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Production of Extracellular Slime by Coryneforms Colonizing Hydrocephalus Shunts

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***Corynebacterium* spp. are responsible for an important minority of cases of colonization of cerebrospinal fluid shunts used for the treatment of hydrocephalus. In common with coagulase-negative staphylococci, they present a serious therapeutic problem because they are often resistant to multiple antibiotics. We studied the morphologies of coryneforms in colonized hydrocephalus shunts removed from patients and observed extracellular slime similar in appearance to that seen in coagulase-negative staphylococci. We also studied a series of such isolates from other cases of hydrocephalus shunt colonization using an established laboratory model and consistently observed slime production in these shunts as well. We propose that this might be a further reason for failure to eradicate these organisms without shunt removal as well as a factor in their pathogenesis in device-related infections.**

Coryneforms have been incriminated in a range of implant-associated infections, although they are less common than coagulase-negative staphylococci. The coryneforms, and especially *Corynebacterium jeikeium*, can exhibit resistance to multiple antimicrobial agents (7, 13, 23, 26, 28), and as in the case of coagulase-negative staphylococci, there is evidence that removal of the colonized device is important in the management of infected patients, especially if they are immunocompromised (2, 21, 25, 27, 31). Rozdzinski et al. (29) reported 23 patients, all immunocompromised, with *C. jeikeium* infections of their intravascular catheters. The intravascular catheters were left in place in all patients, and the organisms were eradicated from the catheters of 18 patients, but relapses occurred in 5 patients, and 3 of these patients died of the infection. Infections of hydrocephalus shunts caused by coryneforms have also been reported (1, 4, 10, 13, 19, 20, 22, 23, 26, 27). Some of these infections have resulted in serious complications such as renal failure.

We studied eight coryneforms isolated from patients with proven hydrocephalus shunt infections, and we examined the shunts removed from four additional patients by scanning electron microscopy (SEM). The eight isolates were also tested for their abilities to colonize silicone shunt catheters in an in vitro model, and further morphological studies were carried out on these isolates in order to determine the mode of colonization of implants by these organisms.

MATERIALS AND METHODS

Sources of the organisms. The eight strains were isolated from shunts removed from patients with clinically and microbiologically proven hydrocephalus shunt infections. The same organisms were also isolated from cerebrospinal fluid and a culture of blood from one patient. Seven of the shunts were ventriculoperitoneal and one was ventriculoatrial. Four of the patients were female children, one was a male child, two were adult males, and one was an adult female.

Within 18 h of removal of the shunts from the patient, samples were obtained from the shunts by a previously published protocol (8), and the luminal fluid was used to prepare Gram films and cultures. All strains were isolated on Columbia agar (Unipath, Basingstoke, United Kingdom) containing 7% defibrinated horse blood (Unipath) incubated aerobically at 37°C. Up to 4 days was required for the growth of some strains, although less time was required after several subcultures.

Identification. Strains were identified by using the API-Coryne test gallery (Biomérieux, Basingstoke, United Kingdom). In some cases, additional tests were also carried out, and those which gave equivocal results were checked for purity and were sent to the Central Public Health Laboratory, London, United Kingdom, for confirmation of the results.

Antimicrobial susceptibility. The antibiograms of the strains were determined by using a modified Stokes' method (32) on DST agar (Unipath) containing 7% lysed horse blood (Unipath). Fifteen antimicrobial agents were tested, and methicillin susceptibility was tested separately by incorporation of the agent into agar at 30°C. The antibiograms were converted into a five-digit code (Table 1) (7). This system was used primarily as part of the characterization of strains, but it also gave an indication of resistance to multiple antimicrobial agents.

Electron microscopy of removed shunts. Four removed shunts were available for SEM. These were fixed as 5-mm segments in buffered glutaraldehyde. The segments were then carefully cut in half along the long axis and were gently rinsed in deionized water to remove the buffer and fixative. They were then immersed in progressively higher concentrations of ethanol in water and were dehydrated in absolute ethanol, following which they were dried in a critical point dryer (Polaron) by using carbon dioxide and were sputter coated with gold before examination by SEM (Jeol JFM 35).

