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Characterization of clinically significant isolates of *Staphylococcus epidermidis* from patients with cerebrospinal fluid shunt infections

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SUMMARY

Biotyping, slime production, antibiograms, extrachromosomal DNA banding and total DNA restriction analysis were used to characterize *Staphylococcus epidermidis* strains causing cerebrospinal fluid shunt infections in 11 patients. Infections considered to be community acquired and those acquired in the first 2 weeks of hospital admission were due to oxacillin-susceptible isolates. Multiply resistant strains were isolated from patients who were in hospital for more than 1 month before tube implantation. Slime was detected in staphylococci for 54% of cases, but its expression varied. Strains from different patients could be differentiated from one another by the extrachromosomal DNA bandings and total DNA restriction patterns, but isolates from the same patient were usually similar. During the period of external drainage, epidemiological markers were useful in differentiating persistence of infection from contamination or re-infection by a new strain.

INTRODUCTION

Coagulase-negative staphylococci, specially *Staphylococcus epidermidis*, are the predominant cause of cerebrospinal fluid (CSF) shunt infection but can also be frequent contaminants of CSF specimens [1–5]. The distinction between infective and contaminating *S. epidermidis* isolates is not easy because the signs and symptoms of CSF shunt infections are varied and often non-specific: only a minority of patients present with signs and symptoms clearly suggestive of CSF infection (severe headache, high fever, photophobia, neck stiffness, and lethargy). The CSF cellular reaction to shunt infection is usually minimal and the glucose

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concentration is usually slightly reduced. Fever is the only reliable manifestation of shunt infection [3].

Although no specific determinant can be used to identify clinically significant isolates of coagulase-negative staphylococci, several typing methods can be used to determine whether the strains are similar. Typing methods include biochemical profiles, antimicrobial susceptibility profiles, bacteriophage susceptibility patterns, serological typing, protein profiles, immunoblot fingerprinting, extrachromosomal DNA patterns, total DNA restriction endonuclease analysis and DNA hybridization [6]. Some markers provide more conclusive data than others, and a combination of different markers is generally used [7].

This study, based on a retrospective review of episodes of CSF shunt infections caused by *S. epidermidis* in 11 patients, was undertaken to determine the characteristics of the 30 isolates of *S. epidermidis* isolated from these patients using biotyping, antimicrobial susceptibility testing, slime production detection, extrachromosomal DNA banding and total DNA restriction endonuclease analysis.

MATERIALS AND METHODS

Patients. Eleven cases of CSF shunt ventriculitis or meningitis were diagnosed at the Pierre Wertheimer Hospital, Lyon, between 1986 and 1989. The infections occurred in five children aged 6 months to 12 years and six adults aged 42–75 years (Table 1). Antibiotic prophylaxis with oxacillin or teichoplanin was given to five patients when the shunt was inserted. The site of infection is listed in Table 1.

Two cases of meningitis (cases 1 and 2) were considered to be community-acquired as the patients had been discharged from hospital for 16 and 4 months respectively when the infections occurred; two of them (cases 3 and 4) had not been in hospital before implantation of the shunt and developed meningitis within 2 weeks of the procedure; the other seven patients had been in hospital for at least a month before implantation of the shunt and all received antibiotics before they developed meningitis.

The shunt was assumed to be infected when fever occurred with or without symptoms suggestive of CSF infection. CSF cultures were obtained by lumbar or ventricular puncture. Isolates of *S. epidermidis* were considered significant when Gram-positive cocci were detected on Gram-stained smear of the spun deposit of CSF, when the CSF white blood cell count was over 100×10^6 cells/l and when it was the sole micro-organism isolated. In the absence of pleocytosis, *S. epidermidis* was considered significant if repeated cultures yielded isolates with similar identification and antibiogram patterns.

Bacteria. Thirty strains (1–5 per patient) were selected for study from all the isolates considered significant: 21 were recovered from CSF, seven from shunt tubing and 2 from blood cultures. All the isolates were picked from agar plates, grown in brain heart infusion broth, and frozen at −20 °C.

