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

ANNA MALM, ANNA BIERNASIUK, RENATA ŁOŚ, URSZULA KOSIKOWSKA, MAREK JUDA, IZABELA KORONA-GŁOWNIAK and GRZEGORZ GÓRNIEWSKI*

Department of Pharmaceutical Microbiology, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland
*Department of Thoracic Surgery, Medical University of Lublin, Jaczewskiego 8, 20-954 Lublin, Poland

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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.

Key words: 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 glycocalix, 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 assesed 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 $(NH_4)_2SO_4$ – as strong hydrophobic, at 1.2–1.6 M $(NH_4)_2SO_4$ – as hydrophobic, at >1.8 M $(NH_4)_2SO_4$ – as hydrophilic.

Statistical analysis. Statistical analyses of adhesion properties of the isolated staphylococci were made using the nonparametric tests (Chi^2 test, Chi^2 test with Yates correction or V^2 test), depending on the total (observed) or expected frequences. 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 (r_{pb}).

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 subcollections 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

The group of staphylococci	Species	Number of strains		
		Nasal swabs	Throat swabs	Skin swabs
Coagulase-positive staphylococci	*S. aureus* (n = 142)	77	38	27
Coagulase-negative staphylococci (n =172)	*S. epidermidis* (n = 67)	56	6	5
	S. xylosus (n = 16)	9	4	3
	S. hominis (n = 11)	7	2	2
	S. haemolyticus (n = 14)	10	1	3
	S. capitis (n = 2)	1	0	1
	S. saprophiticus (n = 4)	2	2	–
	S. sciuri (n = 3)	1	2	–
	S. warneri (n = 9)	–	–	9
	S. lentus (n = 2)	–	–	2
	S. simulans (n = 1)	–	–	1
	Novobiocin-sensitive *Staphylococcus* spp.*	15	1	18
	(n = 34)			
	Novobiocin-resistant *Staphylococcus* spp.* (n = 9)	3	5	1

* definitive identification of these CNS strains was impossible by using API 20 STAPH

Table II
Slime production by staphylococcal strains isolated from skin or mucous membranes of nasopharynx of healthy people

Species (total number of strains)	The extent of slime production	Number (percent) of strains		
		Nasal swabs	Throat swabs	Skin swabs
S. aureus (n = 142)	+++	41 (53.3)	23 (60.5)	24 (88.9)
	++	36 (46.7)	13 (34.2)	2 (7.4)
	–	0 (0)	2 (5.3)	1 (3.7)
Coagulase-negative staphylococci (n = 172)	+++	29 (27.9)	8 (34.8)	10 (22.2)
	++	69 (66.3)	14 (60.9)	28 (62.2)
	–	6 (5.8)	1 (4.3)	7 (15.6)

+++ intensive slime production, ++ moderate slime production, – lack of slime production

Table III
The relative cell surface hydrophobicity of staphylococcal strains isolated from skin or mucous membranes of nasopharynx of healthy people

Species (total number of strains)	The relative cell surface hydrophobicity	Number (percent) of strains		
		Nasal swabs	Throat swabs	Skin swabs
S. aureus (n = 142)	Very strong hydrophobic	0 (0)	0 (0)	1 (3.7)
	Strong hydrophobic	1 (1.3)	1 (2.6)	0 (0)
	Hydrophobic	11 (14.3)	2 (5.3)	8 (29.6)
	Hydrophilic	65 (84.4)	35 (92.1)	18 (66.7)
Coagulase-negative staphylococci (n = 172)	Very strong hydrophobic	2 (1.9)	4 (17.4)	0 (0)
	Strong hydrophobic	10 (9.6)	4 (17.4)	6 (13.3)
	Hydrophobic	7 (6.7)	4 (17.4)	10 (22.2)
	Hydrophilic	85 (81.7)	11 (47.8)	29 (64.5)

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 ($r_{pb}=-0.33$); this correlation was comparable to nasopharyngeal isolates ($r_{pb}=-0.38$) and to skin strains ($r_{pb}=-0.32$). In contrast, there was no correlation between the extent of slime production and the nature of cell surface among *S. aureus* strains ($r_{pb}<-0.1$).

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).

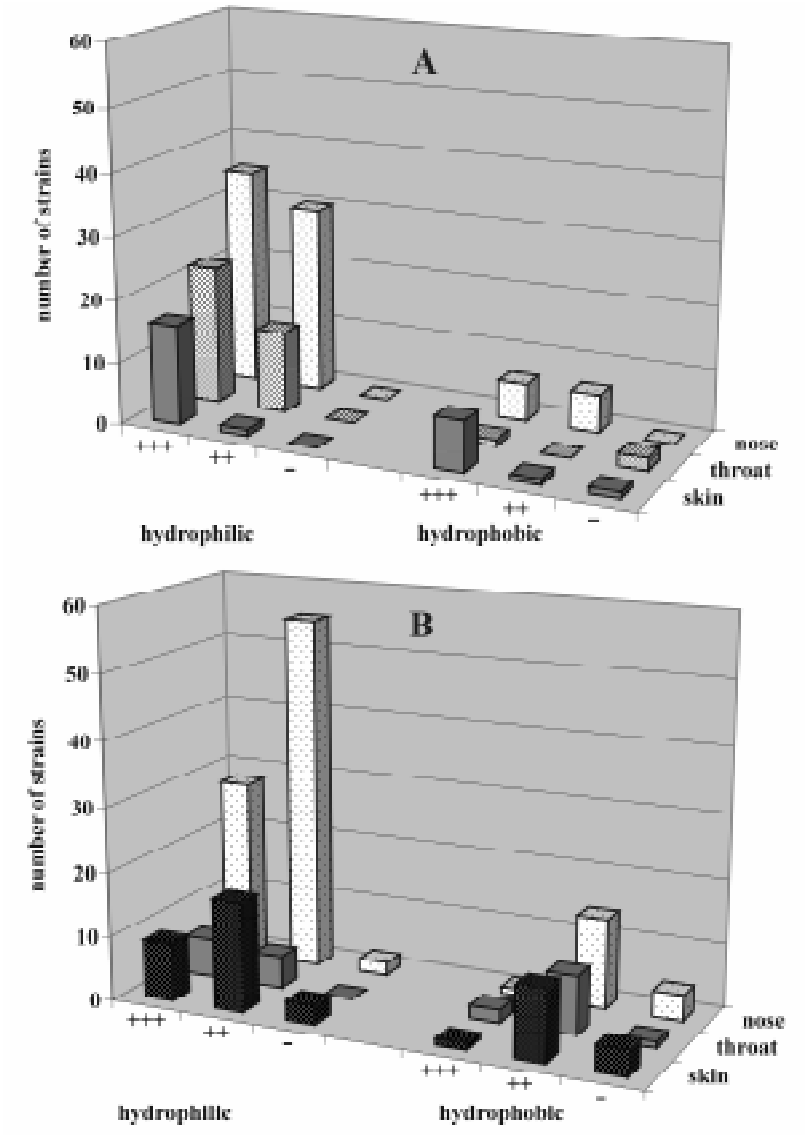

Fig. 1. Correlation between slime production and cell surface hydrophobicity in *Staphylococcus aureus* strains (A) and coagulase-negative (CNS) strains (B) isolated from skin or mucous membranes of nasopharynx of healthy people. The extent of slime production: +++ intensive slime production; ++ moderate slime production; – lack of slime production

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 personel (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; Łoś *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 (Drago *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).

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