Comparative Virulence of *Staphylococcus epidermidis* Isolates in a Murine Catheter Model

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ABSTRACT

Among coagulase-negative staphylococci, *Staphylococcus epidermidis* is the species most commonly implicated in catheter-related infections. Whether some staphylococcal organisms are inherently more virulent than others, or whether their ability to infect relates more to the sheer numbers of organisms at the catheter site, remains unclear. We therefore compared eight *S. epidermidis* isolates and two other coagulase-negative staphylococci using a murine model that allowed us to quantify catheter colonization and abscess formation in the same animal. The organisms were isolated from different clinically relevant settings and were classified according to their slime phenotype. The ability to evoke abscesses or colonize catheters in half of the animals (ID_{50}) was assessed. ID_{50} inoculum titers ($\log_{10}$ data $\pm$ SD) ranged widely, from 8.5 ± 0.3 to 10.2 ± 0.2 for abscess formation ($p < 0.005$) and from 7.5 ± 0.5 to 10.3 ± 1.0 for catheter colonization ($p < 0.005$). ID_{50} values by statistical

criteria suggested variability among organisms in the ability to induce abscess formation. High slime production correlated with both parameters, but not with the clinical source of the isolate. Our findings demonstrate impressive heterogeneity in the ability of a representative group of *S. epidermidis* isolates to colonize catheters and to evoke abscess formation and implicate slime productivity as a major virulence factor. The murine model used permitted simultaneous analysis of multiple factors involved in pathogenesis and should be useful in establishing the basis of *S. epidermidis* pathogenicity. (*Pediatr Res* 37: 70-74, 1995)

Abbreviations

ConS, coagulase-negative staphylococci
CVC, central venous catheter
ID, infective dose
cfu, colony-forming unit

ConS are the most common cause of infections in patients with foreign body devices (1-3). Most of these infections occur in patients with CVC, although a substantial number develop in patients with cerebrospinal shunts or with devices required for continuous ambulatory peritoneal dialysis (4-8). Among ConS organisms, *Staphylococcus epidermidis* is the species with the greatest pathogenic potential (3). The basis for *S. epidermidis*' ability to cause infections has been studied for at least two decades, without a definitive conclusion. Inasmuch as *S. epidermidis* is a commensal skin and mucous membrane organism (9), its pathogenicity may be related to sheer numbers of the organism and their proximity to a medical device. Biochemical properties intrinsic to *S. epidermidis* include the production of a slime layer (glycocalyx), adhesins, and exoproteins as well as resistance to multiple antibiotics; all have been proposed as virulence factors (4, 10-17). Yet only the slime layer pheno-

type has been shown by a number of independent investigators to correlate with the clinical features of infection (4, 18, 19).

One of the difficulties in assessing slime production in the pathogenesis of CVC-related staphylococcal infections has been the lack of suitable experimental systems. In this report, we describe studies with an immunocompetent mouse catheter model, adapted from Christensen *et al.* (20) and Patrick *et al.* (21), which permitted evaluation of the slime phenotype by two distinct criteria: abscess formation and catheter colony counts. The results suggest wide variability in *S. epidermidis* isolates as assessed by these two parameters and support a prominent role for the slime layer in pathogenesis.

METHODS

Bacterial strains and preparations. We assessed eight *S. epidermidis* isolates [MB-1, RP62A (American Type Culture Collection, ATCC, Rockville, MD), MP, MB-4, HZ-1, HM-1, MLC, and MLL] and one isolate each of *Staphylococcus haemolyticus* (ATCC 29968) and *Staphylococcus Genus capitis* (ATCC 27840). These *S. epidermidis* organisms were from confirmed infections (22) in patients with or without a CVC or with a cerebrospinal fluid shunt (Table 1) because the organism

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did not grow in clumps facilitating cfu quantification. Organisms were not serially passaged but were stored in buffered tryptic soy broth with 10% glycerol at -70°C . A commercially available identification system (American Microscan; Baxter, West Sacramento, CA) was used for biotype analysis and antibiogram susceptibility testing.

Slime layer quantitation. To classify the organisms by their slime-producing ability, we used the method of Christensen *et al.* (23). Measurements were performed in quadruplicate on three occasions and then averaged.