Testing of isolates for ability to colonize catheters in vitro. A slightly modified version of the model of catheter colonization described previously (5) was used. This consists of the use of a vertically mounted silicone catheter in a controlled environment chamber so that temperature, humidity, and partial O_2 pressure can be standardized and kept constant over long periods. A separate circuit enables long-term perfusion of the

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TABLE 1. Derivation of numerical antibiogram

Antimicrobial agent	Resistance score[a]	Five-digit codon
Penicillin	4	7
Tetracycline	2	
Chloramphenicol	1	
Erythromycin	4	7
Methicillin	2	
Trimethoprim	1	
Clindamycin	4	7
Gentamicin	2	
Rifampin	1	
Amikacin	4	7
Netilmicin	2	
Cefuroxime	1	
Vancomycin	4	7
Spiramycin	2	
Fusidic acid	1	

[a] Resistance is scored as indicated in the column, and the scores for each drug in each triplet were added to give the score in the last column. Susceptibility receives a score of zero. The numerical antibiogram therefore consists of a five-digit codon representing data for 15 antimicrobial agents. The codon exemplified here would represent a pattern of total resistance.

catheter with nutrient solutions, indefinitely if necessary. For the work described here, each catheter was inoculated aseptically by using a port and a sterile 26-gauge needle. All eight strains were tested. After inoculation of approximately 10^7 CFU of each strain, the silicone catheters were perfused with brain heart infusion (Unipath) or a medium consisting of yeast extract (Unipath) at 1.25 g/liter, sodium chloride at 4.3 g/liter, glucose at 0.8 g/liter, urea at 0.2 g/liter, and creatinine at 0.03 g/liter. This latter medium was found by experimentation to be the medium with the least amount of nutrients which would still support growth in the perfusion system. It was used because cerebrospinal fluid is itself poor in nutrients, and a nutrient-limited situation therefore needed to be tested. A flow rate of approximately 3.5 ml/h was used, and two catheters were perfused with each medium. Effluent samples from the output end of the model were cultured daily. After 4 days the catheters were aseptically removed from the model, processed as described above for shunts removed from patients, and prepared for examination by SEM.

RESULTS

Identification of strains. The identities of the strains are given in Table 2. The identification of coryneforms is notoriously difficult, even though several schemes have been devised (3, 7, 16, 28). The API-Coryne system suffices for many strains, but it leaves some strains unidentified (11). Further tests were carried out on some strains. Although the identity of strain F186 could not be completely certified, it was nonhemolytic on sheep blood agar but was weakly positive by the CAMP test and, on first isolation, produced characteristic pink colonies. The API-Coryne system profile of strain F1122 was not typical of *C. jeikeium* but it was distinguished from *Corynebacterium minutissimum* by its colonial morphology and the ability to grow in 6.5% NaCl. One strain from a removed shunt which was examined by SEM (the strain died in storage) was unidentifiable in the API-Coryne system beyond *Corynebacterium* sp. Two of the remaining three shunts which were examined by SEM grew *Corynebacterium xerosis* (not available for further investigation), and the other grew an organism identified as either *Corynebacterium striatum* or CDC group G-1 (strain F436).

TABLE 2. Sources, characteristics, and identification of the strains studied

Strain no.	Source[a]	Antibiogram	API-Coryne profile	Identity
F171	VP shunt, CSF	61242	2100304	*C. jeikeium*
F184	VP shunt, CSF	77622	3100324	CDC group I-2
F185	VP shunt, CSF	77413	2100304	*C. jeikeium*
F186	VP shunt, CSF	31001	3150004	*Rhodococcus equi*
F276	VA shunt, CSF, B/C	01000	3100324	CDC group I-2
F436	VP shunt, CSF	15502	3100305	*C. striatum* or CDC group G-1
F1009	VP shunt, CSF	53023	6112004	*Brevibacterium* sp., CDC group B
F1122	VP shunt, CSF	01000	2100324	*C. jeikeium*

[a] VP, ventriculoperitoneal; CSF, cerebrospinal fluid; VA, ventriculoatrial; B/C, blood culture.

