Slime Production and Expression of the Slime-Associated Antigen by Staphylococcal Clinical Isolates

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The ability to produce slime and to express a slime-associated antigen was examined in a collection of staphylococcal clinical isolates. Slime-producing strains were found among coagulase-negative staphylococci in percentages comparable to those reported in other studies; surprisingly, a high percentage of Staphylococcus aureus strains also were able to produce this extracellular material. In the latter case, this ability was strongly dependent on the presence of an additional carbohydrate source in the growth medium. Expression of the slime-associated antigen appeared to be species specific and confined to the Staphylococcus epidermidis sensu stricto isolates; its strong association with the ability of these strains to produce thicker biofilms indicated slime-associated antigen as a possible virulence marker for S. epidermidis.

Both Staphylococcus aureus and coagulase-negative staphylococci (CoagNS), particularly Staphylococcus epidermidis, are an important cause of infections associated with indwelling medical devices. Among factors possibly explaining the frequent colonization of indwelling devices by staphylococci, the microbial production of mucoid exopolymERIC substances and the presence of receptors to plasma proteins absorbed onto the biomaterial surface have been considered.

As far as CoagNS are concerned, extracellular polysaccharides seem to be the most important factor, and biofilm production (slime) has been investigated (reviewed in reference 8). However, the debate is still open on this point; as a matter of fact, controversial results have been reported in terms of the association between slime production and clinically significant CoagNS infections (6, 11, 12, 18, 19, 24, 28) or differences in pathogenic potential between slime-producing and slime-negative strains (1, 23). No definitive evidence is available on the role of host protein receptors expressed by CoagNS. In fact, while some reports clearly demonstrated the ability of S. epidermidis to adhere to immobilized plasma proteins (4, 22), others have reported such proteins decrease or have a blocking effect on staphylococcal adherence (20). Such contrasting results have been ascribed to a masking action of slime covering protein receptors in in vitro assays (4).

As far as S. aureus is concerned, slime production has never been considered as a virulence factor. The importance of coagulase-positive staphylococci in medical device-associated infections has mostly been ascribed to their ability to express molecules that recognize host matrix proteins (15).

In this study, we wanted to evaluate the occurrence of slime production among clinical isolates of both CoagNS and S. aureus; moreover, we analyzed the expression of a slime-associated antigen (SAA) (7), which preliminary results obtained by our group suggested is strictly correlated with the expression of slime and possibly represents a marker of virulence for staphylococci (3).

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to produce slime, with a mean biofilm thickness (0.653 ± 0.08) comparable to that of CoagNS other than *S. epidermidis*.

Almost all of the strong-slime-producing *S. aureus* strains were those of human origin, while of the nine animal strains, only three produced a detectable biofilm (mean OD, 0.300 ± 0.05). The others were all non-slime producers. The three *S. intermedius* strains from canine dermatitis were weak slime producers.

The ability of the human strains to produce slime, when considered with regard to the source of isolation, is shown in Table 1. Although the number of isolates was not always comparable among the various species and site of isolation, differences were significant for *S. epidermidis* strains isolated from catheter-associated infections, for all isolates from orthopedic implants, and for *S. aureus* from throat and nasal swabs.

The SAA was identified exclusively within members of the *S. epidermidis* sensu stricto group, with almost 60% of the slime-producing strains also expressing SAA (Table 2); slime-producing, SAA-negative strains were also detected, while the phenotype slime-negative, SAA-positive strain was never recovered (Table 2). Almost all SAA-expressing strains were strong slime producers (44 of 45); of those, 12 always showed OD values above 3.00. The differences in the OD of the biofilm produced by each strain. Bars indicate the medians of the plotted values. Dots indicate the average OD of triplicate determinations of the biofilm produced by each strain. Bars indicate the medians of the plotted values.

A casual observation that slime production by *S. aureus* seemed to be affected by the addition of glucose to the medium prompted us to systematically investigate this aspect. The ability of *S. aureus*, of either human or animal origin, to produce slime was dramatically affected by the presence of an additional carbohydrate source in the medium (Fig. 2). The addition of 1% glucose increased the percentage of slime-producing *S. aureus* from 34.7% to 83.3%, with a mean OD (±SE) rise from 0.19±0.05 to 0.67±0.09 (*P* < 0.001); only six of such slime-producing strains did not show a significant difference in biofilm production measured after growth in the presence or absence of glucose. The “carbohydrate effect” was never detected for other staphylococcal species, with the exception of a few nonsignificant value fluctuations for strains with a border-line classification.

### TABLE 1. Slime production of the 201 staphylococcal strains of human origin examined in this study with regard to the source of isolation

<table>
<thead>
<tr>
<th>Source</th>
<th><em>S. epidermidis</em></th>
<th><em>S. aureus</em></th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slime+</td>
<td>Slime−</td>
<td>Slime+</td>
<td>Slime−</td>
</tr>
<tr>
<td>Catheters</td>
<td>43 (63.2)</td>
<td>25 (36.8)</td>
<td>2 (40)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>Blood</td>
<td>16 (66.7)</td>
<td>8 (33.3)</td>
<td>32 (97)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Orthopedic implants</td>
<td>12 (75)</td>
<td>4 (25)</td>
<td>2 (100)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>CAPD</td>
<td>5 (71.4)</td>
<td>2 (28.6)</td>
<td>1 (100)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Urine</td>
<td>7 (77.8)</td>
<td>2 (22.3)</td>
<td>7 (75)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Throat and nasal swabs</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>3 (33.3)</td>
<td>2 (25)</td>
</tr>
<tr>
<td>Wounds</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Total</td>
<td>76 (66.1)</td>
<td>39 (33.9)</td>
<td>56 (88.9)</td>
<td>7 (11.1)</td>
</tr>
</tbody>
</table>

