Species identification, antibiotic sensitivity and slime production of coagulase-negative staphylococci isolated from clinical specimens

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SUMMARY

Two hundred and seventy-five consecutive clinical isolates of coagulase-negative staphylococci, including strains associated with disease, contaminants and skin colonizers, were speciated, tested for slime production and for their sensitivity to a range of antibiotics. *Staphylococcus epidermidis* was the most commonly identified species, comprising 63 % of all isolates. Slime production was detected in half the strains of *Staph. epidermidis*, *Staph. haemolyticus* and *Staph. saprophyticus* but was rare in other species. Most *Staph. haemolyticus* strains and approximately half of the *Staph. epidermidis* strains were resistant to five or more antibiotics. A significant association was found between slime production and multiple antibiotic resistance. For catheter-associated strains, clinical relevance was predictable by species i.e. *Staph. epidermidis*. Multi-resistant slime-positive *Staph. haemolyticus* strains, although infrequently associated with disease, were common skin colonizers, presumably acquired from the hospital environment. We describe a practical and inexpensive scheme for the speciation of human coagulase-negative staphylococcal isolates.

INTRODUCTION

Coagulase-negative staphylococci (CNS) are commensals of the human skin and mucous membranes. Although formerly regarded as contaminants of clinical specimens, they have been increasingly recognized as nosocomial pathogens in patients whose defences are compromised by an implanted foreign body or by the administration of immunosuppressive drugs (Wade *et al.* 1982; Lidwell *et al.* 1983; Eykyn, 1984; Mickelsen *et al.* 1985). Since the medical and surgical management

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of patients increasingly relies on these forms of treatment, it is to be expected that hospital-acquired infections with CNS will be an ongoing and increasing problem.

Until recently, speciation of CNS by the clinical laboratory was seldom necessary, but the recognition of these organisms as significant human pathogens has increased the need for more exact identification. Conventional biochemical tests are accurate, but too time consuming for most clinical laboratories. Commercial kits are simple to use and provide results quickly, but expense limits their widespread use. There is an urgent need for a practical and reliable scheme for the routine speciation of CNS.

Several species of CNS inhabit the human skin, but most infections are caused by *Staph. epidermidis* (Gemmell & Dawson, 1982; Marsik & Brake, 1982; Sewell *et al.* 1982; Gruer, Bartlett & Ayliffe, 1984). Strains causing infection are frequently resistant to many anti-staphylococcal antibiotics (Gruer, Bartlett & Ayliffe, 1984; Richardson, Marples & de Saxe, 1984; Davies *et al.* 1986; McAllister *et al.* 1987).

The virulence of CNS is not well understood but is probably multi-factorial. Strains associated with disease produce a wider range of extracellular toxins and enzymes than commensal strains (Gemmell, 1983). Many CNS produce an extracellular polysaccharide (slime), which is believed to facilitate the establishment of infection on the surfaces of implanted foreign bodies (Bayston & Penny, 1972; Christensen *et al.* 1982*a*, *b*; Marrie & Costerton, 1984; Davenport *et al.* 1986). Slime production appears to depend on the species of CNS (Christensen *et al.* 1983; Christensen, Simpson & Beachey, 1985) and is associated with multiple antibiotic resistance (Christensen *et al.* 1983).

This paper describes a collection of CNS isolated from specimens received by the Bacteriology Laboratory of a large teaching hospital in Australia, with adult and paediatric but no obstetric or neonatal beds. The collection includes clinically relevant isolates and skin colonizers as well as contaminants of blood cultures and other specimens. The aims of this study are to develop a practical and inexpensive scheme for the routine identification of human staphylococcal species in the hospital laboratory and to examine the relationship between species, antimicrobial sensitivity pattern, slime production and clinical relevance of CNS.

