Slime Production and Cell Surface Hydrophobicity of Nasopharyngeal and Skin Staphylococci Isolated from Healthy People

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Abstract

The collection of 314 staphylococcal strains including Staphylococcus aureus and coagulase-negative staphylococci (CNS) was isolated from skin or nasopharynx of healthy people. It was found that the majority of staphylococci possessed the ability to produce slime intensively or moderately, irrespective of ecological niche-nose, throat or skin. Most of them showed the hydrophilic cell surface. However, among S. aureus skin isolates or CNS throat isolates predominated strains with hydrophobic cell surface. There was a slight correlation between slime production and the nature of cell surface among CNS isolates but not among S. aureus strains. It was found that most of slime-producing CNS strains showed hydrophilic cell surface, while slime-negative isolates usually possessed hydrophobic cell surface. Our data suggest that slime production but not cell surface hydrophobicity can be regarded as an essential colonization factor responsible for staphylococci adherence to skin or mucous membranes of upper respiratory tract. These data also suggest that slime production seems to be a general feature of staphylococci isolated from various niches of healthy people.

Keywords: slime, cell surface hydrophobicity, staphylococci.

Introduction

Adhesion of bacterial cells, i.e. their attachment to epithelial cells of skin or mucous membranes of the respiratory, alimentary or genitourinary tract is the first step of colonization. This is due to nonspecific or specific cell-cell interactions, involving several microbial and host factors. The cell surface hydrophobicity of bacteria is an important non-specific adhesion factor, while production of extracellular mucoid substances of polysaccharide nature, so-called slime or glycocalyx, may enhance the ability of bacterial cells to adhere specifically to host tissue (Howard and Rees, 1994; Wilson et al., 1996).

Staphylococci colonize several niches of the human body. These microorganisms usually existing as a resident or as a transient member of the normal flora of skin and upper respiratory tract, can be regarded as a potential reservoir of endogenous infections under predisposing conditions (Howard and Kloos, 1994; Wilson et al., 1996). Phage typing and antibiotic resistance patterns suggest that the colonizing and invading strains are usually identical (Cree et al., 1994). In the light of controversial or conflicting literature data regarding adhesion properties of staphylococcal strains (Baldassarri et al., 1997; Ammendolia et al., 1999), the aim of this paper was to compare the extent of slime production and the relative cell surface hydrophobicity of potentially pathogenic Staphylococcus aureus and coagulase-negative staphylococci (CNS) strains colonizing skin or mucous membranes of nasopharynx in healthy people.

Experimental

Materials and Methods

Bacterial strains. Staphylococcal strains were isolated from January to March 2003 from skin (forehead), nasal or throat swabs of both children (aged from 3 to 10 years) and adults (aged from 21 to 60 years) with no clinical symptoms of skin or respiratory tract infections. Isolated species were identified by conventional methods (macroscopic, microscopic or biochemical assays) or by
rapid commercial latex test – Slidex Staph-Kit (bioMerieux). API 20 STAPH was used to determine species of CNS strains. All isolates were classified as methicillin-sensitive staphylococci according to NCCLS standards (Cuny et al., 2002).

Assay of slime production. Slime production by isolated staphylococcal strains was assessed by the visual method described by Freeman et al. (1989), using solid medium containing 5% sucrose and 0.08% Congo red. After inoculation, agar plates were incubated for 24 hrs at 37°C. The extent of slime production was assessed on the basis of colour of staphylococcal colonies, according to criteria presented by Freeman et al. (1989).

Assay of cell surface hydrophobicity. The relative cell surface hydrophobicity of isolated staphylococcal strains was assessed using modified ammonium sulfate salt aggregation test (Lindahl et al., 1981). It was assumed that strains autoaggregated were described as very strong hydrophobic, aggregated at 0.4–1.0 M (NH₄)₂SO₄ – as strong hydrophobic, at 1.2–1.6 M (NH₄)₂SO₄ – as hydrophobic, at >1.8 M (NH₄)₂SO₄ – as hydrophilic.

Statistical analysis. Statistical analyses of adhesion properties of the isolated staphylococci were made using the nonparametric tests (Chi² test, Chi² test with Yates correction or V² test), depending on the total (observed) or expected frequencies. The difference at the 5% level was considered statistically significant. Correlation between slime production and cell surface hydrophobicity was assessed by the point-biserial coefficient (rₚₑ).

Results

The collection of 314 staphylococcal strains, including Staphylococcus aureus and coagulase-negative staphylococci (CNS), isolated from skin or nasopharynx in healthy people was presented in Table I. Table II shows data concerning slime production by the isolated staphylococcal species. The majority of them were able to produce slime intensively or moderately – 139/142 (97.9%) vs 158/172 (91.9%) among S. aureus or CNS isolates, respectively. However, slime-negative organisms were isolated more frequently among CNS strains than those belonging to S. aureus (p<0.0190). It was also found that, irrespective of ecological niche, CNS strains showed more frequently ability to produce slime moderately than S. aureus isolates – 111/172 (64.54%) vs 51/142 (35.92%), respectively (p<0.0000). Moreover, it was observed that intensive slime production was detected more frequently among skin S. aureus strains compared to nasopharyngeal isolates of this species – 24/26 (92.31%) vs 64/113 (56.64%), respectively (p<0.0007).

