA) as described previously [21]. A difference of three bands or more was used for strain discrimination [21, 22]. Isolates belonging to the same type or strain had identical PFGE patterns, isolates that differed by one to three fragments were considered subtypes and isolates that differed by more than three fragments were considered to represent different strains [22].

Interpretation of isolate identification and typing results

Bacteria isolated from different sites (stopcocks, skin swabs, outer and inner surfaces of CVC tips and IDCs, parenteral fluid and blood) of each patient were considered identical if they belonged to the same species, and for CNS, to the same biotype, antibiogram, plasmid DNA profile and PFGE type. The discriminatory power of any typing method was considered superior to other methods when it distinguished more types for the same collection of isolates. An episode of sepsis was considered catheter-related if micro-organisms isolated from peripheral blood cultures were the same species, and for CNS, were the same type as those recovered from the catheter tip [23–27].

Statistical analysis

Comparison of numerical results for groups A and B was performed with the unpaired Student's *t* test. Comparison of attributive results was done with the χ^2 test.

Results

Two hundred and ninety-four specimens (392 sites) were cultured from 49 neonates on parenteral nutrition with clinical signs of sepsis (Tables 1 and 2). Subsequently the micro-organisms from these sites were identified and CNS isolates were compared by the four typing methods (Tables 3 and 4).

The stopcocks were contaminated in 18 (36%) of 49 neonates in group A (Table 1), and were sterile in 31 (64%) of 49 patients in group B (Table 2). In 10 patients the stopcocks were contaminated with >1000 cfu and in eight with 15–1000 cfu. Prolonged duration of catheterisation was a risk factor for stopcock contamination ($t = 3.15$, $p = 0.002$). Stopcocks of CVCs inserted into a jugular, subclavian or femoral vein were more likely to be contaminated than stopcocks of CVCs inserted into an umbilical vein ($\chi^2 = 8.26$, $p = 0.005$). However, umbilical catheters were inserted for a shorter period of time. The number of CVC insertions was also a risk factor for stopcock contamination ($t = 3.96$, $p = 0.0003$).

The catheter tips were colonised in 25 patients (51%),

Table 1. Bacteria cultured from the stopcock, outer surface of the catheter tip and intradermal segment, inner surface of the catheter tip and intradermal segment, parenteral fluid, skin and blood of 18 patients with contaminated stopcocks (group A)

Patient no.	Catheter/stopcock isolates	Stopcock	To	IDCo	Ti	IDCi	Fluid	Skin isolates	Bc isolates	Hospitalised before
1	SE	+++	++	+	+++	0	+++	0	SE*	+
2	SE,En	+	++	++	+	++	0	+++SE	SE*	+
3	SE	+	+	0	0	0	0	++SE	0	–
4	SE,SH	+++	0	+	0	0	+++	++SE	SE,SH*	+
5	SE,SH	+++	0	0	++	++	+++	0	Ssp*	+
6	SE	+++	+++	0	+++	0	NP	+++SE	SA	+
7	SE,SH	++	+	0	+	0	0	0	SE*	–
8	SL	+++	++	0	+	0	0	+++SE	0	+
9	SA,SH	+++	++	+	+++	+++	+++†	+++SE	SASh*	–
10	Kl,EB	+++	0	0	+	+++	NP	0	Kl,EB*	+
11	Cand,En	+++	+++	+++	+++	+++	+++	+++En	Cand*	–
12	Cand	+	++	++	+++	NP	+++	+++Cand	Cand*	–
13	Bacil,Cor	+++	+	0	0	+	+++	0	Bacil*	–
14	Bacil	+++	+++	+++	+++	+++	+++	0	Bacil*	–
15	Cand	++	++	++	++	++	NP	0	Cand*	+
16	Cand	+	0	0	0	0	0	0	0	+
17	SE	++	0	0	0	0	0	0	0	+
18	SH	+	0	0	0	0	0	+SE	0	+
Total	15/18	18	12	8	12	8	8	9	13	11