MATERIALS AND METHODS

Staphylococcal isolates

Coagulase-negative staphylococci isolated over a 6-month period were included in this study. The collection finally contained 275 strains recovered from catheter tips and prosthetic devices (80 strains), blood and other normally sterile body fluids and tissues (83 strains), urine (27 strains), skin and wounds (68 strains), peritoneal dialysis fluid (7 strains) and sputum (10 strains). Isolates were determined to be CNS and included in the study if they were Gram-positive cocci, catalase-positive and coagulase-negative by slide and tube tests. The ability to ferment glycerol in the presence of erythromycin and quantitative determination of DNAase activity were used as supplementary tests to distinguish CNS from micrococci and coagulase-positive cocci respectively. Duplicate isolates from the

same site were excluded if they belonged to the same species, and had the same antibiotic sensitivity pattern and capacity for slime production.

Identification procedure

After observation of colonial size and morphology, speciation was performed using a combination of conventional biochemical tests based on Kloos & Schleifer's simplified scheme for routine identification of human staphylococcal species (Kloos & Schleifer, 1975) and the API Staph System (API Systems SA, La Balme les Grottes, France). Because we wished to develop a simple and practical method for routine identification of staphylococci from clinical material in a busy hospital laboratory, we adapted Kloos & Schleifer's scheme so that all tests were performed on agar plates and incubation times were preferably overnight but did not exceed 48 h. Strains which failed to give clear-cut results in that period underwent supplementary testing with the API Staph System. The following American Type Culture Collection (ATCC) strains were included as controls: *Staph. saprophyticus*, ATCC 15305; *Staph. warneri*, ATCC 27836; *Staph. capitis*, ATCC 27840; *Staph. simulans*, ATCC 27851; *Staph. haemolyticus*, ATCC 29970; *Staph. xylosis* ATCC 29971; *Staph. cohnii*, ATCC 29974; *Staph. hominis*, ATCC 35982 (SP2) and *Staph. epidermidis*, ATCC 35984.

Biochemical tests procedure

A multipoint inoculator was used to deliver staphylococcal strains on to the surface of 13 test plates and one control horse blood agar plate. For most tests, a 4 h broth culture was diluted so that a final inoculum of 10^4 c.f.u. per spot was delivered to the surface of the agar plate. Phosphatase and glycerol-erythromycin plates were inoculated with undiluted cultures to increase the sensitivity of these tests. The number of strains per plate was restricted to 12, to ensure that large zones of acid production did not mask reactions of adjacent strains.

Deoxyribonuclease activity

Deoxyribonuclease (DNAase) was detected on DNAase agar (Oxoid) by the method described by Zeirdt & Golde (1970). After overnight incubation at 35 °C for 18 h, plates were flooded with 1N-HCl to precipitate unhydrolysed DNA. Large clear zones around the growth indicated strong DNAase activity typical of *Staph. aureus*, small zones of clearing were interpreted as weakly positive, and absence of a zone as negative.

Fermentation of glycerol in the presence of erythromycin

The fermentation of glycerol in the presence of erythromycin was determined as described by Schleifer & Kloos (1975) on agar containing 1% glycerol and 0.4 μg erythromycin per ml. After incubation at 35 °C overnight or for 48 h, a yellow zone surrounding or beneath the growth was interpreted as positive. Strains yielding negative results were retested by streak inoculation of an overnight culture.

Aerobic acid production from carbohydrates

Acid production from nine carbohydrates (lactose, maltose, mannitol, mannose, ribose, sucrose, trehalose, xylitol, xylose) was determined by the method of Kloos

& Schleifer (1975) on purple agar (made using Oxoid ingredients) containing 1%
carbohydrate. Plates were inspected after overnight incubation and at 48 h for a
yellow zone surrounding or beneath the area of growth.

Phosphatase

The ability to produce phosphatase was tested by the method of Barber &
Kuper (1951) on Columbia agar (Oxoid) containing 0.01% sodium phenol-
phthalein diphosphate. After overnight incubation at 35 °C, the release of free
phenolphthalein was detected using a drop of 0.880 sp. gr. ammonia placed on the
lid of the petri dish. Growth of enzyme-producing strains turned pink immediately
after exposure to ammonium vapour.

