Epidem. Inf. (1987) **98**, 231–239
Printed in Great Britain

Plasmid analysis as an epidemiological tool in neurosurgical infections with coagulase-negative staphylococci

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(*Accepted 5 January 1987*)

SUMMARY

Coagulase-negative staphylococci isolated from blood or spinal fluid during a period of 1 year in a department of neurosurgery, were analysed by biotyping, antibiotic resistance pattern and plasmid profiles. Altogether 41 isolates from 19 patients were studied. About 90% of the isolates were *Staphylococcus epidermidis*. The antibiotic resistance pattern seemed to be closely related to antibiotic usage in the unit. Most common was resistance to penicillin (63%), trimethoprim-sulphamethoxazole (49%) and cloxacillin (39%) while resistance to gentamicin was seen in only one strain.

In several cases species and antibiograms were identical in isolates from different patients. Plasmid pattern analysis could then be used for identification of different strains. In one instance, plasmid pattern and restriction enzyme analysis confirmed that two patients probably were infected by the same strain.

INTRODUCTION

Coagulase-negative staphylococci have recently come into focus as the cause of serious hospital-acquired infections (Tuazon & Miller, 1983; Christensen *et al.* 1982; Wade *et al.* 1982; Davies *et al.* 1984). *Staphylococcus epidermidis* infections are the predominant cause of cerebrospinal fluid shunt infection (Price, 1984). To determine the epidemiology of such infections, a simple and versatile typing system is needed (Parisi, 1985); phage-typing has been used e.g. in the Netherlands (Van-Boven, 1976) and Britain (Richardson, Marples & de Saxe, 1984) but has not yet come into general use. Biotyping (Kloos & Schleifer, 1975) is not sensitive enough for this purpose, since some 80% of clinical isolates of coagulase-negative staphylococci belong to the species *S. epidermidis*.

It has long been known that coagulase-negative staphylococci carry plasmids that can be used for typing (Laufs, Heczko & Pulverer, 1978; Parisi & Hecht, 1980; Kloos, Orbarn & Walker, 1981; Archer, Vishniavsky & Stiver, 1982; Cohen, Wong & Falkow, 1982; Weinstein *et al.* 1982; Farrar, 1983; Archer *et al.* 1984; Parisi, 1985).

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Table 1. *Diagnoses in the 19 patients from whom coagulase-negative staphylococci were isolated*

Diagnosis	No.	Coagulase-neg. staph. isolated from: Spinal fluid	Blood	Both
Infected ventriculo atrial shunt	5	1	1	3
Infected ventriculo peritoneal shunt	4	2	1	1
Infections after neurosurgical operation[a]	7	2	5	—
No clinical sign of infection[b]	3	2	1	—
Total	19	7	8	4

[a] Other than shunt operations.
[b] No patient with a shunt.

We have studied the plasmid patterns of all coagulase-negative staphylococci isolated in blood or cerebrospinal fluid from patients in a neurosurgical department during a period of 1 year.

MATERIALS AND METHODS

Bacterial cultures and identification

All cultures of coagulase-negative staphylococci isolated from spinal fluid or blood during 1982 from patients in the department of Neurosurgery, Karolinska Hospital, Stockholm, Sweden, were studied. Altogether 38 positive cultures, 23 from spinal fluid and 15 from blood, were obtained from 19 patients. In cultures from two patients more than one strain of coagulase-negative staphylococcus was found, making the total 41 strains. Three of eight cultures from one of the patients were carried out in the department of Pediatrics, division of Neurology, but were included because of their epidemiological interest. The diagnoses of the 19 patients were obtained by chart review (Table 1).

Coagulase-negative staphylococci were identified and biotyped according to the methods of Kloos & Schleifer (1975). Determination of antibiotic susceptibility and minimum inhibitory concentration by disk diffusion and agar dilution methods were done as described by Ericsson & Sherris (1971).

Lysis procedure and gel electrophoresis

Plasmid DNA was extracted by a method modified after Birnboim & Doly (1979) and Flock & Lindberg (personal communication).

Reagents. Solution I: 0·3 M sucrose, 25 M disodium EDTA and 25 M Tris (pH 8) as stock solution and, added fresh before use, lysostaphin 250 μm/ml and lysozyme 2 mg/ml. Solution II: 0·3 M-NaOH, 2% sodium dodecyl sulfate (SDS); stable for about 1 week in room temperature. Solution III: 50 g phenol (solid), 50 ml chloroform and 20 ml H_2O.