Animal model. A murine catheter model (21) as modified from Christensen *et al.* (20) was used in all experiments. Briefly, a 0.5-cm section of a 14-g Teflon catheter (Quick-Cath; Travenol Laboratories, Inc., Deerfield, IL) was inserted s.c. through a 0.5-cm incision in the interscapular area of 6- to 8-wk-old CD-1 mice (Charles River Laboratories, Raleigh, NC). The wound was closed with a single stitch of 4.0 silk thread. (Control mice lacked surgical incisions.) The animals were allowed to rest for 3 d, at which time they were examined for poor wound healing or infection. Animals with adequate healing received injections with titrated organisms at concentrations of 10^7 to 10^{11} cfu/mL. Each isolate was studied at four dilutions with a minimum of four animals tested per dilution. After the approximate ID was determined in separate experiments, additional animals were tested at selected titers to find the ID₅₀ (see "Statistical design").

The organisms at the five concentrations were injected in 100 μL of PBS along the length of the catheter; a second cohort of mice with catheters received injections of PBS as a control. After 8 d, all animals were examined by one of the investigators without knowledge of the inoculum for the presence and location of abscesses. Catheters were removed and cultured in 1 mL of Columbia broth (Edge Biological, Inc., Memphis, TN). Quantitative cultures were performed as per

Sherertz *et al.* (7) using a 30 W sonicator (RAI, New York, NY) for 1 min and plated on sheep red blood agar. The plates were incubated for 48 h at 37°C , after which the colonies were counted. Catheter colonization was defined as the growth of organisms with an identical phenotype as the inoculating organism with quantitative counts of ≥ 5 cfu. Abscess formation was defined as any grossly visible collection of purulent material. Organisms isolated from the catheter or wound site were evaluated for biotype, antibiogram, and quantity of slime production compared with the original isolate.

Statistical design. Inoculum titers required to evoke abscess formation or catheter colonization in 50% of the animals (ID₅₀) were estimated with a probit model with the SAS statistical software package (SAS Institute, Inc., Cary, NC). Differences in log₁₀ ID₅₀ estimates ($\pm$ SD) were tested for statistical significance using normal distribution approximations for the maximum likelihood estimates. The Wald χ^2 test was used to compare differences in abscess formation and catheter colonization according to the level of slime production. Comparison of catheter adherence as defined by cfu per centimeter squares of catheter were evaluated by a Mann-Whitney U test.

RESULTS

The phenotypes and sites of origin of the eight *S. epidermidis* isolates are shown in Table 1. MB-1, RP62A, MP, and MB-4 were blood isolates from patients with CVC-associated bacteremia; HZ-1 and HM-1 were blood isolates from neonates with persistent bacteremia in the absence of indwelling catheters or other medical devices and MLC and MLL were cerebrospinal fluids isolates from patients with cerebrospinal fluid shunt infections. For comparison, there were also single isolates of *S. capitis* and *S. haemolyticus* (ATCC 27840 and ATCC 29968). The characteristics of organisms recovered

Table 1. Origin and phenotype of *ConS* isolates

Isolate	Origin	Phenotypes		
		Biotype*	Antibiotic susceptibility†	Slime production (mean OD _{570 nm} $\pm$ SD)‡
MB-1	Patient with CVC-related bacteremia	307164	Cp, Rif, V	1.084 $\pm$ 0.233
RP62A (ATCC 35984)	Patient with CVC-related bacteremia	395164	Cp, Rif, Tet, V	0.478 $\pm$ 0.042
MP	Patient with CVC-related bacteremia	717164	V	0.036 $\pm$ 0.014
MB-4	Patient with CVC-related bacteremia	307064	Cd, Cp, E, Rif, Tet, V	0.088 $\pm$ 0.001
HZ-1	Neonate with bacteremia in the absence of CVC	703124	Cp, Rif, Tet, TS, V	0.620 $\pm$ 0.40
HM-1	Neonate with bacteremia in the absence of CVC	707064	Cd, Cp, E, Rif, Tet, TS, V	0.219 $\pm$ 0.005
MLC	Patient with CSF shunt infections	207064	Cd, Cp, E, Rif, Tet, TS, V	0.316 $\pm$ 0.013
MLL	Patient with CSF shunt infections	307614	Cd, Cefot, Cp, E, G, I	0.073 $\pm$ 0.014
<i>S. capitis</i> (ATCC 27840)		306100	Cd, Cefot, Cp, E, G, I, Ox, Rif, V	0.060 $\pm$ 0.008
<i>S. haemolyticus</i> (ATCC 29968)		30231	Cd, Cefot, CP, G, I, Ox, Rif, Tet, TS, V	0.025 $\pm$ 0.046

CSF, cerebrospinal fluid.

* By Microscan analysis.

† Cd, clindamycin; Cefot, cefotaxime; Cp, ciprofloxacin; E, erythromycin; G, gentamicin; I, imipenem; Ox, oxacillin; Rif, rifampin; Tet, tetracycline; TS, trimethoprim/sulfamethoxazole; V, vancomycin.