Haemolysis

Haemolysis was determined on Columbia agar containing 5% bovine blood
(Kloos & Schleifer, 1975). After incubation at 35 °C for 48 h, most strains of *Staph.
haemolyticus* produced strong haemolysis, defined as a clear zone extending 1.5
mm or more from the edge of growth. Other species were usually non-haemolytic
or produced only weak or diffuse haemolytic activity.

Slime production

Slime production was determined by examining overnight trypticase soy broth
cultures for a visible film of adherent growth which stained with safranin
(Christensen *et al.* 1982*b*). Test tubes were incubated in a tilted position to
facilitate reading, and slime production was recorded as weak (+), moderate
(+ +) or strong (+ + +), according to the density of the adherent film. Strains
SP2 (ATCC 35982), RP12 (ATCC 35983) and RP62A (ATCC 35984) (Christensen
et al. 1985) kindly supplied by Dr G. Christensen were included as controls.

Antimicrobial sensitivity

Antimicrobial sensitivity tests were performed by the method of agar dilution
(Butcher *et al.* 1986). Eleven antibiotics were incorporated individually into Iso-
Sensitest agar (Oxoid) containing 7·5% v/v sterile defibrinated lysed horse blood.
The antibiotics and the break-point concentrations used to determine sensitivity
or resistance are set out in Table 1. A replicator was used to deliver 10^4 c.f.u. per
spot on to the surface of all antibiotic media except penicillin G and methicillin.
A heavier inoculum of 10^6 c.f.u. was replicated on to penicillin and methicillin
media to aid in the detection of β-lactamase or heteroresistance. Control plates
without antibiotic were also inoculated. Plates containing methicillin were
incubated at 30 °C (Annear, 1968) and all other antibiotic plates at 37 °C. Growth
on antibiotic plates was compared with that on the control plates without
antibiotic. Any reduction, of growth of staphylococci on media containing
sulphadiazine was recorded as 'sensitive'. Apart from a single colony, any growth
detected on any of the other antibiotic plates was considered significant and
recorded as 'resistant'.

Laboratory assessment of clinical relevance

Blood culture isolates were considered clinically relevant if CNS of the same
species, having the same antibiogram, and capacity for slime production were

Table 1. *Antibiotics and break-point concentrations used to determine sensitivity or resistance*

Antibiotic	Concentration (mg/l)	Antibiotic	Concentration (mg/l)
Penicillin G	0·06	Gentamicin	1
Methicillin	4	Tobramycin	1
Erythromycin	1	Amikacin	4
Sulphadiazine	128	Chloramphenicol	20
Trimethoprim	8	Vancomycin	4
Tetracycline	5		

recovered from more than one blood culture set. All other blood culture isolates were called contaminants. Catheter tips were cultured by the method of Maki, Weise & Sarafin (1977). Growth of 15 or more staphylococcal colonies was interpreted as heavy colonization of the catheter tip (clinically relevant), whereas growth of fewer than 15 colonies indicated contamination. Conventional quantitative microscopic and cultural criteria were used to evaluate urine isolates which were classified as clinically relevant (significant bacteriuria), or as contaminating skin or enteric flora. Isolates recovered from miscellaneous skin sites, sputum, peritoneal dialysis fluids and other specimens were assessed on the basis of some or all of the following criteria: clinical notes, numbers of polymorphs and staphylococci observed in smears or wet preparations, the number of staphylococcal colonies isolated on horse blood agar, whether staphylococci were recovered in pure or mixed culture and results of other cultures from the same site.

Statistical methods

The analysis of all simple comparisons was carried out using chi-square tests. For multiple comparisons where associations between species group, multiple resistance, slime production and clinical relevance were sought, log-linear modelling (Dobson, 1983; McCullagh & Nelder, 1983) was used on the resulting four-way contingency tables. The GLIM program (Payne, 1985) was used for this analysis. This type of analysis allows the effects of all four factors to be considered and the strongest associations identified in a sequential manner. It is more appropriate than pairwise analysis of factors which may give spurious associations which arise secondarily to their relationship to other factors.

