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Molecular epidemiology of catheter-related bloodstream infections caused by coagulase-negative staphylococci in haematological patients with neutropenia

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SUMMARY

Catheter-related bloodstream infection (CRBSI) caused by coagulase-negative staphylococci (CNS) is common in haematological patients with febrile neutropenia. As the clinical signs of CRBSI are usually scarce and it is difficult to differentiate from blood culture contamination, we tried to confirm CRBSI by molecular typing of CNS isolated from paired blood cultures (one from a peripheral vein and another from the central venous catheter hub). Blood cultures were positive in 59 (36%) out of 163 patients. CNS were isolated in 24 (40%) patients; in 14 from paired blood cultures (28 isolates) and in 10 from a single blood culture. CNS from paired blood cultures were identified as *Staphylococcus epidermidis*. Antimicrobial susceptibility was determined and bacteria were typed by pulsed-field gel electrophoresis (PFGE) of bacterial genomic DNA. In 13 patients, the antibiotic susceptibility of isolates was identical. The PFGE patterns from paired blood cultures were identical or closely related in 10 patients, thus confirming the presence of CRBSI. In the remaining four patients they were unrelated, and suggested a mixed infection or contamination. Since CNS isolates from three patients had identical PFGE patterns, they were probably nosocomially spread amongst them.

INTRODUCTION

Catheter-related bloodstream infection (CRBSI) is among the most frequent of hospital-acquired bloodstream infections. In CRBSI, microorganisms gain access to the extraluminal or intraluminal surface of the catheter by one of the following mechanisms: (1) skin organisms invade the percutaneous tract, which is probably facilitated by capillary action at the time of insertion or later, (2) microorganisms contaminate the catheter hub and lumen when the catheter is manipulated, and (3) organisms are carried haematogenously to the central venous catheter (CVC) from a remote source of infection [1–3].

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Coagulase-negative staphylococci (CNS) account for 40% of all isolated microorganisms in primary bloodstream infections [4–6].

The diagnosis of CRBSI remains difficult, especially in haematological patients with febrile neutropenia. Clinical signs such as tenderness or purulent discharge at the insertion site, implicating the catheter as the source of infection, are frequently absent [7]. Nowadays, clinicians usually avoid removal of the catheter in patients with febrile neutropenia, which would permit semi-quantitative or quantitative catheter-tip culture, because reinsertion of a new CVC carries a substantial risk of bleeding [7, 8]. Currently, blood culture techniques involving paired blood cultures obtained simultaneously from a peripheral vein and the CVC hub

are frequently used for diagnosis of CRBSI if catheter removal is undesirable or impossible [9, 10].

As local signs of CRBSI in haematological patients with febrile neutropenia are usually absent and it is impossible to differentiate CRBSI caused by CNS from blood culture contamination without molecular typing, we tried to confirm that patients with CNS isolated from peripheral blood and from CVC hub blood had CRBSI by pulsed-field gel electrophoresis (PFGE) of bacterial genomic DNA.

METHODS

Sampling

In 2001, blood cultures were obtained from 163 patients (90 males, 73 females) with one or more episodes of febrile neutropenia (a neutrophil count of $<500/\mu l$) and an in-dwelling short-term non-tunelled CVC (placed into the subclavian vein or into the internal jugular vein) in place in the course of treatment of acute myelogenous leukaemia, acute lymphoblastic leukaemia, multiple myeloma and similar conditions, hospitalized at the Department of Haematology of the University Medical Centre in Ljubljana. Patients received prophylaxis with ciprofloxacin. During febrile episodes empirical therapy with ceftazidime and aminoglycoside was started and eventually modified according to culture results. All patients had two or more blood cultures sent; if CRBSI was suspected, paired blood cultures were obtained from a peripheral vein and from the CVC hub. Blood cultures were incubated in the automated blood culture system Vital (bioMérieux, Marcy l'Etoile, France).

Isolation of bacteria

Positive blood cultures were plated onto bacteriological media. The identity of isolates from peripheral blood cultures and CVC hub blood cultures was assessed on the basis of colonial morphology, species identification and an identical antibiogram. In CNS isolates, identity was additionally confirmed by PFGE [11, 12]. Bacterial isolates were identified to species level using conventional methods. CNS were identified by the ID 32 Staph System (bioMérieux) as specified by the manufacturer. The antimicrobial susceptibility of CNS to penicillin, oxacillin, erythromycin, clindamycin, trimethoprim/sulphamethoxazole, vancomycin, teicoplanin, rifampin, gentamicin and ciprofloxacin (Becton Dickinson, Sparks, MD, USA) was determined by the disk diffusion method as recommended by NCCLS [13].