Biotyping. The infecting organism was identified as *S. epidermidis* according to the classification of Kloos and Schleifer [8] and using the API Staph gallery (API-

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Table 1. *History of patients and time between shunt surgery and CSF infections*

Patient No.	Age	Initial disease	Time of hospitalization before shunt	Antibiotic treatment before surgery	Type of shunt*	Antibio-prophylaxis	Time between surgery and infection
1	66	Subarachnoid haemorrhage	4 months	Penicillin G	VPS	−	16 months
2	52	Cranio-pharyngiomia	1 day	None	VPS	−	4 months
3	12	Arachnoïd cyst	1 day	None	CPS	+	13 days
4	42	Subarachnoid haemorrhage	1 day	None	VES	−	5 days
5	2	Posterior fossa tumour	1 month	Spiramycin	VPS	+	2,5 months
6	54	Subarachnoid haemorrhage	5 weeks	Amoxycillin +calvulanic acid	VPS	−	3 days
7	57	Subarachnoid haemorrhage	2 months	Amoxycillin +pefloxacin	VPS	+	5 days
8	12	Arnold–Chiari malformation	1 month	Oxacillin	VAS	+	20 days
9	75	Suprasella meningioma	1 month	Pipemidic acid	VAS	−	11 days
10	6 mo	Intraventricular haemorrhage	6 months	Amoxycillin piperacillin +aminoglycosides	VPS	+	2 days
11	6	Subarachnoid haemorrhage	3 weeks	None	VAS	−	11 days

* VPS, ventriculoperitoneal shunt; VAS, ventriculoatrial shunt; CPS, cystoperitoneal shunt; VES, ventriculoexternal shunt.

system, Montalieu-Vercieu, France). The 19 biochemical reactions were used to generate a seven-digit biotype code.

Antimicrobial susceptibility testing. Sensitivity to antibiotics was determined by the agar diffusion method on commercially prepared Mueller–Hinton medium (BioMérieux, Marcy l'Etoile, France) using antimicrobial disks (BioMérieux) containing: penicillin G (6 μg), oxacillin (5 μg), gentamicin (15 μg), tetracycline (30 IU), chloramphenicol (30 μg), erythromycin (15 IU), pristinamycin (15 μg), rifampicin (30 μg), co-trimoxazole (1·25+23·75 μg), vancomycin (30 μg), fosfomycin (50 μg), fusidic acid (10 μg), pefloxacin (5 μg). Plates were incubated at 35 °C (30 °C for oxacillin) for 18–24 h. The results were expressed as susceptible, intermediate, or resistant according to the criteria of the Comité Français de l'Antibiogramme [9]. β-lactamase production was detected with nitrocefin (BioMérieux) according to the manufacturer's instructions.

Slime production. Strains were examined for slime production as described by Christensen and co-workers [10].

Extrachromosomal DNA banding. Extrachromosomal DNA was extracted from *S. epidermidis* cultures by rapid boiling technique of Holmes and Quigley [11] modified as described previously [12].

Restriction endonuclease analysis (*REA*). The restriction enzyme pattern of chromosomal DNA from 12 isolates was compared. A single strain considered to represent true infection was selected from 10 of the 11 cases, and two strains were selected for case No. 4. DNA was recovered by the action of lysostaphin as previously described [13]. The DNA was completely digested with *Eco*R I restriction enzyme (Boehringer Mannheim, Meylan, France) under the conditions recommended by the manufacturer.

RESULTS

Biotyping. The seven-digit biotype numbers generated from the 19 biochemical tests in the API Staph kit are shown in Table 2. Four different biotype codes, all consistent with identification as *S. epidermidis*, were obtained. The isolates from each individual patient had an identical biotype, except for patient No. 4 for whom two biotypes were detected. There was no correlation between a particular biotype and a history of meningitis.