RESULTS

Speciation

By conventional biochemical tests, 150 strains gave reactions which were typical of *Staph. epidermidis*; they were phosphatase-positive, failed to ferment trehalose and mannitol, and gave reactions in other fermentation tests consistent with *Staph. epidermidis*. Nineteen of the 22 remaining *Staph. epidermidis* isolates were typical except for a negative plate phosphatase reaction. Retesting with API Staph System confirmed that these strains were indeed *Staph. epidermidis*. Presumably the plate method was insufficiently sensitive to detect their weak phosphatase activity, since they were positive in the API test for phosphatase.

The majority of *Staph. haemolyticus* strains (46 of 58) were strongly haemolytic on bovine blood agar and had biochemical profiles typical of this species. The 12 remaining strains were identified by the API Staph System, with the plate method providing confirmatory information, such as sensitivity to novobiocin.

The less frequently encountered species were most conveniently identified using a combination of conventional tests and the API Staph System. Supplementary testing with the API Staph System was most useful for strains which fermented carbohydrates slowly, and species with several variable reactions in the Kloos & Schleifer scheme. Although *Staph. saprophyticus* is the only common novobiocin-resistant species, we used API Staph as a back-up for novobiocin-resistant strains. This may not be necessary with urinary strains in a diagnostic laboratory.

Eight of the nine ATCC control strains were satisfactorily identified with our scheme. *Staph. hominis*, ATCC 35982, was incorrectly identified by the API Staph System as *Staph. haemolyticus*.

Clinical relevance

Eighty strains (29%) were assessed to be clinically relevant, including 13 of 83 (16%) blood strains, 35 of 80 (44%) catheter isolates, 25 of 27 (93%) urinary strains and all 7 peritonitis strains. Conversely, all CNS recovered from the skin and from sputum, after assessment by the criteria given in the Methods section, were classified as colonizers.

Distribution of species

The distribution of species is shown in Table 2. *Staph. epidermidis* was the most common species identified, accounting for 63% of isolates. *Staph. haemolyticus* was the second most common species, followed by *Staph. hominis*, *Staph. simulans* and *Staph. saprophyticus*. Although *Staph. epidermidis* was the species most commonly recovered from all sources, the distribution of species differed between sources ($P < 0.001$) (Table 3). *Staph. epidermidis* was by far the most common species recovered from catheter tips, comprising 60 of 80 (75%) isolates from all catheters, and 31 of 35 (89%) isolates from colonized (clinically relevant) catheters. *Staph. haemolyticus*, on the other hand, was most commonly isolated from skin (33% of *Staph. haemolyticus* isolates), contaminated catheters (19% of isolates) and contaminated blood cultures (21% of isolates). The vast majority of other species (89%) were recovered from the skin or were regarded as contaminants of catheters and blood cultures.

Capacity for slime production

Approximately one-half of the strains in this collection formed an adherent film of slime on the sides of glass tubes (Table 2). Slime production was commonly observed for strains of *Staph. epidermidis*, *Staph. haemolyticus* and *Staph. saprophyticus*, but was rare or not detected among the remaining species, i.e. *Staph. hominis*, *Staph. warneri*, *Staph. capitis*, *Staph. cohnii* and *Staph. xylosis*. The percentages of *Staph. epidermidis*, *Staph., haemolyticus* and 'other' species recovered from skin were 40%, 28% and 32% respectively (Table 3), but if only slime-positive strains are considered, these percentages become 53%, 37% and 10%. On the skin, slime production appears to be an advantage for *Staph.*

Table 2. *Capacity for slime production and multiple antibiotic resistance of the various species of coagulase-negative staphylococci*

Staphylococcus species	Number tested	Number (%) positive for slime production	Number (%) resistant to five or more antibiotics
epidermidis	172	92 (53)	84 (49)
haemolyticus	58	30 (52)	48 (83)
hominis	18	2 (11)	1 (6)
simulans	10	3 (30)	2 (20)
saprophyticus	7	4 (57)	2 (29)
warneri	5	0 (0)	0 (0)
capitis	3	0 (0)	0 (0)
cohnii	1	0 (0)	0 (0)
xylosis	1	0 (0)	0 (0)
All species	275	131 (48)	137 (50)