Analysis of chromosomal DNA by PFGE

Bacteria were grown on nutrient agar plates and resuspended in SE buffer (75 mM NaCl, 25 mM EDTA; pH 7·5). Chromosomal DNA was isolated as previously described, digested with *Sma*I and analysed by PFGE in a contour-clamped homogenous electric field CHEF II apparatus (Bio-Rad Laboratories, Hercules, CA, USA) with pulse times from 1 to 45 s in a 45-h period [11]. A difference of three bands or more was used for strain discrimination. Isolates of CNS having identical PFGE patterns were identical and belonged to the same type or strain; isolates that differed by 1–3 fragments were subtypes and were considered to be closely related. Isolates that differed by more than three fragments were different strains and considered unrelated [12]. CRBSI was defined as proven identity or close relatedness of isolates from a peripheral vein and the CVC hub as confirmed by PFGE.

RESULTS

In 59 (36%) patients (31 males, 28 females), blood cultures were positive. Forty-nine patients had monomicrobial bacteraemia and 10 patients had polymicrobial bacteraemia. CNS were isolated in 24 (40%) patients (14 patients from a peripheral vein and CVC hub, 10 patients from a single blood culture), *Streptococcus* spp. in 10, *Staphylococcus aureus* in 8, *Enterococcus* spp. in 5, *Corynebacterium* spp. in 3, *Stomatococcus mucilaginosus* in 2, *Escherichia coli* in 9, *Pseudomonas aeruginosa* in 3, *Klebsiella* spp. in 2 patients, and other enterobacteria in 3 patients.

In 14 patients (7 males, 7 females), CNS were isolated from paired blood cultures (28 isolates) obtained from a peripheral vein (14 isolates) and the CVC hub (14 isolates). All isolates belonged to the species *S. epidermidis*. In all patients, isolates were susceptible to vancomycin and teicoplanin. One patient was susceptible to oxacillin, 3 to erythromycin and 4 to clindamycin, 3 to trimethoprim–sulphamethoxazole, 10 to rifampin, 3 to gentamicin, 2 to ciprofloxacin and none to penicillin. In 13 patients, antibiotic susceptibility of isolates from a peripheral vein was identical to antibiotic susceptibility of isolates from the CVC hub, and in 1 (patient no. 7) it

Table. *Antimicrobial susceptibility and PFGE patterns of 28* S. epidermidis *isolates from a peripheral vein and the CVC hub in 14 haematological patients with febrile neutropenia*

Patient no.	Isolate	PEN	OXA	E	CC	SXT	VA	TEIC	RIF	GM	CIP	Identical or related PFGE pattern
1	1 P	R	R	R	R	R	S	S	S	R	R	Yes
	2 C	R	R	R	R	R	S	S	S	R	R	
2	3 P	R	R	R	R	R	S	S	R	R	R	Yes
	4 C	R	R	R	R	R	S	S	R	R	R	
3	5 P	R	R	S	S	R	S	S	R	R	S	No
	6 C	R	R	S	S	R	S	S	R	R	S	
4	7 P	R	R	R	R	S	S	S	R	S	R	No
	8 C	R	R	R	R	S	S	S	R	S	R	
5	9 P	R	R	S	S	R	S	S	S	R	R	Yes
	10 C	R	R	S	S	R	S	S	S	R	R	
6	11 P	R	R	R	R	R	S	S	S	R	R	Yes
	12 C	R	R	R	R	R	S	S	S	R	R	
7*	13 P	R	R	R	R	R	S	S	S	R	R	No
	14 C	R	R	R	R	R	S	S	R	R	R	
8	15 P	R	R	R	R	R	S	S	S	R	R	No
	16 C	R	R	R	R	R	S	S	S	R	R	
9	17 P	R	S	R	R	S	S	S	S	S	S	Yes
	18 C	R	S	R	R	S	S	S	S	S	S	
10	19 P	R	R	R	R	R	S	S	S	R	R	Yes
	20 C	R	R	R	R	R	S	S	S	R	R	
11	21 P	R	R	R	R	R	S	S	S	R	R	Yes
	22 C	R	R	R	R	R	S	S	S	R	R	
12	23 P	R	R	R	S	R	S	S	S	R	R	Yes
	24 C	R	R	R	S	R	S	S	S	R	R	
13	25 P	R	R	R	R	R	S	S	R	R	R	Yes
	26 C	R	R	R	R	R	S	S	R	R	R	
14	27 P	R	R	S	S	S	S	S	S	S	R	Yes
	28 C	R	R	S	S	S	S	S	S	S	R	

P, peripheral blood; C, CVC hub blood; PEN, penicillin 10 IU; OXA, oxacillin 1 μg; E, erythromycin 15 μg; CC, clindamycin 2 μg; SXT, trimethoprim–sulphamethoxazole 1·25/23·75 μg; VA, vancomycin 30 μg; TEIC, teicoplanin 30 μg; RIF, rifampin 5 μg; GM, gentamicin 10 μg; CIP, ciprofloxacin 5 μg.