‡ Measured by the method of Christensen *et al.* (23). Samples were tested in quadruplicate on three occasions, and the results averaged.

Figure 1. Log₁₀ inoculum titers (SD) causing abscesses in 50% of the animals (ID₅₀). Bars at the top of the figure indicate results that were not statistically different from each other. Asterisks denote high slime-producing isolates.

from the mouse model, either on catheters or on swabs of the catheter site, were identical to those of the original isolates.

Figure 1 shows the inoculum titers at which the *S. epidermidis* and other ConS isolates produced abscesses in 50% of the animals. The log₁₀ ($\pm$ SD) values ranged from a low of 8.5 ± 0.3 (HZ-1) to a high of 10.2 ± 0.2 (*S. capitis* and *S. haemolyticus*) (Table 2, $p < 0.005$). Isolates classified as high slime producers (HZ-1, MLC, RP62A, and MB-1) caused abscess formation at significantly lower titers than did their nonslime-producing counterparts ($p < 0.001$). By grouping the isolates according to nonsignificant changes in their ability to

Table 2. Infective dose of coagulase-negative staphylococci resulting in abscesses and catheter colonization in 50% of mice

Isolate*	n†	ID ₅₀ for abscess formation‡	ID ₅₀ for catheter colonization‡
RP62A	23	8.8 $\pm$ 0.2	9.1 $\pm$ 0.3
MB-1	35	9.3 $\pm$ 0.2	9.9 $\pm$ 0.8
MP	23	9.6 $\pm$ 0.4	9.4 $\pm$ 0.5
MB-4	25	9.7 $\pm$ 0.2	9.9 $\pm$ 0.3
HZ-1	31	8.5 $\pm$ 0.3	8.8 $\pm$ 0.2
HM-1	22	9.5 $\pm$ 0.2	10.3 $\pm$ 1.0
MLC	18	8.6 $\pm$ 0.3	7.5 $\pm$ 0.5
MLL	22	9.4 $\pm$ 0.3	9.4 $\pm$ 0.4
<i>S. capitis</i>	33	10.2 $\pm$ 0.2	10.0 $\pm$ 0.4
<i>S. haemolyticus</i>	31	10.2 $\pm$ 0.2	10.3 $\pm$ 0.3

* Source described in Table 1.

† n = sample size.

‡ Maximum likelihood estimate.

induce abscesses (horizontal bars at the top of Fig. 1), it was possible to identify two subsets whose ability did not overlap: HZ-1, MLC, and RP62A versus MP, MB-4, *S. capitis*, and *S. haemolyticus*.

With catheter colonization as the endpoint, the ID₅₀ values again ranged widely (Fig. 2, $p < 0.05$); however, by statistical criteria, the majority of isolates showed overlapping abilities to colonize. Three of four high slime producers (MLC, HZ-1, and RP62A) required significantly lower inoculum titers to colonize 50% of the catheters, compared with the nonslime producers ($p < 0.025$). Isolate MLC had a higher cfu per cm² of catheter than isolate HM-1 ($p = 0.05$). MLC also had a higher cfu per cm² than MB-1 and MB-4, but the difference was not quite significant (MLC versus MB-1, $p = 0.08$; MLC versus MB-4, $p = 0.09$).

There was no evidence to implicate the clinical origin of an isolate as a determinant of virulence in this model system.

DISCUSSION

This study demonstrates marked differences in the ability of *S. epidermidis* organisms isolated from clinically relevant samples to colonize catheters and to evoke abscesses *in vivo*. The results were obtained with a mouse model of CVC infection that permitted quantification of two indicators of bacterial virulence: abscess formation and colonization of catheters.

Others have also observed a spectrum of virulence for ConS isolates. Namavar *et al.* (24) compared the virulence of five *Staphylococcus aureus* and 11 *S. epidermidis* isolates injected intracerebrally into neonatal mice. The mean dose lethal to

Figure 2. Log₁₀ inoculum titers (SD) resulting in colonization of s.c. catheters in 50% of mice (ID₅₀). Bars at the top of the figure indicate results that were not statistically different from each other. Asterisks denote high slime-producing isolates.

50% of the animals was 9.4×10^4 organisms (range, 2×10^4 to 2×10^5) for *S. aureus* contrasted with 3.0×10^7 (range 1.4×10^6 to 7.8×10^7) for *S. epidermidis*. Recently, Gunn (25) evaluated 60 strains (eight species) of ConS in a neonatal mouse weight retardation model, finding that *S. haemolyticus*, *S. epidermidis*, and *Staphylococcus saprophyticus* were the most virulent species. Lambe *et al.* (26) evaluated 30 ConS isolates from five different species in a catheter-related mouse model. *S. epidermidis*, *Staphylococcus schleiferi*, and *Staphylococcus lugdunensis* were the most virulent species, producing abscesses in 76–91% of the mice with positive cultures obtained from infected foreign bodies and tissues in 80–100% of animals. Thus, these studies allude to *S. epidermidis* as one of the most virulent species of ConS but not as virulent as *S. aureus*.