Table 3. *Species distribution of coagulase-negative staphylococci isolated from different sources*

Species	Number (%) of isolates from					
	Blood	Catheter	Skin	Urine	Other	All sources
Staph. epidermidis	55 (66)	60 (75)	27 (40)	19 (70)	11 (65)	172 (63)
Staph. haemolyticus	16 (19)	14 (18)	19 (28)	4 (15)	5 (29)	58 (21)
'Other' species	12 (14)	6 (8)	22 (32)	4 (15)	1 (6)	45 (16)

epidermidis and *Staph. haemolyticus*, but not for 'other' species. Further associations between slime production and the other factors are described in the section headed 'Multivariate analysis'.

Resistance to antibiotics

Fifty per cent of all isolates (Table 2) and 56% of clinically relevant isolates were classified as multi-resistant. The majority of strains of *Staph. haemolyticus* and about half the *Staph. epidermidis* strains were resistant to five or more antibiotics, but multi-resistance was uncommon among 'other' species. Further associations between multi-resistance and other factors will be discussed under the section headed 'Multivariate analysis'.

There was also a species difference in the pattern of antibiotic resistance (Table 4) with $P < 0.001$ for all of the following comparisons. Strains of *Staph. haemolyticus* were more often resistant to methicillin, trimethoprim, tetracycline, gentamicin and tobramycin than *Staph. epidermidis* and 'other' species; resistance to chloramphenicol was more common among *Staph. epidermidis* strains. 'Other' species were more sensitive to all antibiotics than *Staph. epidermidis* and *Staph. haemolyticus*. Although these 'other' species were frequently resistant to penicillin (67% of strains) and sometimes resistant to sulphonamides or tetracycline, resistance to other antibiotics was rare.

The 30 slime-positive strains of *Staph. haemolyticus* are of interest. All but two

Table 4. *Resistance to antibiotics of the various species of coagulase-negative staphylococci*

	Number (%) of resistant strains			
Antibiotic	*Staph. epidermidis* ($n = 172$)	*Staph. haemolyticus* ($n = 58$)	'Other' species ($n = 45$)	All species ($n = 275$)
Penicillin G	157 (91)	53 (91)	30 (67)	240 (87)
Methicillin	45 (26)	36 (62)	6 (13)	87 (32)
Erythromycin	54 (31)	22 (38)	2 (4)	78 (28)
Sulphadiazine	126 (73)	40 (69)	12 (27)	178 (65)
Trimethoprim	98 (57)	45 (78)	6 (13)	149 (54)
Tetracycline	66 (38)	44 (76)	10 (22)	120 (44)
Gentamicin	78 (45)	47 (81)	6 (13)	131 (48)
Tobramycin	80 (47)	46 (79)	6 (13)	132 (48)
Amikacin	0 (0)	0 (0)	0 (0)	0 (0)
Chloramphenicol	49 (28)	6 (10)	0 (0)	55 (20)
Vancomycin	0 (0)	0 (0)	0 (0)	0 (0)

were multi-resistant and over half displayed a characteristic sensitivity pattern with resistance to penicillin, methicillin, sulphadiazine, trimethoprim, tetracycline, gentamicin, tobramycin, and sometimes erythromycin. The same pattern was uncommon among slime-negative strains of *Staph. haemolyticus* (4 of 28) and among *Staph. epidermidis* strains (11 of 172).

Multivariate analysis

Because this collection includes all CNS submitted for antibiotic sensitivity testing over a given period, selection criteria differed between sources. The data were therefore divided into sources, and associations between species, slime, multi-resistance and clinical relevance were sought using multi-way contingency tables and the GLIM statistical package.