Generally, the majority of the isolated staphylococcal strains showed hydrophilic cell surface – 118/142 (83.1%) vs 125/172 (72.67%) among S. aureus or CNS isolates, respectively (Table III). However, strains with hydrophobic cell surface were isolated more frequently among CNS isolates than S. aureus isolates (p<0.0280), particularly strains possessing strong hydrophobic cell surface – 20/172 (11.63%) vs 2/142 (1.41%), respectively (p<0.0009). It was also found that specific situation regarding the nature of cell surface took place among S. aureus isolates from skin and CNS isolates from throat, since in both sub-collections strains with hydrophobic cell surface predominated (p<0.025 or p<0.0042, respectively).

Table I

The collection of tested staphylococcal strains isolated from skin or mucous membranes of nasopharynx in healthy people

<table>
<thead>
<tr>
<th>The group of staphylococci</th>
<th>Species</th>
<th>Number of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-positive staphylococci (n = 142)</td>
<td>S. aureus</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>S. epidermidis (n = 67)</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>S. xylosus (n = 16)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>S. hominis (n = 11)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>S. haemolyticus (n = 14)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>S. capitis (n = 2)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>S. saprophiticus (n = 4)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>S. sciuri (n = 3)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>S. warneri (n = 9)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>S. lentus (n = 2)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>S. simulans (n = 1)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Novobiocin-sensitive Staphylococcus spp.* (n = 34)</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Novobiocin-resistant Staphylococcus spp.* (n = 9)</td>
<td>3</td>
</tr>
</tbody>
</table>

* definitive identification of these CNS strains was impossible by using API 20 STAPH
There was no correlation between the biochemical phenotype (API numerical code) and cell surface hydrophobicity or ability to slime production within individual species of isolated staphylococci (data not show). According to Figs. 1 A and B, most of slime-producing CNS strains showed hydrophilic cell surface – 120/158 (75.95%), while those with no ability to produce slime usually possessed hydrophobic cell surface – 9/14 (64.29%) (p<0.0034). Besides, most of slime-producing, hydrophobic CNS strains were classified as moderately slime-producing organisms – 34/38 (89.47%) (p<0.0030). Despite this, there was only a slight correlation between the extent of slime production and the nature of cell surface among isolated CNS (rpb = –0.33); this correlation was comparable to nasopharyngeal isolates (rpb = –0.38) and to skin strains (rpb = –0.32). In contrast, there was no correlation between the extent of slime production and the nature of cell surface among S. aureus strains (rpb < –0.1).

### Discussion

Slime production appears to be one of virulence factors of staphylococci which provides not only a permanent binding to the host tissue, but also protects bacteria against phagocytosis interfering with specific acquired immune response and impairs an access of antibacterial agents to targets within bacterial cell. In addition, slime-producing bacteria, including staphylococci, have the ability to form in the host organism a structure, so-called biofilm, which consists of an elastic gel containing exopolysaccharide within which microcolonies can develop. Formation of this structure and its extreme inherent resistance to antimicrobial agents and to host defense mechanisms is the basis for many persistent and chronic bacterial infections, which causes difficulty in eradication of biofilm-producing bacteria (Costerton et al., 1999; Donlan and Costerton, 2002).
Production of slime plays an important role in staphylococcal diseases, especially in hospital-acquired infections caused by *S. aureus* or CNS associated with indwelling medical devices, since this allows bacteria to adhere to smooth surfaces of foreign body-biomaterials. The source of these microorganisms is usually the skin or mucosal surfaces of the patient or the hospital personnel (von Eiff *et al.*, 1999). Data obtained in this paper suggest that despite some differences in the extent of slime production by particular strains this phenomenon seems to be a general feature of staphylococci isolated from healthy people, irrespective of ecological niche. According to the literature (Cree *et al.*, 1994), there is also no correlation between adhesion properties of staphylococci and their phage-type, plasmid profile or antibiotic resistance patterns.

Among non-specific mechanisms of bacterial adherence to host tissues, hydrophobicity of cell surface of bacteria seems to be an essential adhesion factor. Our data suggest that in contrast to Gram-negative bacteria (Jankowski *et al.*, 1997; Mikucka *et al.*, 2000; Janicka *et al.*, 2002; Loś *et al.*, 2004), the adherence of staphylococci to skin or mucous membranes of nose or throat in healthy people is rather not determined by cell surface hydrophobicity. However, it should be taken into account that slime production by staphylococci may be responsible for masking of the real nature of cell surface, since it may interfere with our ability to detect cell surface hydrophobicity in *vitro*. Our observations showed that a slight correlation between the extent of slime production and the nature of cell surface among isolated CNS is in agreement with data presented by other authors that slime production seems to be discriminative factor between hydro-
phobic and hydrophilic CNS strains (Baldassarri et al., 1997). However, such correlation was not found among S. aureus strains.

Extensive studies on non-specific or specific interactions between staphylococci and skin or airways mucous membranes provide information about microbial factors responsible for the carrier state, which may be a precursor to localized or invasive infections, including those associated with biomaterials (Drako et al., 2002; Wu et al., 2003). Our data suggest that slime production but not cell surface hydrophobicity can be regarded as an essential colonization factor responsible for staphylococci adherence to skin or mucous membranes of upper respiratory tract due to presence of specific extracellular matrix slime-reactive adhesions (Shuter et al., 1996; Baldassarri et al., 1997).

Literature