Antimicrobial susceptibility pattern. Four strains (nos. 1–4) were susceptible to oxacillin and all but one to the other agents tested (Table 2). These strains were from patients with community-acquired meningitis and those who had not been in hospital before shunt implantation. Eight strains (nos. 4′–11) were resistant to multiple antibiotics, including oxacillin and all but one were resistant to gentamicin. Seven of these eight strains were from patients with hospital-acquired infections who had been in hospital for more than 1 month before shunt implantation. Infection in patient No. 4 was initially caused by a fully sensitive strain that was replaced after 1 month of treatment by a multiply resistant

Table 2. *Characteristics of 40* S. epidermidis *isolates responsible for 11 CSF shunt infections*

Isolate no.	Biotype	Antibiotic resistance*													Slime production	Plasmid in kilobases					
		PG	OX	GM	TE	CL	ER	PR	RF	CT	VA	FF	FA	PF							
1A	6706112	S	S	S	S	S	S	S	S	S	S	R	S	R	−					1·8	
1B	6706112	S	S	S	S	S	S	S	S	S	S	R	S	R	+					1·8	
1C	6706112	S	S	S	S	S	S	S	S	S	S	R	S	R	−					1·8	
2A	6706112	R	S	S	S	S	S	S	S	S	S	S	S	S	−				4		
2B	6706112	R	S	S	S	S	S	S	S	S	S	S	S	S	−				4		
3A	6706113	R	S	S	S	S	S	S	S	S	S	S	S	S	+		7·8				
3B	6706113	R	S	S	S	S	S	S	S	S	S	S	S	S	+		7·8				
3C	6706113	R	S	S	S	S	S	S	S	S	S	S	S	S	+		7·8				
4A	6706112	S	S	S	S	S	S	S	S	S	S	S	S	S	+	> 40	9·1			2·5	
4B	6706112	S	S	S	S	S	S	S	S	S	S	S	S	S	−	> 40	9·1			2·5	
4'A	6704112	R	R	R	S	S	S	S	R	R	S	R	S	R	+	> 40		4·6			
4'B	6704112	R	R	R	S	S	S	S	R	R	S	R	S	R	−	> 40		4·6			
4'C	6704112	R	R	R	S	S	S	S	R	R	S	R	S	R	−	> 40		4·6			
5A	6704112	R	R	R	R	S	S	S	R	R	S	R	S	R	−	> 40			4·2, 4		
5B	6704112	R	R	R	S	S	S	S	R	R	S	R	S	R	−	> 40					
5C	6704112	R	R	R	R	S	S	S	R	R	S	R	S	R	−	> 40			4·2, 4		
6A	6706112	R	R	S	S	R	R	S	R	R	S	R	S	R	+	> 40	7·8			1·8	
6B	6706112	R	R	S	S	R	R	S	R	R	S	R	S	R	−	> 40	8 7·8			1·8	
6C	6706112	R	R	S	S	R	R	S	S	R	S	R	S	R	+	> 40	7·8			1·8	
7A	6706112	R	R	R	S	S	R	S	R	R	S	R	S	R	+	> 40	7·8			1·8	
7B	6706112	R	R	R	S	S	R	S	R	R	S	S	S	R	+	> 40	7·8				
8A	6706112	R	R	R	R	S	S	S	S	R	S	R	S	S	−		7·8	4·6,	4		
8B	6706112	R	R	R	R	S	S	S	S	R	S	R	S	S	−		7·8	4·6,	4		
8C	6706112	R	R	R	R	S	S	S	S	R	S	R	S	S	−		7·8	4·6,	4		
9A	6706112	R	R	R	S	S	R	S	R	R	S	R	S	R	−	> 40				2·5	
9B	6706112	R	R	R	S	S	R	S	R	R	S	R	S	R	−	> 40				2·5	
9C	6706112	R	R	R	S	S	R	S	R	R	S	R	S	R	+	> 40				2·5	
10A	6706112	R	R	R	R	S	R	S	S	R	S	S	S	S	−			4·4		2·5	
10B	6706112	R	R	R	R	S	R	S	S	R	S	S	S	S	−			4·4		2·5	
11	6706112	R	R	R	S	R	R	S	R	S	S	S	S	S	−	> 40				2·5	

* PG, penicillin G; OX, oxacillin; GM, gentamicin; TE, tetracycline; CL, chloramphenicol; ER, erythromycin; PR, pristinamycin; RF, rifampicin; CT, co-trimoxazole; VA, vancomycin; FF, fosfomycin; FA, fusidic acid, PF, pefloxacin.