For blood isolates, the strongest association was between slime and multi-resistance ($P < 0.005$), with multi-resistance and slime occurring together. No other associations were statistically significant. With catheter strains, species and clinical relevance were associated ($P < 0.025$), with *Staph. epidermidis* being more often clinically relevant than *Staph. haemolyticus* and 'other' species. After adjusting for this effect, slime and resistance showed an association ($P < 0.05$), again with resistance and slime occurring together. No further associations were noted.

For skin, there was no clinically relevant isolate. The strongest effect noted was between species and multi-resistance ($P < 0.0005$), with *Staph. haemolyticus* being more resistant than *Staph. epidermidis*, which was more resistant than 'other' species. After adjusting for this effect, slime and multi-resistance showed an association ($P < 0.001$) with resistance and slime occurring together. A weaker association remained between species and slime ($P < 0.05$), *Staph. epidermidis* being a better slime producer than *Staph. haemolyticus* and 'other' species.

There were insufficient numbers of isolates from other sources for meaningful analysis.

Thus the only association found for the three most common sources was

between slime and multi-resistance. Clinical relevance was predictable by species for catheter strains, but was not associated with other factors. Skin strains of *Staph. haemolyticus* were more resistant than strains of *Staph. epidermidis* and 'other' species. For blood cultures, only two species were clinically relevant; *Staph. epidermidis* and *Staph. haemolyticus*.

DISCUSSION

Because of the increasing recognition of CNS as nosocomial pathogens, there is an urgent need for an identification scheme which is practical and inexpensive. Kloos & Schleifer (1975) have proposed a scheme for the routine identification of human staphylococcal species, but incubation periods of up to 5 days and the need to use a variety of solid and liquid media make this scheme impractical for most clinical laboratories. The API Staph System comprises 19 biochemical tests which are read after overnight incubation. More than 88 % of staphylococci from clinical sources can be identified to species level with this system (Gemmell & Dawson, 1982; Giger, Charilaou & Cundy, 1984), but expense precludes its routine use by many laboratories. Recently Reuther (1986) described a method based on Kloos & Schleifer's simplified scheme, in which strains were multipoint-inoculated on to a range of agar media and results read after 18 h of incubation. There was 90·7 % agreement between identifications with this system and the API Staph System. We believe the most convenient scheme combines both these methods. Multipoint inoculation of a range of agar media identified most clinical isolates of CNS within 24 h. The remaining strains could be identified by additional testing with the API Staph System. This scheme is both inexpensive and convenient, particularly for clinical laboratories using the agar dilution method of antibiotic sensitivity testing. Indeed, a screen consisting of only five key tests (mannitol fermentation, trehalose fermentation, alkaline phosphatase, novobiocin sensitivity and hae- molysis of bovine blood agar) would be sufficient to identify, with reasonable accuracy, most strains of *Staph. epidermidis*, *Staph. haemolyticus* and *Staph. saphrophyticus* which comprise, in the present study, over 80 % of clinical isolates.

In this study, the ability to ferment glycerol in the presence of erythromycin and strong DNAase activity were used as supplementary tests to confirm the identity of isolates. Although a number of tests have been recommended for the separation of staphylococci from micrococci, no single test is both reliable and practical (Goodfellow, 1987). Schleifer & Kloos (1975), using staphylococci from a variety of sources, including human skin, animals, food and the environment, found that less than 2 % of staphylococci were glycerol/erythromycin-negative. In a study in our laboratory, only 1·2 % of 166 human strains were glycerol/ erythromycin-negative. One objection might be that some coagulase-negative staphylococcal strains were excluded from this study because only DNAase- negative strains were included. However, in another study in our laboratory only 1·8 % of 166 CNS strains were DNAase-positive by our plate method, and even these produced this enzyme in small amounts.

Although several species of CNS inhabit the skin (Kloos & Musselwhite, 1975), studies of clinical isolates show that from 57 to 85 % are *Staph. epidermidis* (Eng *et al.* 1982; Marsik & Brake, 1982; Sewell *et al.* 1982; Needham & Stempsey, 1984).