To, catheter tip, outer surface; IDCo, intradermal segment, outer surface; Ti, catheter tip, inner surface; IDCi, intradermal segment, inner surface; Fluid, parenteral fluid; Skin, skin at catheter puncture site; Bc, blood culture from peripheral vein; 0, no growth; +, 15–100 cfu; ++, 100–1000 cfu; +++, 1000 or more cfu/catheter segment or in 1 ml fluid; SE, *S. epidermidis*; SH, *S. haemolyticus*; SL, *S. lugdunensis*; SA, *S. aureus*; Ssp, *Staphylococcus* sp.; Kl, *Klebsiella* sp.; EB, *Enterobacter* sp.; En, *Enterococcus* sp.; Cor, *Corynebacterium* sp.; Bacil, *Bacillus cereus*; Cand, *Candida albicans*; NP, not performed; Hospitalised before: +, before infection control measures; –, after infection control measures.

*Sepsis catheter-related.

†No lipids given.

Table 2. Bacteria cultured from outer surface of the catheter tip and intradermal segment, inner surface of the catheter tip and intradermal segment, parenteral fluid, skin and blood of 30 patients with sterile stopcocks (group B)

Patient no.	Catheter isolate	To	IDCo	Ti	IDCi	Fluid	Skin	Bc	Hospitalised before
1	SE,EC	0	+++	+++	+++	NP	+++SE	SE,EC*	+
2	SH	++	++	+++	+++	0†	+++SE	0	+
3	SE	++	+	0	0	0	+++SE	SE*	+
4	SE	++	0	+	0	0†	0	SE*	—
5	En	+++	0	0	0	0	+++SE	SE	+
6	En	+++	0	0	0	0†	+++En,Kl	0	+
7	SA	0	0	+	0	0	+SA	Ac	—
8	Cor	+	0	0	0	0	++SH	0	—
9	Ps	++	0	+	0	0	+++Ps	0	—
10	Str	+	0	0	0	0	0	0	—
11	0	0	0	0	0	0	+++SE	SE	+
12	0	0	0	0	0	NP	0	0	+
13	0	0	0	0	0	0	++SE	0	+
14	0	0	0	0	0	0	+++Ps	SE,Cand	+
15	0	0	0	0	0	0	+En,SE	0	+
16	0	0	0	0	0	0	0	0	—
17	0	0	0	0	0	0	0	0	—
18	0	0	0	0	0	0	0	0	—
19	0	0	0	0	0	0	++SH	0	—
20	0	0	0	0	0	0	+SE,En	SE	—
21	0	0	0	0	0	0†	+SH	0	—
22	0	0	0	0	0	0	+++SE	0	—
23	0	0	0	0	0	0	0	0	—
24	0	0	0	0	0	0	0	0	—
25	0	0	0	0	0	0	0	0	—
26	0	0	0	0	0	0	0	0	—
27	0	0	0	0	0	0	++SH	0	—
28	0	0	0	0	0	0	0	0	—
29	0	0	0	0	0	0†	+SE	SE	—
30	0	0	0	0	0	0	0	Bacil	—
31	0	0	0	0	0	0†	0	G-bac	—
Total positive	10	8	3	5	2	0	18	11	10

See footnotes to Table 1.

15 (83%) in group A and 10 (32%) in group B, and this difference was statistically significant ($\chi^2 = 11.6$, $p = 0.0005$). Twenty-six bacterial isolates were isolated from 15 CVC tips in group A and 11 from 10 CVC tips in group B. Mixed bacterial colonisation of the CVC tip was present in eight patients in group A and in one patient in group B ($\chi^2 = 18.3$, $p = 0.001$). Twelve patients in group A had bacteria on the outer CVC tip surface, two only on the inner CVC tip surface and one only on the IDC. Eight patients in group B had bacteria on the outer CVC tip surface and two only on the inner surface. Group A had significantly higher colonisation rates for outer ($\chi^2 = 9.5$, $p = 0.02$) and inner catheter tip surfaces ($\chi^2 = 16$, $p = 0.01$) than group B. The rate of IDC contaminations was comparable to the rate of catheter tip colonisation. In all 15 patients in group A, the catheter tips were colonised with bacteria of the same species as those from the stopcocks. Of the 25 colonised CVCs, 18 were jugular, subclavian or femoral (68% colonised), and seven were umbilical (30% colonised). Parenteral fluid mixture was contaminated in eight patients in group A and in none in group B (Tables 1 and 2). Bacteria were found on the skin surrounding the puncture site of 27 patients (9 in group A and 18 in group B); the difference between groups A and B was significant ($\chi^2 = 3$, $p = 0.001$).