* CVC hub blood isolate differed from peripheral blood isolate in resistance to rifampin.

was not (the isolate from the peripheral vein was susceptible to rifampin, while the isolate from the CVC hub was not, although there was no history of receiving rifampin) (see Table). PFGE patterns of *S. epidermidis* from a peripheral vein and from the CVC hub were identical or closely related in 10 patients (patient nos. 1, 2, 5, 6, 9–14), while in 4 patients (patient nos. 3, 4, 7, 8) they were unrelated (Table, Figs 1 and 2). CNS isolates from patient nos. 1, 3 and 11 (isolates 1, 2, 6, 21 and 22) had identical PFGE patterns and were probably nosocomially spread amongst them (Figs 1 and 2).

DISCUSSION

Our understanding of the epidemiology and pathophysiology of CRBSI has increased greatly during the past two decades. Dissemination of such knowledge and increased awareness of effective measures for the prevention of CRBSI (maximal barrier precautions during insertion of central venous lines and less manipulation during use) have led to decreased incidence of these infections [14]. Clinical criteria alone are unreliable for establishing the diagnosis of intravascular device-related infections. This is especially true for neutropenic cancer patients, in whom fever of unknown origin is frequent, clinical findings implicating the catheter as the source of infection are often absent, and early antimicrobial therapy is usually instituted without, or long before, the removal of the catheter. The diagnosis of CRBSI in patients with febrile neutropenia is, therefore, difficult or even impossible, unless the catheter tip is removed or paired blood cultures (obtained from

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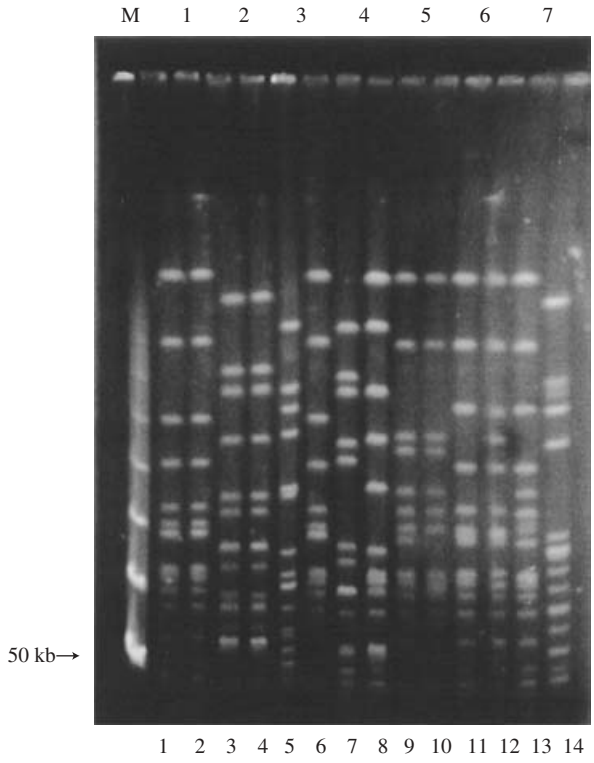

Fig. 1. PFGE patterns of *S. epidermidis* isolates 1–14 paired from a peripheral vein and from the CVC hub of patient nos. 1–7.