### TABLE 2. Combinations of expression of SAA and slime production among the 115 *S. epidermidis* isolates from different sources

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>No. (%) of isolates from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Catheters</td>
</tr>
<tr>
<td>Slime+ SAA+</td>
<td>26 (38.2)</td>
</tr>
<tr>
<td>Slime+ SAA−</td>
<td>17 (25)</td>
</tr>
<tr>
<td>Slime− SAA+</td>
<td>0</td>
</tr>
<tr>
<td>Slime− SAA−</td>
<td>25 (36.7)</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
</tr>
</tbody>
</table>

**FIG. 1.** Slime production of SAA-positive and SAA-negative *S. epidermidis* clinical isolates. Dots indicate the average OD of triplicate determinations of the biofilm produced by each strain. Bars indicate the medians of the plotted values.
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cantly different from that produced by SAA-negative strains, is
of the biofilm produced by SAA-expressing strains, signifi-
ance among clinically significant isolates. Also, the thickness
found to be expressed frequently by
non-slime-producing strains. Different from SAA, PS/A was
erved from the skin of either patients or hospital personnel
majority of clinically important staphylococcal strains are de-
ever, we believe that, as indicated by other authors (21), the
observation is the high frequency of the SAA-positive slime
among S. epidermidis strains. The exclusive association of such
antigen with slime, with almost 60% of the slime-producing S.
epidermidis strains expressing SAA, is indicative of its impor-
tance among clinically significant isolates. Also, the thickness
of the biofilm produced by SAA-expressing strains, signifi-
cantly different from that produced by SAA-negative strains, is
strongly suggestive of SAA as a virulence marker for S. epider-
midis. We did not examine skin isolates to analyze the associ-
ation between SAA and severity of clinical infections. How-
ever, we believe that, as indicated by other authors (21), the
majority of clinically important staphylococcal strains are de-
ived from the skin of either patients or hospital personnel
involved in their care. The only previous report describing the
occurrence of a supposedly slime-associated antigen (PS/A)
(26) in a collection of clinical isolates was based on detection
of a molecule associated with slime but also expressed by
non-slime-producing strains. Different from SAA, PS/A was
found to be expressed frequently by S. epidermidis and S. ca-
pitis and rarely by other CoagNS species, indicating it was an
antigen common among microorganisms belonging to the ge-
nus Staphylococcus (21). On the other hand, SAA was defi-
nitely slime specific and possibly confined to S. epidermidis,
although the number of other CoagNS studied was limited.

As far as S. aureus is concerned, there are no epidemiolog-
ical studies available describing slime production in human
isolates. A few studies analyzed strains causing bovine mastitis,
reporting 12% showed slime production with a strong tendency
to phenotypic variation and rapid loss of the slime layer by in
vitro subculture (5), compared to 80% of strains reported as
slime producing in vivo (27).

The pivotal role of a medium richer in glucose we observed
may represent an explanation of why slime production has
never been thoroughly investigated before as a possible viru-
ence factor for S. aureus. In fact, the addition of glucose
dramatically increased the number of slime-producing strains,
suggesting that the gene for slime production is inducible by a
suitable metabolic source. It has already been reported that the
expression of other characteristics such as the capsule produc-
tion of serotypes 5 and 8 is greatly influenced by environmental
and bacterial growth conditions (9, 17, 25). To verify whether
the addition of glucose truly stimulated the production of slime
or supported the adherence to plastic through a different
mechanism, we examined 10 randomly chosen S. aureus strains
by transmission electron microscopy and observed that strains
grown in TSB-G presented larger amounts of extracellular
poly saccharide material enclosing numerous bacterial cells
(data not shown). The observation reported in this study
should stimulate investigation into the importance of slime
production in S. aureus. Fattom et al. (13) described the use of
a vaccine based on polysaccharides with specific antibodies
protecting mice against bacterial challenge; this report may
suggest the importance of the polysaccharide material, in ad-
tion to the capsular one, in eliciting protective antibodies.

As suggested by the data reported by Flock et al. (14),
molecules with binding functions may be necessary for coloni-
zation when fibronectin-binding proteins and other extracellu-
lar matrix-binding proteins are lacking. Because the majority of
S. aureus strains examined in this study were from orthopedic
implants, a definitive conclusion about the occurrence of slime
production among clinical isolates cannot be drawn. However,
the large prevalence of slime production among strains iso-
lated from such specific infection sites (infected knee and hip
prostheses) may be suggestive of an important role for slime in
biomaterial-associated infections supported by S. aureus as is
the case for CoagNS biomaterial-centered infections.

Slime production by S. epidermidis and the other staphylo-
coccal strains was only slightly affected by glucose, since the
amount of this sugar available in the commercial medium was
sufficient for slime production. Only a few strains with border-
line values of OD (none to weak or weak to strong) sometimes
showed nonsignificant fluctuations (data not shown), suggest-
ing the presence of a gene system for slime production differ-
ent from that in S. aureus.

The data reported here indicate an important role of SAA as
a virulence marker for clinically significant S. epidermidis iso-
lates. Its occurrence among a majority of clinical isolates and
its association with the strains’ ability to produce thicker bio-
films strongly suggest a role of SAA in pathogenesis. The
purified antigen might be considered, together with other an-
tigens suggested to elicit protective antibodies, such as PS/A,
for the production of a vaccine to be used in patients at risk for
biomaterial-associated infections.

Also, the indication of the predominance of slime-producing
S. aureus strains, particularly among prosthesis-centered infec-
tions, should stimulate research on this topic, to evaluate the
possible role of this factor in biomaterial-associated S. aureus
infections.
ACKNOWLEDGMENTS

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REFERENCES