If strains associated with disease are selected, *Staph. epidermidis* accounts for 65–93 % of isolates (Eng *et al.* 1982; Sewell *et al.* 1982; Gruer, Bartlett & Ayliffe, 1984; Needham & Stempsey, 1984; Richardson, Marples & de Saxe, 1984). *In vitro* studies using the rat model of endocarditis and *in vivo* studies of phagocytic killing indicate the greater virulence of *Staph. epidermis* compared with other CNS (Baddour *et al.* 1984). In the present study *Staph. epidermidis* accounted for 63 % of clinical isolates and 89 % of clinically relevant catheter isolates, confirming that this species is the most common clinical isolate and supporting a pathogenic role for *Staph. epidermidis* in catheter-related sepsis.

The results presented in this paper agree with several other studies, which have shown a high incidence of antibiotic resistance in CNS isolated from both colonized and infected patients in hospital (Marsik & Brake, 1982; Richardson & Marples, 1982; Gruer, Bartlett & Ayliffe, 1984; Varaldo *et al.* 1984; Davies *et al.* 1986; McAllister *et al.* 1987; Younger *et al.* 1987). Antibiotic-resistance profiles reported from different parts of the world and from different hospitals vary (Richardson & Marples, 1982; Varaldo *et al.* 1984), possibly reflecting different patterns of antibiotic use. Several investigators have reported that strains of *Staph. haemolyticus* are significantly more resistant to antimicrobial agents than other staphylococcal species (Price & Flournoy, 1982; Gill, Selepak & Williams, 1983; Hamilton-Miller & Illiffe, 1985; Davies *et al.* 1986; Del Bene *et al.* 1986). In the present study, strains of *Staph. haemolyticus* were more resistant to methicillin, gentamicin, tobramycin, trimethoprim and tetracycline than were strains of *Staph. epidermidis*. *Staph. epidermidis* strains fell into two equal groups: those resistant to five or more antibiotics, and relatively sensitive strains. 'Other' coagulase-negative staphylococcal species were usually resistant to penicillin, sometimes to sulphonamides or tetracycline, but rarely to other antibiotics. Thus there were two distinct populations of CNS: multi-resistant *Staph. epidermidis* and *Staph. haemolyticus* strains – which were presumably acquired from the hospital environment – and sensitive community-acquired strains of *Staph. epidermidis* and several other species. Because this study took place in one hospital over a 6-month period, it is possible that the multi-resistant *Staph. haemolyticus* and *Staph. epidermidis* strains represented a small number of clones that spread throughout the hospital. Since plasmid analysis was not performed, this possibility cannot be discounted. In the 3 years since the collection of strains for this study, however, the small changes in antibiotic sensitivity patterns of CNS do not suggest the appearance or disappearance of clones of resistant CNS.

Slime production has been detected in most species of CNS (Christensen *et al.* 1983; Needham & Stempsey, 1984; Christensen, Simpson & Beachey, 1985). In this study, over half of the strains of *Staph. epidermidis*, *Staph. haemolyticus* and *Staph. saprophyticus* adhered to the sides of glass test tubes compared with 30 % of *Staph. simulans* and 10 % of *Staph. hominis* strains. Christensen, Simpson & Beachey (1985), using a microtitre plate adherence method, also noted that the most strongly adherent strains were *Staph. epidermidis*, *Staph. haemolyticus* and *Staph. saprophyticus* but in their study, only 42 % of 33 *Staph. hominis* strains were non-adherent. The hypothesis that slime production by some CNS may play a part in enabling staphylococcal microcolonies to adhere to Holter shunts was first postulated by Bayston & Penny (1972). Subsequently Christensen *et al.*

(1982*b*), investigating an outbreak of catheter-related sepsis, recovered a significantly greater proportion of slime-producing strains from patients with sepsis than from contaminated blood cultures. Two recent studies (Davenport *et al.* 1986; Ishak *et al.* 1985) support the view that extracellular slime production is an important colonizing and virulence factor in coagulase-negative staphylococcal infections associated with a prosthetic device, but others (Needham & Stempsey, 1984; West *et al.* 1986) have been unable to confirm these findings. There is recent evidence, however, suggesting that peritonitis (Kristinsson, Spencer & Brown, 1986) and shunt infections (Diaz-Mitoma *et al.* 1987; Younger *et al.* 1987), with slime-producing strains are more difficult to eradicate than similar infections due to non-slime-producing CNS.