They were similar to those isolated from catheters in six patients in group A and in five in group B (Tables 1 and 2).

Blood cultures were positive in 24 (49%) of the 49 patients. All the patients had systemic signs of sepsis, which responded to catheter removal and antibiotics. Sepsis was significantly more common in group A (13 patients, 72%) than in group B (11 patients, 32%) ($\chi^2 = 7.8$, $p = 0.005$). It was catheter-related in 15 patients (63%) (12 in group A and three in group B) ($\chi^2 = 46.2$, $p = 0.0005$). In the remaining nine (37%) patients (one in group A and eight in group B) it was not catheter-related; in three patients there were different species on the CVC tip and in six patients, CVCs were not colonised. In these patients the bacteria in blood could have been the consequence of haematogenous seeding from another, distant source, or blood cultures may have been contaminated (Tables 1 and 2).

CNS caused catheter tip infections in 13 neonates (52%), nine in group A and four in group B. Catheter tip infections with CNS were significantly more common in group A than in group B ($\chi^2 = 38$, $p = 0.0005$). CNS were isolated from the blood of 14 patients (58%) with sepsis, six in group A and eight

in group B. Sixty-five CNS isolates were obtained from 53 of 104 cultured sites of 13 neonates with CNS infection of CVC tip, and these belonged to 14 biotypes, 22 antibiograms, 22 plasmid profiles and 26 PFGE types (from A to Y) (Tables 1–4). Fifty CNS isolates – belonging to 12 biotypes, 19 antibiograms,

17 plasmid profiles and 20 PFGE types – were cultured from nine patients in group A (Table 3). Fifteen CNS isolates – belonging to four biotypes, seven antibiograms, six plasmid profiles and seven PFGE types – were from four patients in group B; some of them were identical to those in group A

Table 3. Biotypes, antibiograms, plasmid profiles and PFGE types of CNS cultured from stopcocks, catheters, parenteral fluid, skin and blood of nine patients in group A (source shown in Table 1)