Susceptibilities to antimicrobial agents. Four of the eight strains were resistant to penicillin and four were resistant to methicillin. Only one strain, strain F436, was resistant to rifampin, and all strains were susceptible to vancomycin. Two of the *C. jeikeium* strains (strains F171 and F185) and the very similar CDC group I-2 strain (strain F184) were resistant to multiple antimicrobial agents (Table 2).

Ability to Colonize catheters in vitro. All eight strains produced effluent viable counts of $\geq 10^7$ CFU/ml within 3 days of inoculation of the catheters, and visible biofilms were produced on the luminal surfaces in all cases. On definitive culture of aspirates of the catheters after their removal from the model, the same organisms as those inoculated were retrieved, with no evidence of contamination.

SEM of catheters from the in vitro model. Clear evidence of slime production, visually very similar to that seen in catheters colonized by coagulase-negative staphylococci (Fig. 1), was seen with all strains (Fig. 2 to 4). There were no major differences in slime production between the two perfusion media. The extent of colonization found in low-magnification views of catheters from the model resembled those found in low-magnification views of catheters freshly removed from infected patients (Fig. 5 and 6).

SEM of shunts removed from patients. Large coalescent colonies were present in all four shunts removed from patients (Fig. 6), with clear evidence of slime production (Fig. 7 and 8) that was visually very similar to that seen in the catheters colonized in vitro.

DISCUSSION

The two major causes of failure of antimicrobial therapy of hydrocephalus shunt infections are the difficulty in achieving effective concentrations of an antimicrobial agent(s) in the cerebrospinal fluid and the resistance of the infecting organism to antimicrobial agents (6, 12). Four of the organisms tested in the present study were resistant to penicillin, and three were resistant to multiple antimicrobial agents. However, all strains were susceptible to vancomycin and all but one were susceptible to rifampin, as has been found previously (7, 17, 33). Another potential cause of the failure of patients with implants

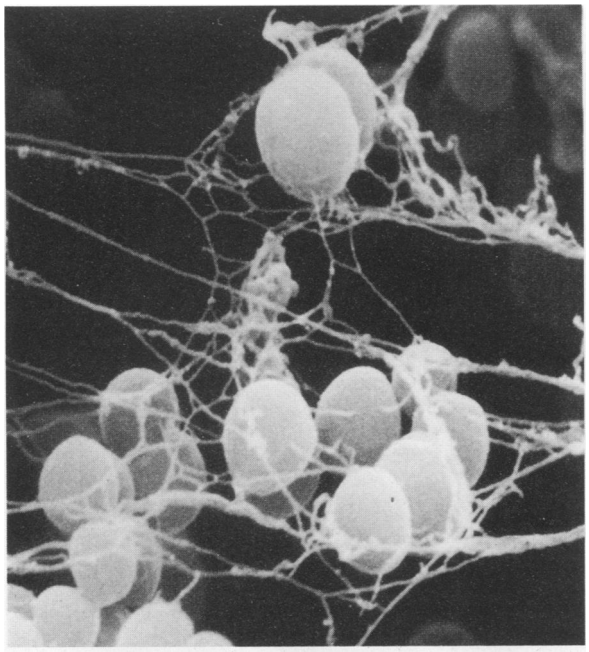

FIG. 1. Scanning electron micrograph of *S. epidermidis* in a colonized cerebrospinal fluid shunt showing slime production. Magnification, ×15,000.