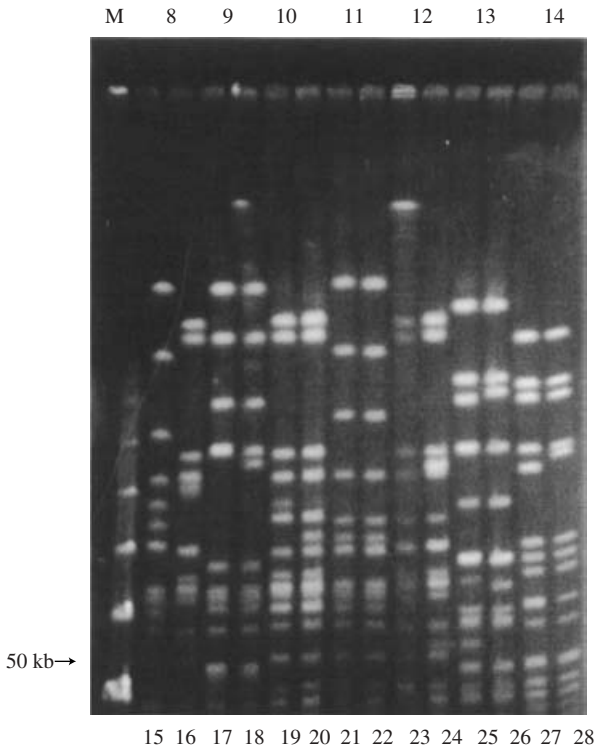

Fig. 2. PFGE patterns of *S. epidermidis* isolates 15–28 paired from a peripheral vein and from the CVC hub of patient nos. 8–14.

peripheral vein and CVC hub) are performed, and bacteria-typed. There are only a few studies that used PFGE for the typing of strains from blood cultures obtained for the diagnosis of CRBSI. In the study of Seifert et al. [7] PFGE of all available isolates recovered from peripheral blood cultures and CVC hub cultures yielded an identical PFGE pattern. In our study, CRBSI caused by CNS was suspected in 14 patients with febrile neutropenia without other foci of infection and was confirmed by identical PFGE patterns of *S. epidermidis* from peripheral blood and CVC hub blood in 10 out of 14 patients, while antibiotic susceptibility was identical in 13 patients (Table, Figs 1 and 2). It is known that identical antimicrobial susceptibility is not a reliable marker of strain identity. In four patients, whose PFGE patterns were different, there was a possibility of mixed infection; in these patients, the isolate chosen for PFGE from the CVC hub was not genetically identical to the isolate from a peripheral vein, or there was another source of bloodstream infection. In these patients and in 10 patients who had CNS from a single blood culture, there was also the possibility of blood culture contamination. CNS isolates from three patients had identical PFGE patterns. It was concluded that they were probably nosocomially spread among these patients.

REFERENCES

1. Crnich CJ, Maki DG. The promise of novel technology for the prevention of intravascular device-related bloodstream infection. CID 2002; **34**: 1232–1242.
2. Bouza E, Burillo A, Munoz P. Catheter-related infections: diagnosis and intravascular treatment. Clin Microbiol Infect 2002; **8**: 265–274.
3. Mueller-Premru M, Gubina M, Kaufmann ME, Primožič J, Cookson BD. 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. J Med Microbiol 1999; **48**: 451–460.
4. Brun-Buisson C. New technologies and infection control practices to prevent intravascular catheter-related infections. Am J Respir Crit Care Med 2001; **164**: 1557–1558.
5. Weinstein MP. Blood culture contamination: persisting problems and partial progress. J Clin Microbiol 2003; **41**: 2275–2278.
6. Weinbaum FI, Lavie S, Danek M, Sixsmith D, Heinrich GF, Mills SS. Doing it right the first time: quality

https://doi.org/10.1017/S0950268804002584 Published online by Cambridge University Press

improvement and the contaminant blood culture. J Clin Microbiol 1997; **35**: 563–565.
7. Seifert H, Cornely O, Seggewiss K, et al. Bloodstream infection in neutropenic cancer patients related to short-term nontunnelled catheters determined by quantitative blood cultures, differential time to positivity, and molecular epidemiological typing with pulsed-field gel electrophoresis. J Clin Microbiol 2003; **41**: 118–123.
8. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous catheter-related infection. N Engl J Med 1977; **296**: 1305–1308.
9. Siegman-Igra Y, Anglim AM, Shapiro DE, Adal KA, Strain BA, Farr BM. Diagnosis of vascular catheter-related bloodstream infection: a meta-analysis. J Clin Microbiol 1997; **35**: 928–936.
10. Bouza E, Perez-Molina J, Munoz P. Report of ESGNI-001 and ESGNI-002 studies. Bloodstream infections in Europe. Clin Microbiol Infect 1999; **5** (Suppl 2): 1–12.
11. Lina B, Vandenesch F, Etienne J, Kreiswirth B, Fleurette J. Comparison of coagulase-negative staphylococci by pulsed-field gel electrophoresis. FEMS Microbiol Lett 1992; **92**: 133–137.
12. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; **33**: 2233–2239.
13. Performance standards for antimicrobial disk susceptibility tests, 7th edn [Editorial]. National Committee for Clinical Laboratory Standards, 2000: 1–48.
14. Guidelines for the prevention of intravascular catheter-related infections [Editorial]. MMWR 2002: 1–29.