Patient no.	Isolate, site, species	Biotype (type)	Antibiogram	Plasmids in kb (profile)	PFGE pattern-type
1	1 To, SE	16203220	(1) PGKF	(1) >23,8,4,2	(1) A
	2 IDCo, SE	16203220	(1) PGKF	(1) >23,8,4,2	(1) A
	3 Ti, SE	16203220	(1) PGKF	(1) >23,8,4,2	(1) A
	4 Stopc., SE	16203220	(1) PGKF	(1) >23,8,4,2	(1) A
	5 Fluid, SE	16203220	(1) PGKF	(1) >23,8,4,2	(1) A
2	6 Bc, SE	16203220	(1) PGKF	(1) >23,8,4,2	(1) A
	7 IDCo, SE	16603220	(2) CLEPGKM	(2) 16,6,4,2	(2) B
	8 Ti, SE	16603220	(2) CLEPGKM	(2) 16,6,4,2	(2) B
	9 IDCi, SE	16603220	(2) CLEPGKM	(2) 16,6,4,2	(2) B
	10 Stopc., SE	16603220	(2) CLEPGKM	(2) 16,6,4,2	(2) B
3	11 Bc, SE	16603220	(2) CLEPGKM	(2) 16,6,4,2	(2) B
	12 Skin, SE	16603220	(2) CPGKM	(3) >23,2	(3) C
	13 To, SE	16603220	(2) LEPGKM	(4) >23,2	(3) C
	14 Skin, SE	16603220	(2) LEPGKM	(4) >23,2	(3) C
	15 Stopc., SE	16603220	(2) PM	(5) 0	(0) D
4	16 Bc, SE	16603220	(2) PGKM	(6) 16,2	(4) E
	17 Bcc., SH	26213460	(3) LESGKRM	(7) 12,7	(5) F
	18 IDCo, SE	36603261	(4) P	(8) 12	(6) G
	19 Skin, SE	16603220	(2) LEPGK	(9) 16,2	(4) H
	20 Stopc., SE	16603220	(2) PGKM	(6) 16,2	(4) E
5	21 Fluid, SE	16603220	(2) PGKM	(6) 16,2	(4) E
	22 Ti, SH	26632464	(5) ESPGKNRM	(10) 2	(7) I
	23 IDCi,SH	26632464	(5) ESPGKNRM	(10) 2	(7) I
	24 IDCi,SE	16603220	(2) PGKRF	(11) >23,>23,<23	(8) J
	25 Stopc., SC	06232210	(6) CLTPFNM	(12) >23,>23,<23	(9) NP
6	26 Stopc., SE	36603220	(7) LEPGKNM	(13) 23,7,2	(10) K
	27 Fluid, SH	26632464	(5) PGKNM	(14) 2	(7) L
	28 Fluid, SE	36603220	(7) LEPGKNM	(13) 23,7,2	(10) K
	29 Bc, Ssp	16636021	(8) P	(8) 4	(11) M
	30 Skin, SE	16603220	(2) CLEPGKM	(2) 18,16,6,4,2	(12) N
7	31 To, SE	16603220	(2) CLEPGKM	(2) 16,6,4,2	(2) B
	32 Stopc., SE	16603220	(2) CLEPGKM	(2) 9,4,3,2	(13) N
	33 Ti, SE	16603220	(2) CLEPGKM	(2) 9,4,3,2	(13) NP
	34 To, SE	16601221	(9) LETPGKNM	(15) >23,<23,2	(14) O
	35 Ti, SE	16601221	(9) LETPGKNM	(15) >23,<23,2	(14) O
8	36 Stopc., SE	16601221	(9) LETPGKNM	(15) >23,<23,2	(14) O
	37 Bc, SE	16601221	(9) LETPGKNM	(15) >23,<23,2	(14) O
	38 Ti, SH	06613461	(10) LETP	(16) 5,2	(15) P
	39 To, SL	56713460	(11) C	(17) 3	(16) Q
	40 Ti, SL	56713460	(11) C	(17) 3	(16) Q
9	41 Stopc., SL	56713460	(11) C	(17) 3	(16) Q
	42 Skin, SE	16601221	(9) LETPGKNM	(15) >23,<23,2	(14) R
	43 To, SH	26733460	(12) CESPKNFM	(18) 2	(7) S
	44 IDCo,SH	26733460	(12) CESPKNFM	(18) 2	(7) S
	45 Ti, SH	26733460	(12) CESPKNFM	(18) 2	(7) S
Total	46 IDCi,SH	26733460	(12) CESPKNFM	(18) 2	(7) S
	47 Stopc.,SH	26733460	(12) CESPKNFM	(18) 2	(7) S
	48 Fluid, SH	26733460	(12) CESPKNFM	(18) 2	(7) S
	49 Bc, SH	26733460	(12) CESPKNFM	(18) 2	(7) S
	50 Skin, SE	16603220	(2) PGNM	(19) 8,3,2	(17) T
Total		50	12	19	17 20