Baddour *et al.* (27) compared the virulence of *S. epidermidis* isolates to another coagulase-negative staphylococci, *Staphylococcus hominis*, using a rat model of endocarditis. A marked species difference was noted at an inoculum of 10^7 cfu with *S. epidermidis* causing endocarditis and significantly more positive blood cultures than *S. hominis*. *S. epidermidis* appeared to possess certain traits allowing an enhanced virulence in this system. The authors noted that slime-producing strains of *S. epidermidis* had a higher percentage of positive blood cultures than slime-negative strains, but they were unable to discern a difference in the ability of organisms with the slime phenotype to cause endocarditis.

The murine model used in this study made it possible to assess abscess formation and catheter colonization in the same system. In a previous study (21), we observed a high frequency of abscess formation in sham-operated control mice inoculated with *S. epidermidis*. This suggests that colonization of a foreign body is not an absolute requirement for ConS infection of traumatized tissue. Although ID₅₀ doses for these two endpoints corresponded well in our study (Table 2), there were instances in which abscesses were produced by organisms that did not readily colonize catheters. The reverse situation was also seen: the MLC isolate, which showed the greatest affinity for catheters, was only able to induce abscess formation at a much higher ID₅₀.

Thus, certain *S. epidermidis* organisms possess traits that confer a pathogenetic advantage in the model system. The slime layer of *S. epidermidis*, which may inhibit host defenses while allowing the organism to procure nutrition (10, 15, 28), was related to pathogenesis. Each of the four isolates that caused infection at relative low titers were high slime producers (OD_{570 nm} > 0.240), whereas the less pathogenic organisms uniformly produced lower amounts of this substance.

Numerous investigators have attempted to correlate slime production by ConS with the clinical outcome of infection or response to antibiotic therapy (4, 18, 19). Three studies showed an association of slime production with a poor outcome (4, 18, 19). Christensen *et al.* (12), using a mouse model similar to ours, evaluated the virulence of isogenic phenotypic variants of a slime-producing, pathogenic isolate of *S. epidermidis*. These authors showed a 3 times greater ID₅₀ in a rat model of catheter-related endocarditis by the Reed and Muench method for the slime-producing isolate compared with its non-slime-

producing variants. However, there was a considerable instability of these spontaneous variants.

While studying phase variation, Christensen *et al.* (11) noted that slime production appears to be one of a panel of virulence factors that could be coregulated. Muller *et al.* (14) in an elegant study used transposon mutagenesis to show that slime production is closely associated with another virulence factor, the elaboration of a capsular polysaccharide/adhesin. These authors have recently shown that transposon mutants deficient in the production of the polysaccharide/adhesin and slime antigen are associated with decreased virulence in a rabbit model of endocarditis (16).

Our present results conflict somewhat with our previous report showing that the slime-producing capability of *S. epidermidis* did not increase the risk of catheter-related infection (21). However, in the earlier evaluation, we used a fixed inoculum of 10^9 cfu/mL, the threshold dose for inducing abscesses with the majority of isolates in the present study. Thus, only at higher concentrations of the organism was it possible to recognize any pathogenic contribution from the slime phenotype. Additionally, Teflon catheters have been shown to resist colonization (29); thus, a higher inoculum may have been needed to achieve colonization. In both our present and previous study, slime-producing isolates showed a greater ability to colonize catheters, regardless of the size of the inoculum.

Other factors that may have contributed to the virulence of *S. epidermidis* include a polysaccharide or protein adhesin required for adherence of some organisms to plastic (17, 30, 31). Thus, potentially pathogenic organisms classified as high slime producers could be relatively inefficient colonizers of catheter tubing due to differences in adherence properties under *in vivo* conditions (14, 17, 31, 32). This mechanism might account for the anomalous results obtained with the MB-1 isolate (Fig. 2 and Table 2). The study of Muller *et al.* (14) did show an association between an adherence factor and slime, but this association is not always observed (33). Clearly, factors other than slime-mediated colonization bear on the infectivity of *S. epidermidis* and should be evaluated in more detail in the future.

These findings implicate the slime phenotype of *S. epidermidis* as a major virulence factor. The murine model should prove valuable for identifying factors that distinguish highly virulent slime-producing organisms from those that merely contaminate catheters and traumatized tissue.

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