Procedure. One or two loopfuls of overnight culture on blood-agar, was added to 500 μl of solution I in an Eppendorf tube. After 20 min at 37 °C, 250 μl of solution II was added and gently mixed by vortex mixer. The tube was maintained for

https://doi.org/10.1017/S0950268800061987 Published online by Cambridge University Press

30 min at 65 °C and after cooling to room temperature 100 μl of solution III was added and again mixed. After centrifugation the supernatant was carefully removed. To the supernatant was added 5 M ammonium acetate to a final concentration of 0·3 M and then one volume of isopropanol. The tube was maintained at −70 °C for 30 min and centrifuged; the supernatant was then discarded. To wash the DNA pellet 1 ml of 70% ethanol and 60 μl 5 M ammonium acetate was added. The tube contents were mixed and then centrifuged again. The DNA was dissolved in 20–40 μl water and 10–20 μl of the dissolved DNA was mixed with a solution containing glycerol, Ficoll and bromphenol blue and heated at 60 °C for 3 min. Samples of 15–30 μl were then applied to a horizontal slab gel. Agarose type II medium EEO, was used, dissolved in electrophoresis buffer (89 mM Tris, 79 mM boric oxide, 2·5 M EDTA) to a concentration of 0·8%. Ethidium bromide was added to the electrophoresis buffer or to the agarose gel at a concentration of 0·5 mg/l. Electrophoresis was performed either on a 'mini-gel', 5 cm × 10 cm × 33 mm, (Biorad Mini Sub Bio-Rad, Richmond, California) usually for 2 h at 90 V, or on a horizontal 'standard'-gel 15 × 25 cm (BMC verkstad, Uppsala, Sweden) for 16–20 h at 40 V. DNA bands were visualized by shortwave ultraviolet light (Ultraviolet Products Inc. San Gabriel, California) and photographed with a Polaroid camera (Polaroid CN-5 Land camera) with a Wratten No. 9 filter.

Plasmid DNA from some strains were digested, without further purification, with restriction endonuclease *Eco* RI according to the supplying company's specification.

As molecular weight standard plasmid DNA from *Escherichia coli* V517 (Macrina *et al.* 1978) or *Hind* III digested lambda DNA were used.

Chemicals. Lysostaphin, lysozyme, Agarose type II medium EEO and ethidium bromide were purchased from Sigma Chemical Co. St Louis, Missouri. *Hind* III digested lambda DNA and *Eco* RI were from New England Biolabs, Beverly, Massachussets.

RESULTS

Patient characteristics

The 38 cultures were isolated during 22 occasions of suspected infection. About half the 19 patients had infected neurosurgical shunts (Table 1). Nine patients were previously essentially healthy and were admitted to the hospital because of an acute or subacute neurological disease. Among these, all except one required one or more neurosurgical operations. Coagulase-negative staphylococci were first isolated on average 13 days (range 4-22 days) after the patients' first operation.

Use of antibiotics

During September 1982–September 1983 antibiotic prophylaxis was registered during all neurosurgical operations at the unit, as well as the total use of antibiotics in the department of neurosurgery (Table 2). In 90% of all prophylaxis, trimethoprim-sulphamethoxazole (TMP-SMZ) (65%) or isoxazolyl penicillins (Clox) (24%) were used. These two drugs, in addition to penicillin (Pc), were the most commonly used for therapy. Use of aminoglycosides was rare.

Table 2. *Antimicrobial resistance of coagulase-negative staphylococci isolated from blood or spinal fluid during 1982 and antibiotic usage September 1982–September 1983 in the department of Neurosurgery*

Antibiotics	Isolates resistant to antibiotic (n = 41)(%)	Total DDD (%)	Antibiotics used in prophylaxis (%)
Penicillin	63	32	5
Clox/Diclox Flucloxacillin	39	30	24
Trimethoprim/ Sulphmethoxazole	49	23	65
Cephalosporins	ND	8	2
Gentamicin	2	5	1
Others	—	2	3

ND, not done, DDD, defined daily doses (Nordic Council on Medicines, 1982).

Antibiogram and species

Table 3 shows antibiograms and species of the 41 isolates of coagulase-negative staphylococci. Twenty-six of 41 isolates were resistant to Pc (MIC > 8 mg/l) and of those, 16 were also resistant to Clox (MIC > 1 mg/l). Resistance to TMP-SMZ (TMP > 2 mg/l, SMZ > 32 mg/l) was the second most common, occurring in 20 of 41 isolates.