To, catheter tip, outer surface; IDCo, intradermal segment, outer surface; Ti, catheter tip, inner surface; Ii, intradermal segment, inner surface; Stopc., catheter stopcock; Fluid, parenteral fluid; Skin, skin at the catheter puncture site; Bc, blood culture from peripheral vein; SE, *S. epidermidis*; SH, *S. haemolyticus*; SC, *S. cohnii*; Ssp, *Staphylococcus* spp.; SL, *S. lugdunensis*; antibiogram indicated by the initials of the antibiotics to which bacteria were resistant: C, chloramphenicol; L, lincomycin; E, erythromycin; T, tetracycline; S, streptomycin; P, penicillin G; G, gentamicin; K, kanamycin; N, neomycin; Nov, novobiocin; F, fusidic acid; R, rifampicin; Va, vancomycin; Cip, ciprofloxacin; Mup, mupirocin; M, methicillin. Plasmids in kb (profile), number and mol. wt of plasmids; PFGE pattern-type, type obtained by PFGE of chromosomal DNA based on more than three-band difference rule; NP, not performed.

(Tables 3 and 4). PFGE provided the best discrimination among strains of the same species.

A single CNS species was present at different sites in six patients in group A (nos. 1, 2, 3, 6, 8, 9): four patients had *S. epidermidis*, one *S. lugdunensis* and one *S. haemolyticus*. Three patients (nos. 4, 5, 7) had two or more CNS species. Four patients with a single CNS species (nos. 1, 2, 8, 9) had a single type, whereas two patients (nos. 3 and 6) had several genotypes. In six patients (nos. 1, 2, 4, 7, 8, 9) CNS on the CVC tips were of the same type as those present in stopcocks, and in one patient (no. 3) this was the same type as on the skin. Six patients (nos. 1, 2, 4, 5, 7, 9) had CNS in blood and in five of them they were the same type as in the stopcocks (Tables 1 and 3, Figs 1 and 2).

In group B, all four patients with CNS tip colonisation had a single species of bacteria: three patients had *S. epidermidis* and one *S. haemolyticus*. In three patients (nos. 2–4), a single type was present at different sites. One patient (no. 1) with a single species had two different CNS types. CNS on CVC tips were different from those on the skin. Of three patients who had CNS in blood (nos. 1, 3, 4), all had *S. epidermidis*. In all three, bacteria isolated from blood were of the same type as on catheter tips. In five patients in group B, CNS were isolated from blood in the absence of CVC infection with CNS (Tables 2 and 4, Fig. 1).

More neonates with stopcock contamination, CVC tip infection and CRS were hospitalised before enhanced infection control measures were introduced. Following this, there was a significant decrease in the rate of stopcock contamination ($\chi^2 = 9.7$, $p = 0.005$) and catheter tip infection ($\chi^2 = 5.3$, $p = 0.05$), but the decrease in the rate of sepsis was not statistically significant ($\chi^2 = 0.59$, $p > 0.05$) (Tables 1 and 2).

Discussion

There are many different theories on the mechanisms of CVC infection caused by CNS. Maki and colleagues [11], who mainly studied peripheral venous catheters in adults, described the migration of bacteria, especially CNS, from the patient's skin along the outer surface of the catheter, with subsequent colonisation. Later Collignon [24], Johnson [25] and others suggested that the mechanisms of CVC infection might be different from those involved in peripheral venous catheter infection. In 1985, Linares and colleagues [13] identified the catheter hub as the origin of CVC infection. Tebbs *et al.* [8] showed that CVC infection may originate in the stopcock, which can be contaminated as a result of frequent manipulations. In neonates, Salzman and colleagues [7] found CNS in catheter hubs and concluded that this might be the source of infection in 70% of neonates with CRS. However, none of these studies used the molecular or genotypic methods required to prove that CNS isolated from a stopcock, patient's skin or CVC tip were clonally related. Widmer [6] pointed out the need to type bacteria from different catheter segments to determine the importance of catheter hub and skin as the origin of catheter sepsis. Also, few studies published to date have used both SQ and Q methods for the cultivation of CVC, and typing of CNS from different catheter segments has rarely been employed to identify the source of CRS in neonatal units [4, 26–29].