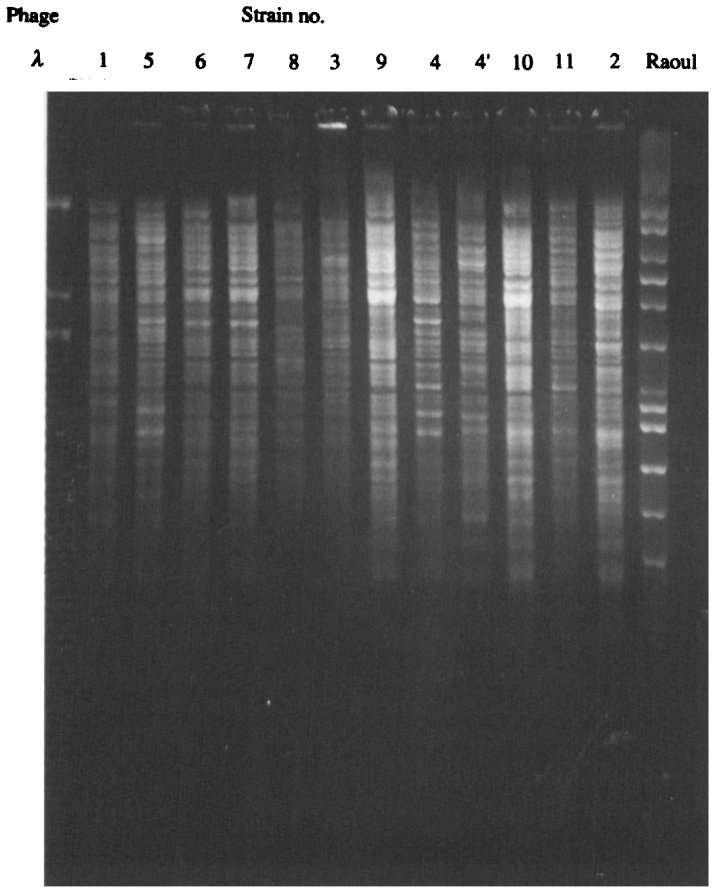

Fig. 1. *Eco*R I restriction profiles of 12 *S. epidermidis* strains. Raoul and bacteriophage λ digested with *Eco*R I were used as sized markers.

organism. The antibiotic resistance profiles of the strains were different from one another but isolates from a single patient had similar resistance patterns with occasional discrepancies (see Table 2).

Slime production. At least one slime producing isolate was detected in 6 of the 11 cases (54%). The isolates from a single patient varied in their slime production capacity (Table 2).

Extrachromosomal DNA banding. Analysis of extrachromosomal DNA bands demonstrated the presence of 1–4 bands for all the isolates (Table 2). No correlation was found between the extrachromosomal DNA-banding patterns and the origin or antibiotic susceptibility of the strains. The patterns for isolates from a given patient were distinguishable, and in six cases were identical for all the isolates obtained. In a further two cases (Nos. 5 and 6), minor modifications – usually the loss of 1–2 bands – were observed. For case No. 4, there was a noticeable difference in the banding pattern of the initial isolates (nos. 4A and 4B) and those obtained later (nos. 4′A, 4′B and 4′C); this correlated with other major

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changes in the typing characteristics of the organism isolated from the patient. This suggested that one infecting organism had been replaced by another.

Restriction endonuclease analysis. The total DNA restriction patterns of the 12 isolates were distinguishable (Fig. 1); in two cases, only by the presence of 1–2 additional fragments (nos. 6 and 7, and nos. 9 and 10): these strains were multiply-resistant and responsible for hospital-acquired meningitis.