Resistance to gentamicin (Gm) (MIC > 16 mg/l) was found in only one isolate (patient 1:6, Table 3), and this strain was also resistant to tobramycin (MIC > 16 mg/l) but sensitive to netilmicin and amikacin. Resistance to the remaining antibiotics tested was also rare; clindamycin (Cl) (MIC > 4 mg/l) 2 isolates, chloramphenicol (Cm) (MIC > 8 mg/l) 3 isolates, erythromycin (Em) (MIC > 2 mg/l) 5 isolates and fusidic acid (Fu) (MIC > 2 mg/l) 6 isolates.

The majority, 36 of 41 isolates, belonged to the species *S. epidermidis*. There were two isolates of *S. capitis* and one each of *S. hominis*, *S. warneri* and *S. simulans*.

Lysis procedure

Small amounts of chromosomal DNA, of uniform mobility, could be seen in all lysates after electrophoresis, indicating lysis but not full purification of plasmid DNA. The amount of plasmid DNA, in repeated lysates from the same isolate, varied slightly depending mostly on the size of the inoculum. Cultures examined after several days storage on agar in a refrigerator showed the same results as when examined after overnight incubation.

Plasmid patterns

Plasmid DNA could be demonstrated in all isolates except one. The 40 isolates contained 2 or more plasmids and up to 11 bands were seen in 1 strain. About 40% of the plasmids had a molecular weight of more than 10 MDa, 20% were 5–10 MDa and 40% were smaller than 5 MDa. In total 24 different plasmid profiles were seen. Plasmid pattern was usually consistent in repeated lysates of the same isolate; additional weak bands could sometimes be seen and were regarded as open circular DNA (OC) bands. Plasmids with a size of more than about 20 MDa or less

Table 3. *Date and place of isolation, biotype, antibiogram and plasmid profile of the 41 isolates (blood or spinal fluid) from 19 patients treated in the department of Neurosurgery, 1982*

Pat.[a]	Isol.[b]	Date of isol.	Isol. on ward	Isol[d] from	Species[e]	Antibiogram[f] resistant to	Plasmid[g] profile
1	1	27.11	outpat[c]	Sp	Cap	0	A
	2	22.iv	outpat.	Sp	Epi	Pc, Clox, TMP-SMZ	B
	3	28.iv	outpat.	Sp	Cap	0	A
	4	21.vi	3	Sp	Epi	Pc, Clox, TMP-SMZ	B
	5	28.vi	2	Sp	Epi	Pc, Clox, TMP-SMZ	B
	6	28.vi	2	Bl	Epi	Pc, Clox, TMP-SMZ, GM	C
	7	1.vii	2	Sp	Epi	Pc, Clox, TMP-SMZ	B
	8	23.vii	2	Sp	Epi	Pc, Clox, TMP-SMZ	B
2	—	14.iii	3	Bl	Epi	Pc	D
3	—	21.iii	2	Bl	Epi	Pc, Clox, TMP-SMZ, Em, Fu	E
4	1	17.iv	3	Bl	Epi	Pc, Dc	F
	2	20.iv	3	Sp	Epi	0	G
5	1	10.iv	2	Bl	Epi	Pc, Clox, TMP-SMZ, Em, Cl	H
	2	15.iv	2	Bl	Epi	Pc, Clox, TMP-SMZ, Em, Cl	H
	3	13.viii	1	Sp	Epi	Pc, Clox, TMP-SMZ	B
	4	1.x	2	Sp	Epi	Pc, Clox, TMP-SMZ	B
6	1	10.iv	3	Sp	Epi	Pc	I
	2	10.iv	3	Bl	Epi	Pc	I
7	1	9.vi	3	Bl	Epi	0	K
	2	22.vi	7	Bl	Epi	0	K
	3	25.vi	2	Bl	Epi	0	K
8	1	26.iv	7	Sp	Epi	0	L
	2	29.iv	7	Sp	Epi	0	L
9	—	1.v	2	Bl	Epi	Pc, Clox, TMP-SMZ, Cm	M
10	1	22.vii	2	Sp	Hom	Pc	—
	2	27.vii	2	Sp	Warn	Pc	N
11	—	24.viii	3	Bl	Epi	Pc, Clox, TMP-SMZ	O
12	—	2.ix	2	Bl	Epi	Pc, Clox, TMP-SMZ, Em, Dc, Fu	P
13	—	12.x	3	Sp	Epi	Pc, Clox, TMP-SMZ, Cm	Q
14	—	15.x	2	Bl	Epi	Pc, TMP-SMZ, Dc	R
15	1	13.xi	3	Sp	Sim	Pc, Cm, Fu	S
	2	25.xi	3	Sp	Epi	TMP-SMZ, Dc, Fu	T
	3	30.xi	3	Sp	Epi	TMP-SMZ, Dc, Fu	T
	4	1.xii	3	Sp	Epi	TMP-SMZ, Dc, Fu	T
16	—	8.xii	7	Sp	Epi	Pc, Em, Dc	U
17	—	9.xii	3	Bl	Epi	0	V
18	1	5.xii	2	Sp	Epi	0	X
	2	5.xii	2	Sp	Epi	0	X
	3	5.xii	2	Sp	Epi	0	X
19	1	29.vii	3	Sp	Epi	Pc, Clox, TMP-SMZ, Em, Fu, Cl	Y
	2	29.vii	3	Sp	Epi	Pc, Dc	Z