The present study investigated the epidemiology of CVC infections and CRS, i.e., whether micro-organisms from contaminated stopcocks or from other potential sites of bacterial access infect catheter tips and cause CRS, and whether infection-control measures can reduce infections. The rate of stopcock contamination observed in this study was 36%, somewhere between those of Tebbs *et al.* [8] and

Table 4. Biotypes, antibiograms, plasmid profiles and PFGE types of CNS cultured from catheters, skin and blood of four patients in group B (source is shown in Table 2)

Patient no.	Isolate, site, species	Biotype (type)	Antibiogram	Plasmids in kb (profile)	PFGE pattern-type
1	51 Bc, SE	16603220	(2) CLEPGKM	(2) 16,6,4,2	(2) B
	52 IDCc, SE	16603220	(2) LEPGKM	(4) 8,2	(18) U
	53 Ti, SE	16603220	(2) CLEPGKM	(2) 16,6,4,2	(2) B
	54 Skin, SE	16603220	(2) LEPGKM	(4) 8,2	(18) V
2	55 To, SH	26613465	(13) ETSPGK	(20) >23	(19) W
	56 IDCc, SH	26613465	(13) ETSPGK	(20) >23	(19) W
	57 Ti, SH	26613465	(13) ETSPGK	(20) >23	(19) W
	58 IDCc, SH	26613465	(13) ETSPGK	(20) >23	(19) W
	59 Skin, SE	16603220	(2) PM	(5) >23,23	(20) Z
3	60 Skin, SE	16603231	(14) LETPKM	(21)	NP NP
	61 Ti, SE	16603220	(2) LETPGKNM	(15) 5,3	(21) X
	62 Bc, SE	16603220	(2) LETPGKNM	(15) 5,3	(21) X
4	63 To, SE	16601221	(9) CLESPGKNM	(22) 12,8,5,2	(22) Y
	64 Ti, SE	16601221	(9) CLESPGKNM	(22) 12,8,5,2	(22) Y
	65 Bc, SE	16601221	(9) CLESPGKNM	(22) 12,8,5,2	(22) Y
Total	15		4	7	6 7

See footnote to Table 3.

Fig. 1. PFGE patterns of *S. epidermidis* and *S. haemolyticus* isolates from patients 1A, 1B and 2B. Lanes 7, 12, 18, *S. epidermidis* control strain which is identical to the strain from patient 1A. The patient numbers and the numbers of isolates refer to those in Tables 1-4. Lanes 1-6, pattern A representing *S. epidermidis* isolates 1-6 from outer catheter tip surface, intradermal segment outside, catheter tip inside, stopcock, parenteral fluid and peripheral blood from patient 1A; 8-11, *S. epidermidis* isolates from patient 1B: 8, pattern B representing isolate 51 from blood; 9, pattern U representing isolate 52 from intradermal catheter outside; 10, pattern B representing isolate 53 from catheter tip inside; 11, pattern V representing isolate 54 from skin; 13-17, *S. haemolyticus* and *S. epidermidis* isolates from patient 2B: 13-16, *S. haemolyticus* isolates 55-58 from catheter tip outside, intradermal segment outside, catheter tip inside and intradermal segment inside; 17, pattern Z representing isolate 59 of *S. epidermidis* from skin; 19, marker λ concatamer.

Brosnan *et al.* [9], who reported stopcock contamination rates of 22% and 51%, respectively. CVC tip infections were more common in the patients with contaminated stopcocks in the present study ($\chi^2 = 11.6$, $p = 0.0005$). The bacterial species found on the catheter tips corresponded more frequently to those found in the stopcocks than to those found on the skin. De Cicco *et al.* [27] observed that 45% of infected CVC tips harboured the same bacterial species as were found in the stopcocks. Infection in the patients with contaminated stopcocks was more frequently due to several species and genotypes than in patients with uncontaminated stopcocks ($\chi^2 = 18.3$, $p = 0.001$) (Tables 1-4). The use of a combination of SQ and Q methods and a cut-off for infection of 15 cfu with both, demonstrated that not all catheter infections are detectable with the SQ roll-plate method alone, confirming the results of Sherertz *et al.* [28]. The parenteral fluid was contaminated in almost half the patients with contaminated stopcocks, probably as a result of retrograde flow, but in none of the patients whose stopcocks were sterile. A similar observation was made by Goldmann and Pier [10].