DISCUSSION

Infection associated with CSF shunts is still a significant source of morbidity and mortality. The incidence of infection after ventriculostomy is 10–15%, with more than half of the cases caused by *S. epidermidis* [14]. Treatment of infected CSF implants is controversial. The highest success rate has been obtained with intravenous vancomycin and the removal of the shunt [4]. In our study, there was a close relationship between hospital admission and the antibiotic resistance of the infecting strain. Community-acquired infections and those acquired in the first 2 weeks in hospital were caused by oxacillin-susceptible *S. epidermidis*; this antibiotic (or other antistaphylococcal β-lactams) can be used for the treatment of these infections in preference to vancomycin, which achieves lower CSF concentrations.

Prolonged hospitalization before shunt implantation allows patients to acquire antibiotic resistant strains of staphylococci from the environment [15, 16]. Seven cases of CSF shunt infections were considered to be hospital-acquired and all were with multiply-resistant strains. Previous antibiotic treatment almost certainly predisposed to infections with these strains.

Slime produced by *S. epidermidis* is viewed as a virulence factor promoting bioprosthetic infection [17] and was detected in organisms from half our cases. Its expression varied. Christensen and colleagues have suggested that phenotypic differences in slime production may occur depending upon the conditions under which the organisms are cultured [18]. In our study, slime production was evaluated visually, and the end point for positivity can vary between different laboratories. The inconstancy of slime production by some isolates and the fact that a large number of pathogenic isolates do not produce slime argues against it being a major determinant of virulence. However, some studies have indicated that slime producing strains are isolated from infections in which removal of the shunt it usually required [19]. One of the four patients infected with strains that failed to produce slime was cured by antimicrobial therapy alone, whereas none of those infected by slime-producing strains was.

The biochemical profile or biotype was of little interest in our epidemiological study due to the small number of phenotypes recognized. Younger [20] studied 85 strains of coagulase-negative staphylococci from CSF and has suggested that phosphatase-negative *S. epidermidis* could be a particularly virulent type, because in his study 20% of the pathogens and none of the contaminants belonged to this subgroup. All our pathogens produced a phosphatase.

A variety of characteristics of *S. epidermidis* strains was demonstrated using epidemiological markers. All strains were isolated from sporadic cases and none

was found to have identical extrachromosomal DNA patterns. The total DNA restriction patterns of 12 representative strains showed that they were generally different from one another. The patterns of two groups of strains (nos. 6 and 7, 9 and 10) appeared to be more closely related; strains nos. 6 and 7 also had similar antibiograms and extrachromosomal DNA banding patterns and were isolated in 1988 from patients hospitalized in the same unit during 6 weeks, but the CSF infections occurred after an interval of 4-months. This suggests the diffusion of a single strain which progressively modified some of its characteristics (for instance, variations in fosfomycin or rifampicin resistance were observed). The second group of strains (nos. 9 and 10) with similar total DNA restriction patterns were isolated in February and June 1988 respectively, from patients who were not hospitalized in the same unit. These two strains had different antibiograms and extrachromosomal DNA patterns.

The variety of strains isolated suggested that the patients were infected by their own flora selected by hospitalization and antibiotic treatment before shunt implantation. Using typing procedures to compare the strains, Bayston [2] found that skin surface strains present in the operative period were indistinguishable from those that subsequently produced shunt infections and suggested that organisms enter the shunt from the patients' skin during surgery.

The epidemiological markers were also useful in differentiating persistence of infection despite antibiotic treatment and shunt removal, from contamination during external drainage. Persistence of infection was demonstrated for patient No. 9, who had similar isolates at 3 weeks' interval. For patient No. 4, replacement of an oxacillin-susceptible by an oxacillin-resistant strain as the sole infecting agent occurred after externalization of the shunt; the two strains were differentiated according to their antibiotic resistance profiles, extrachromosomal DNA profiles and total DNA restriction analysis.

In conclusion, there was a close correlation between the history of the patients and the bacteriological characteristics of the *S. epidermidis* strains responsible for infections on CSF shunts. The longer the patient was hospitalized before the onset of the infection, the higher the risk of infection with multiply resistant strains. The wide range of infecting strains can be assessed by molecular markers, such as plasmid profile or total DNA restriction analysis, and isolates from a single patient show strong correlation between these characteristics. In rare cases, infection was due to strains with similar total DNA restriction patterns, suggesting cross infection.

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