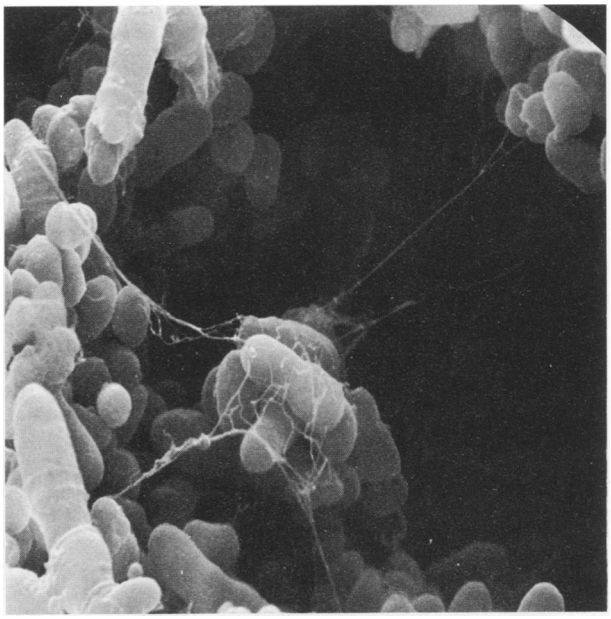

FIG. 3. Scanning electron micrograph of colonized catheter from the in vitro model showing slime production by strain F276 (CDC group I-2). Magnification, ×6,600.

colonized by coagulase-negative staphylococci to respond to antimicrobial therapy is the production of slime (24, 30), a well-known phenomenon in coagulase-negative staphylococci (9, 15, 24). Although corynebacteria have been observed in association with biofilms on a scleral buckling device (14), we think this is the first comprehensive report of slime production by coryneforms associated with implant colonization. It is conceivable that the material seen in Fig. 7 and 8 is host derived (e.g., fibrin), although this is very unlikely in view of the production of apparently identical material seen in vitro (Fig. 2 to 4) in the absence of access to relevant host substances. The appearance of staphylococcal slime on SEM depends on the method of fixation and processing. Some methods result in a high degree of condensation of the matrix, to give a solid covering over the microcolonies, while the

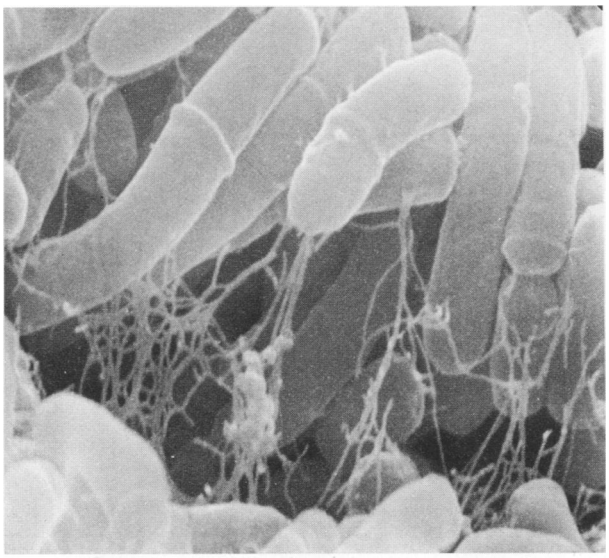

FIG. 2. Scanning electron micrograph of colonized catheter from the in vitro model showing slime production by strain F185 (*C. jeikeium*). Magnification, ×16,000.