[a] Pat., patient no.; [b] Isol.; isolate no. or in case of patient 18 and 19 colonies of different appearance in the same isolate; [c] Outpat. patient cultured during visit to the outpatient department; [d] Sp. spinal fluid; Bl, blood; [e] Cap, capitis, Epi, epidermidis, Hom, hominis, Warn, warneri, Sim, simulans; [f] Pc penicillin; Clox, cloxacillin; TMP-SMZ, trimetoprim-sulphamethoxazole; Em, erythromycin; Dc doxycyclin; Gm, gentamicin; Cm, chloramphenicol; Fu, fusidic acid and Cl, clindamycin; [g] each distinct pattern symbolized with a letter A–Z.

https://doi.org/10.1017/S0950268800061987 Published online by Cambridge University Press

Table 4. *The distribution between patients of four plasmids of approximate weights of 1·2, 1·8, 3·2 and 5–6 MDa commonly seen among the 41 isolates of coagulase negative staphylococci. For antibiograms and biotypes: see Table 3.*

Patient and (isolate No.)	Plasmid composition					Plasmid profile
	1·2 MDa	1·8 MDa	3·2 MDa	5–6 MDa	No. of band of other sizes	
5 (1–2)	X	—	—	—	4	H
16	X	—	—	—	2	U
7 (1–3)	—	X	—	—	3	K
12	—	—	X	—	5	P
15 (1)	—	—	X	—	5	S
1 (1, 3)	X	X	—	—	3	A
3	X	X	—	—	8	D
19 (1)	X	X	—	—	3	Y
11	X	—	X	—	6	0
15 (2–4)	—	X	X	—	5	T
17	—	X	X	—	2	V
2	—	—	X	X	4	D
13	—	—	X	X	0	Q
9	X	X	—	X	6	M
1 (2, 4, 5, 7, 8)	X	—	X	X	1	B
5 (3–4)	X	—	X	X	1	B
6 (1, 2)	X	X	X	X	2	I
14	X	X	X	X	4	R

Isolates from two patients only patient 1 (2, 4, 5, 7, 8) and patient 5 (3, 4) had identical patterns, antibiograms and belonged to the same species.

than about 1·8 MDa were somewhat less reproducible. Twelve isolates from six patients (patient 1:1, 3; 4:2; 7:1–3; 8:1–2; 17; 18:1–3) were sensitive to all antibiotics tested (Table 3). These 12 isolates contained 2–4 'cryptic' plasmids each. While the isolates from patient 1(1:1; 1:3) were of species *S. capitis*, the other five patients isolates were all of species *S. epidermis*, but had plasmid profiles that differed from patient to patient.

Resistance to Pc, Clox and TMP-SMZ was seen in 8 isolates from 3 patients (patient 1:2, 4, 5, 7, 8; 5:3–4; 11) (Table 3). Isolates from patients 1 and 5 showed identical plasmid pattern and also a strong similarity in restriction enzyme analysis (data not shown). The isolate from patient 11 seemed to have two plasmids of about 1·2 and 3·2 MDa in common but differed in others; differences were also seen in restriction enzyme analysis. Patients 1 and 5 had both been treated several times in different wards in the neurosurgical department while patient 11 was treated for the first time at the time of the culture. As can be seen in Table 3 isolation of *S. epidermidis* from the three patients was made on three different wards and one outpatient department during April to October. The three patients were not treated at the same time in the same ward but all had at some time been in ward no. 2. In two further cases isolates from different patients belonged to the same species and had identical antibiograms (patient 2 *vs.* 6 and patient 9 *vs* 13. Table 3). In both cases the plasmid profiles, however, indicated that they were different strains.

