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Extracellular Polysaccharide Production and Biofilm Formation by Coagulase-negative *Staphylococci*

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Abstract: *Coagulase-negative Staphylococci (CoNS) form a part of the skin flora and commonly cause nosocomial bloodstream and catheter- related infections. According to their relatively low virulence, it is important to detect the virulence factors of clinical isolates. The production of extracellular polymers and formation of biofilms belong to virulence factors. We studied clinical isolates of CoNS and compared them with isolates from the healthy skin. The species distribution did not differ significantly. Slime production in clinical strains was more often and more intense. A comparison of methicillin-resistant (MR) and methicillin-sensitive (MS) clinical strains has revealed that slime production is more active in MR strains.*

Keywords: *Slime, Biofilm, Coagulase-negative Staphylococci, Methicillin resistance, Methicillin sensitivity, Infections.*

Intoduction

Coagulase-negative *Staphylococci* (CoNS), particularly *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*, are the most frequent causes of implanted medical device or catheter-related infections, as well as nosocomial bacteremia [2, 6, 7].

They are also identified with a wide variety of other clinical problems like septic arthritis, mediastinitis, peritonitis, osteomyelitis, urinary tract infections, etc. [10, 12].

CoNS now count among the most frequent nosocomial pathogens. Although CoNS had been considered for many years to be non-pathogenic commensal organisms of the skin, now some of them, like *S. epidermidis* and *S. haemolyticus*, are generally accepted as pathogens [10].

The pathogenesis of CoNS infections depends on their ability to first adhere to the substrate and then to form a mucoid biofilm referred to as slime. The initial bacterial adhesion, which is non-specific and reversible, is followed by a specific adhesion, mediated by an adhesion receptor. The critical step in polymer colonization by microorganisms is their ability to produce great amounts of extracellular polysaccharides (slime), in which cells are embedded and covered. Biofilms are sophisticated communities of surface-attached bacteria that exhibit a distinct phenotype. Staphylococcal cells embedded in dense polysaccharide biofilms are inherently resistant to host immune responses and antimicrobial therapy [4, 5].

The aim of this study was to determine the slime production of CoNS and to evaluate it as a virulence factor.

Materials and methods

The study was carried out between January 2004 and June 2005 in a surgical orthopedic hospital in Riga. A total of more than 200 coagulase-negative *Staphylococci* were isolated from clinical samples: purulent fluid from joints, bursa, dwelling artificial devices, abscesses, surgical sites, blood. They were isolated and identified by conventional methods and the Crystal system up to a species level. 198 strains from clinical samples were included in this study. For comparison, 78 strains of CoNS, isolated from the skin of healthy volunteers, were studied.

Antimicrobial susceptibility testing was performed by the standard agar diffusion technique with commercial BBL antibiotic test disks on Mueller-Hinton agar (NCCLS). Methicillin resistance was detected by disk diffusion with oxacillin disk (1 μg) on Mueller-Hinton agar and confirmed by *mec*A detection by PCR, Slidex MRSA latex agglutination test and E-test [11, 13].

For determining the slime production, the qualitative tube test was used. Glass tubes, containing 0,25% glucose (Oxoid) and broth were inoculated with a single colony of a pure culture of a strain of CoNS and incubated for 24 h at 35°C. The content was decanted and 2 ml of a 0,4% solution of trypan blue was added. The tube was rotated to reveal any adherent material on the inner surface. The test was considered positive when there was an adherent layer of stained material on the inner surface of the tube [5].

Results and discussion

The identification of isolated CoNS strains revealed the presence of 9 species of coagulase-negative *Staphylococci* in the examined samples. 8 of them were present on the healthy skin, 7 – in clinical isolates (Table 1).

Table 1. Species distribution of coagulase-negative *Staphylococci* isolated from the healthy skin and clinical samples

Species	Healthy skin	Clinical samples
S. epidermidis	55,0	58,2
S. haemolyticus	6,6	14,1
S. warneri	5,0	5,6
S. capitis	16,6	6,4
S. saprophyticus	5,0	6,7
S. cohnii	5,6	-
S. hominis	3,2	6,8
S. auricularis	3,0	-
S. simulans	-	2,2

There was no significant difference between the species distribution of CoNS between the isolates from the healthy skin and the isolates from clinical samples. The leading species was *S. epidermidis sensu stricto* in both cases. *S. haemolyticus* was more often isolated from clinical samples.

A total of 170 strains of CoNS were tested for the ability to produce extracellular polysaccharide polymer-slime *in vitro* (Table 2). According to our results, slime production occurred more often in clinical isolates. So, it was more typical for clinical strains of *Staphylococci*.

Table 2. Slime production of coagulase-negative *Staphylococci* isolated from the healthy skin and clinical samples

Source of microorganisms	**n**	**Strong production, %**	**Moderate production, %**	**No production, %**
Healthy skin	78	7,69	2,51	89,80
Clinical samples	92	14,13	18,47	67,40

The clinical strains were analyzed separately for the comparison of the slime production in methicillin-sensitive isolates and methicillin-resistant isolates (Table 3).

Our results demonstrate that methicillin-resistant *Staphylococci* produce slime more intensively than sensitive strains, while there is no significant difference between methicillin-sensitive clinical strains and control strains isolated from healthy individuals.

Table 3. Comparison of slime production between methicillin-resistant (MR) and methicillin-sensitive (MS) coagulase-negative *Staphylococci*

Microorganisms	**n**	**Strong production, %**	**Moderate production, %**	**No production, %**
MS CoNS	30	3,40	6,80	89,80
MR CoNS	62	25,80	22,58	51,62

It is generally accepted that coagulase-negative *Staphylococci* possess relatively few virulence factors in comparison with *S. aureus*. The role of biofilm production is crucial for medical device infections and nosocomial bacteremia. So, extracellular polysaccharide matrix production and the formation of a biofilm may be considered as the major virulence factor for coagulase-negative *Staphylococci* [3, 9]. The correlation between the *ica* ADBC operon activity and biofilm production demonstrates the general basis of Staphylococcal virulence [1, 8].

Conclusions

1. Slime production occurs more often and is more active in strains of coagulase-negative *Staphylococci* isolated from clinical samples, in comparison with the control strains isolated from the healthy skin.
2. Methicillin-resistant *Staphylococci* produce slime more actively than methicillin-sensitive *Staphylococci.*
3. The species distribution of the coagulase-negative *Staphylococci* isolated from clinical samples and the healthy skin does not differ significantly.

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