Twenty-four (49%) of the patients had sepsis, a relatively high rate, but the patients studied were at high risk of infection. Ogata *et al.* [1] also described sepsis in 30% of neonates on parenteral nutrition containing lipids, and Wenzel *et al.* [29] recorded that 50% of neonates on parenteral nutrition had clinical signs of sepsis. In 15 (63%) of the patients with sepsis (12 of 13 in group A and three of 11 in group B), sepsis was catheter-related. CRS was more frequent in group A, confirming that bacteria from stopcocks were involved in sepsis ($\chi^2 = 46.2$, $p = 0.0005$). In nine patients (37%), the bacteria isolated from blood were different from the organisms recovered from the stopcocks or CVC tips. In these patients bacteria in the blood may have arisen from haematogenous seeding from another, distant source, or blood cultures may have been contaminated. Norwood *et al.* [30] found a high rate of haematogenous seeding of catheters in intensive care units, especially with yeasts, enterococci and gram-negative bacteria, but Anaisse *et al.* [31] reported a corresponding rate of only 10%. In the present study, sepsis was polymicrobial in five (20%) patients (three in group A and two in group B).

Fig. 2. Plasmid profile of *S. epidermidis* isolates from patient 1A. Lanes 1–6, plasmid profile 1 consisting of four plasmids and representing *S. epidermidis* isolates 1–6 from outer catheter tip surface, intradermal segment outside, catheter tip inside, stopcock, parenteral fluid and peripheral blood (source shown in Tables 1 and 3); M, λ Hind III marker.

This is similar to the study of Decker and Edwards [5], who found polymicrobial sepsis in 12% of patients with CVC.

The typing methods used for CNS in this study varied in their discriminatory power. The discriminatory power of biotyping was poor, but the antibiogram obtained by extended antibiotic susceptibility testing to 16 antibiotics was much more discriminatory than the routine antibiogram; it was as discriminatory as plasmid analysis (Tables 3 and 4). Plasmid analysis had reasonable discriminatory power and plasmid profiles were described by Ludlam *et al.* as being stable during subsequent preparations [18]. PFGE based on a difference of more than three bands provided the best discrimination among strains of the same species and is recommended as the method of choice for typing species recovered from catheter infections [21, 22].

Speciation and typing of CNS are important in the routine management of neonates with sepsis. In the present study typing revealed the importance of stopcocks in catheter infection and CRS caused by CNS. In group A, CNS on CVC tips originated from stopcocks in six of nine patients and from skin in one, while in two patients the origin remained unknown. In group B, the origin of CVC infection remained unknown in all four patients, as the bacteria cultured

from the CVC tips differed from those found on the skin. It is well known that the skin flora of patients can change during hospital stay and that mixed infections with several types of bacteria of the same species may occur. In some of the patients, CVC contamination at the time of insertion was a possible source of catheter infection and CRS. However, most of the results suggest that the catheter stopcock is a more likely origin of CVC tip infection and CRS than the patient's skin.

CNS cultured from the blood of adults are often regarded as contaminants. In neonates with CVC, St Gemme *et al.* [23] found 65% of CNS blood isolates to be clinically important, while the corresponding rate reported by Nataro *et al.* [32] was 42%. In the present study, nine (64%) of the 14 patients with CNS in their blood had the same bacteria present on their CVC tips, but the remaining five had uninfected CVCs, similar to the results obtained by St Gemme *et al.* [23].

Semi-quantitative and quantitative methods of cultivation and typing demonstrated that in neonates on parenteral nutrition, bacteria from contaminated stopcocks caused CVC infection and CRS, rather than bacteria from the skin. PFGE, based on a difference of more than three bands, provided the best discrimination among strains of the same CNS species, and antibiogram and plasmid analysis were also discriminatory. Finally, the incidence of stopcock contamination, CVC tip infection and sepsis decreased when enhanced infection control measures were implemented.

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