Except for the above mentioned patients 1 and 5 no two patients had identical

plasmid patterns but several had single, and some up to four plasmids of similar appearance. Four plasmids of the approximate weights of 1·2, 1·8, 3·2 and 5–6 MDa were more commonly seen (Table 4). None of these four could, single or in combination, be correlated to resistance to a specific antibiotic. Only in one case, patient 4, could a single plasmid be correlated to antibiotic resistance. In this patient, a 29-year-old woman who had received a ventriculoatrial shunt because of oligodendroglioma, *S. epidermidis* was found in two blood cultures (strain 4:1) 10 days after operation. The strain was resistant only to Pc and DC. *S. epidermidis* with the same antibiogram was found both in cultures from the surgical wound and in urine. Cultures from spinal fluid were initially negative, but after 3 days, and during treatment with cloxacillin, a *S. epidermidis* was isolated, sensitive to all antibiotics (strain 4:2). A large plasmid of similar appearance was present both in 4:1 and 4:2, but in addition 4:1 had a small plasmid with a molecular weight of about 3 MDa.

DISCUSSION

All isolates were lysed and although plasmid DNA was not fully purified results were reproducible and acceptable for a screening method. It is probable that some DNA bands on the gels were OC or linear DNA. As the final amount of plasmid DNA, after lysis of each isolate, was sometimes not enough for more than two runs on the gel we did not try to make a closer identification of each band. When in doubt, or if two isolates had similar plasmid profiles, we repeated lysis and sometimes restriction enzyme analysis. Repeated lysis is easily done as the method is rapid, from agar plate to finished gel electrophoresis in 4–5 h, and technically simple. Even manually handled the method could be used in relatively large scale epidemiological studies. Electrophoresis in 'mini-gel' for 1·5 h at 90 V was usually used for screening plasmids, but for best separation electrophoresis in 'standard gel' at 40 V overnight (16–20 h) was preferred. We have previously examined all isolates by a lysis method described by Parisi & Hecht (1980) (unpublished results). This method has the advantage that large amounts of plasmid DNA were obtained but on the other hand it was time-consuming, only a few isolates could be analysed at a time and too much remaining chromosomal DNA made interpretation difficult.

In accordance with earlier reports (Laufs, Heczko & Pulverer, 1978; Archer, Vishniavsky & Stiver, 1982) most of our isolates contained two or more plasmids. Altogether 24 different plasmid patterns were seen. In several patients repeated isolates showed the same plasmid pattern. That the plasmid pattern of an infecting strain can be quite stable for a long time was shown by isolates from patient 1 and 5 in whom the same strain was isolated during more than 5 months. Similarities in single plasmid bands in isolates from different patients were common and some of these may have had a common origin. Isolates from two patients (patients 1 and 5) showed identical plasmid profiles. There was no direct contact between these two patients and they may have been infected by a common hospital strain of coagulase-negative staphylococci. To demonstrate with certainty that these two patients were infected with the same strain, or that spread of plasmids or smaller pieces of DNA between other patients had occurred, the use of DNA:DNA hybridization would have been needed (Farrar, 1983; Noble, 1983).

The antibiotic resistance pattern seemed to be well correlated to the use of antibiotics in the neurosurgery department, which could indicate that many patients were infected by hospital strains or plasmids from hospital strains. TMP-SMZ was by far the most commonly used drug for prophylaxis (Table 2) and also commonly used for treatment of infections; nearly half of the isolates were resistant to this antibiotic. Multiresistance (resistance to four or more antibiotics) was seen in 11 of the 41 isolates while only 1 strain was found to be resistant to gentamicin. Earlier studies have shown that the antibiogram of coagulase-negative staphylococci is variable, probably mostly due to antibiotic usage and selection of antibiotic-resistant subpopulations from the hospital environment (Archer, 1978; Archer & Tenebaum, 1980; Davies *et al.* 1984; Watanakunakorn, 1984).

We conclude that plasmid pattern analysis is a valuable tool for epidemiologic studies of coagulase-negative staphylococci and that the method used is suitable for screening of a relatively large number of isolates.

A reservoir of antibiotic resistant coagulase-negative staphylococci may be present in the hospital. These staphylococci may colonize and sometimes cause clinically manifest infection in patients admitted to the hospital and in addition there may be a risk for the transfer of resistance factors to *S. aureus* (Forbes & Schaberg, 1983; Jaffe *et al.* 1980; Jaffe *et al.* 1982; Cohen, Wong & Falkow, 1982). Little is known, as yet, about when and how such colonizations and infections occur (Parisi, 1985; Davies *et al.* 1984).

The authors are grateful to Sten Håkansson for clincal data, Evelyn Göransson for skilful technical assistance and Ann ter Laag for preparation of the manuscript and figures.

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