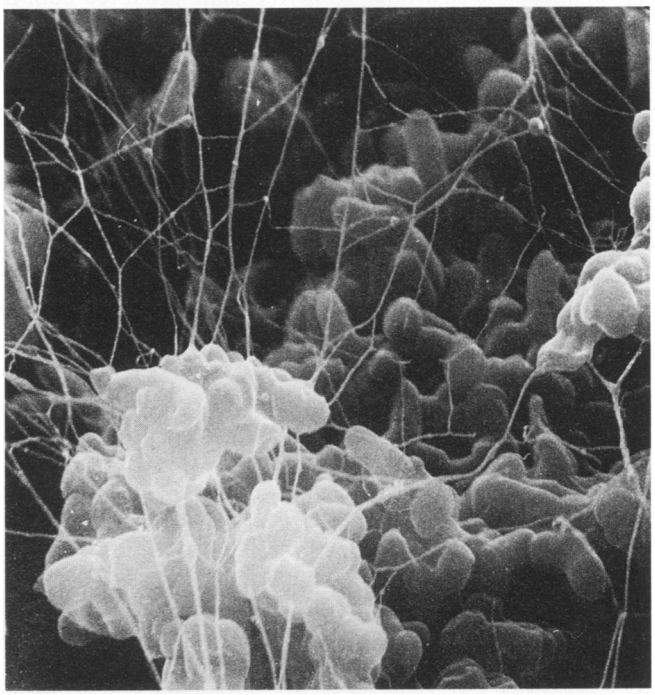

FIG. 4. Scanning electron micrograph of colonized catheter from the in vitro model showing slime production by strain F1009 (*Brevibacterium* sp., CDC group B). Magnification, ×7,800.

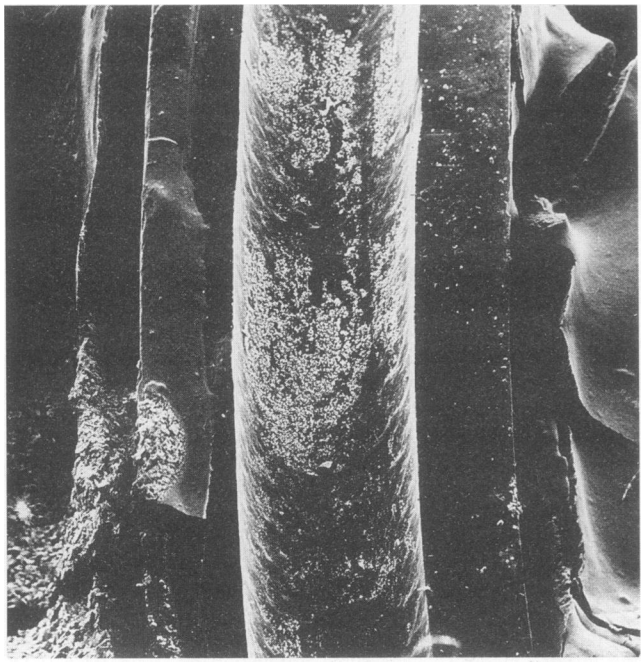

FIG. 5. Scanning electron micrograph of strain F1122 (*C. jeikeium*) colonizing a catheter in the in vitro model. Magnification, ×20.

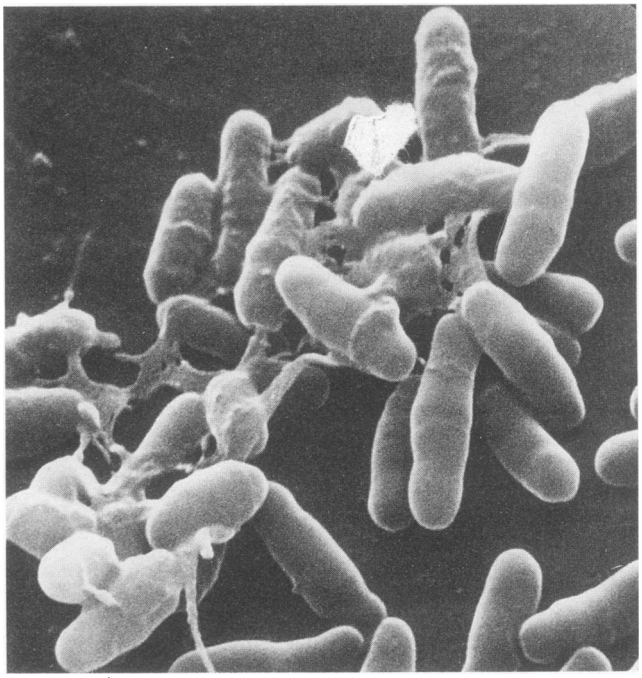

FIG. 7. Scanning electron micrograph of *C. xerosis* colonizing the distal catheter of a surgically removed shunt. Magnification, ×15,000.