There are a number of possible reasons why we were unable to demonstrate a significant association between slime production and disease. First, laboratory criteria, although predictive of heavy colonization or the continued presence of bacteria at a particular site, may not equate with clinical relevance. Indeed, since nosocomial strains of CNS are initially acquired as colonizing flora and subsequently become pathogens only in patients with specific risk factors, it is probable that these two populations of staphylococci have the same characteristics. Moreover, conventional semi-quantitative catheter tip cultures may underestimate the number of slime-producing cells if they remain adherent or are released as micro-colonies. Conversely, the practice of considering two separate blood culture isolates identical if they belong to the same species, have the same antibiotic sensitivity pattern and capacity for slime production, could overestimate the number of clinically relevant isolates.

Secondly, as a measure of the adherence of CNS to smooth surfaces, the tube assay suffers from a number of disadvantages. Some investigators have reported good reproducibility of the tube adherence assay (Davenport *et al.* 1986; Diaz-Mitoma *et al.* 1987), but in our experience reproducibility of tests read by the same investigator was 76 % for strains stored on slopes at 4 °C for periods up to 4 weeks and 68 % for strains stored at -70 °C for 3 years. Because of the subjectivity in reading the tube test and the variation in the amount of slime produced by different strains, the quantitative adherence test (Christensen *et al.* 1985) may be more reliable than the tube test. Another variable which affects the production of extracellular slime is the composition of the growth medium (Christensen *et al.* 1982*b*; Christensen, Simpson & Beachey, 1985; Peters *et al.* 1987). Inded, if suitable media and sensitive assays are used, most CNS can be shown to produce slime (Peters *et al.* 1987).

Finally, it is now accepted that adherence and slime production are separate phenomena, and that quantitative and qualitative assays determining adherence to smooth surfaces are not necessarily proof of the production of extracellular slime. The latter can most reliably be determined by reactivity to mannose-specific lectins (Peters *et al.* 1987). In the light of this recent work, our observations on 'slime production' should perhaps more accurately be described as observations on 'adherence'. Taken together, these observations suggest that, although most CNS, given suitable conditions, are capable of producing slime, they only do so under environmental conditions in which extracellular polysaccharide enhances their virulence or their ability to colonize surfaces. This

explanation would accommodate two apparently opposing findings: that slime production, assessed by qualitative or quantitative adherence assays, is characteristic of strains associated with infection and that most CNS produce slime, given a suitable growth medium.

The association between slime production by CNS and resistance to multiple antibiotics has been noted previously (Bayston & Penny, 1972; Christensen *et al.* 1983). Our studies extend these observations to *Staph. haemolyticus*. The majority of slime-producing strains of this species were resistant to at least seven antibiotics and were recovered most often from the skin, contaminated catheter tips and contaminated blood cultures. *Staph. haemolyticus* favours moist skin sites such as the axilla, groin and perineum (Kloos, 1986), and has been isolated more frequently from the skin of hospitalized patients than from controls, suggesting that this species is commonly acquired by patients after their admission to hospital (Larson *et al.* 1986). Our observations support this view, and also suggest that capacity for slime production may be another selective factor favouring skin colonization by multi-resistant *Staph. haemolyticus*. This is interesting because of the increasing evidence that CNS colonizing the surface of the skin are able to transfer antibiotic resistance to the more virulent *Staph. aureus* (Jaffe *et al.* 1980; Archer & Johnston, 1983) as well as to other species of CNS (Naidoo & Noble, 1987). If slime-producing multi-resistant *Staph. haemolyticus* strains preferentially colonize moist skin sites of hospitalized patients, these organisms might be important reservoirs of resistance genes in the hospital environment.

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