method used in the present study gives a more open appearance, possibly because of a greater amount of conservation of the matrix structure. It should be remembered, however, that the appearance of neither structure is an accurate representation of fully hydrated slime, although both vital preparations (9) and transmission electron micrographs (18) suggest an open, arachnoid structure. While the slime produced both in vivo and in vitro by the coryneforms tested in the present study is visually identical to that of *Staphylococcus epidermidis* (Fig. 1), its physicochemistry, and therefore its interaction with antimicrobial agents, might be quite different. However, it is interesting that four of our isolates and two removed shunts were from patients in whom relapses had occurred after an attempt to treat the infection with seemingly appropriate antimicrobial agents without removal of the shunts. For the remaining patients, the shunt had been removed as part of the initial management, without a trial of a nonsurgical approach.

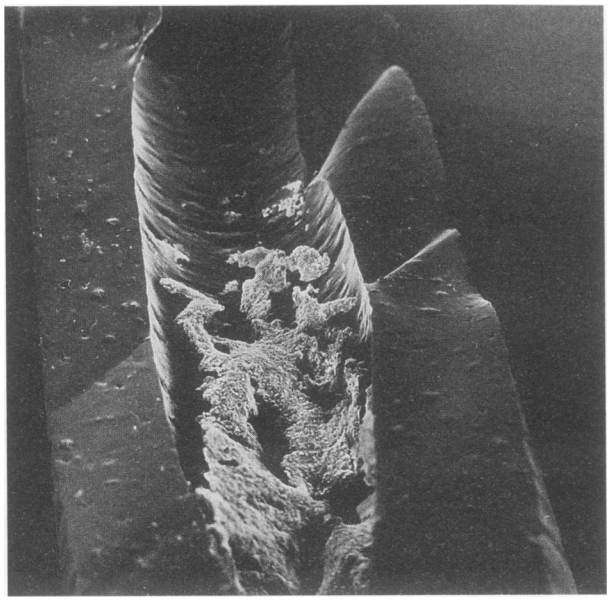

FIG. 6. Scanning electron micrograph of strain F1122 (*C. jeikeium*) colonizing the distal catheter of a surgically removed shunt. Magnification, ×20.

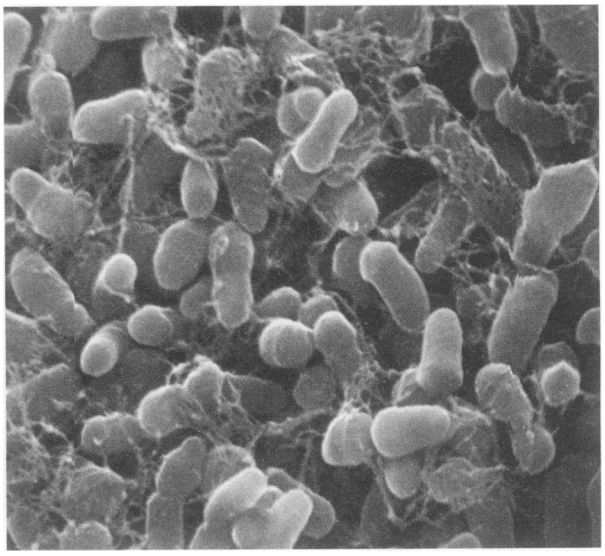

FIG. 8. Scanning electron micrograph of F1122 (*C. jeikeium*) colonizing the distal catheter of a surgically removed shunt. Magnification, ×16,000.

The production of extracellular slime by coryneforms probably contributes to their pathogenesis in device-related infections and might also be responsible for additional therapeutic difficulties, as in the case of infections caused by coagulase-negative staphylococci.

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