Discrimination between Contaminating and Commensal Strains of *Staphylococcus epidermidis* (Search for Phenotypic Virulence Criteria in *Staphylococcus epidermidis*)

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Abstract: *Staphylococcus epidermidis strains are diverse in their pathogenicity. Some are invasive virulent strains causing serious nosocomial infections; some are skin commensals with a low virulence. The discrimination of them is of great clinical significance. To analyze the implication of different factors in virulence, phenotypical methods were applied. Comparative assessment of the enzymatic activity of nosocomial and commensal strains of S. epidermidis was performed: alkaline phosphatase (PHA), beta-glucosidase (LAC), arginine dehydrolase (ARG), urease (URE), maltosidase (PAM), N-acetylglutamic acid hydrolase (FPY), N-acetylglucosaminehydrolase (FGA) were studied. Differences were found in the urease activity, i.e. 93% and 77% of commensal and nosocomial strains, respectively, were positive.*

In sheep blood agar, the difference was more significant – only 16.7% of commensal strains demonstrated the haemolytic activity, but 52.4% of nosocomial strains occurred to be haemolytic.

Keywords: *Nosocomial strains, Commensals, Gram-positive microorganisms, Virulence, Staphylococcus epidermidis.*

Introduction

Staphylococcus epidermidis is the leading representative of the diverse group of coagulase-negative staphylococci (CNS). It is a commensal bacterium of the human body and is referred to as a normal inhabitant of the healthy skin and mucous membranes. As a commensal microorganism, it has a low pathogenic potential [1, 4, 18], but during the last decades, like *Staphylococcus aureus*, it has evolved to become a highly adaptable human pathogen. Since the early 1980s, it has emerged as an important nosocomial pathogen and is among the most frequent health-care associated pathogens, featuring prominently among blood culture isolates and especially causing infections associated with implanted devices such as intravascular catheters and deep-seated prosthetic implants [2, 6, 7, 14, 18].

However, *S. epidermidis* infections preferentially affect immunocompromized patients, or in other words, in order to realize the potential pathogenicity and change from normal skin commensal to a pathogenic microorganism, some host predisposition is required.

The virulence factors of coagulase-negative staphylococci, including *S. epidermidis*, are yet not well defined.

Several attempts have been made to investigate this problem. Evidence indicates that the main factor, which determines the virulence of *S. epidermidis*, is the production of extracellular polysaccharides – slime, which results in the formation of a biofilm. It permits microorganisms to adhere to plastic surfaces, protects from phagocytosis and antimicrobials [3, 5, 12, 16].

From other virulence factors, some secreted proteins may be mentioned such as hemolysins, toxins – haemolytic peptide δ toxin and different enzymes – lipases, proteases, lecithinase, elastase, etc [8, 10, 11, 13, 15, 19]. However, no particular virulence markers have been determined.

S. epidermidis is the most frequent gram-positive bacterium, which can be isolated from different clinical samples. Its ubiquitous prevalence as a commensal bacterium on the skin and mucous membranes makes it very difficult to decide, whether an isolated microorganism is the causative agent of a clinical process or an innocuous representative of normal microbial flora [17].

So, one of the major challenges of a daily diagnostic work is to distinguish clinically significant invasive strains from contaminants. The finding of markers allowing the rapid differentiation between virulent and non-virulent microorganisms would be of great clinical value.

The objective of the present work was to search for *S. epidermidis* virulence factors, applying different phenotypical and genotypical methods, and to evaluate the studied features as possible criteria for the discrimination between virulent nosocomial and non-virulent contaminant strains. In the current article, the results of the investigated phenotypical features of *S. epidermidis* are presented. Our attention was focused mainly on the exoenzyme and hemolysin production.

Materials and methods

The study was carried out during 2008-2009 at the Hospital of Traumatology and Orthopaedics, Riga, Latvia. Samples from patients with hospital-acquired purulent surgical infections were examined. Most of the cultures were isolated from wounds, the skin, abscesses, indwelling artificial devices and joints using blood agar. *S. epidermidis* isolates were prospectively collected under the guidance of a clinical infectologist to choose clinically significant strains. The isolated microorganisms were resistant to several antimicrobials. Control cultures were obtained after the collection of samples from anterior nares of healthy volunteers, who had no contact with the hospital environment. The initial samples were cultured on mannitol-salt agar plates. After 24 hours, two colonies of each donor were randomly selected. Standard laboratory techniques were used to identify coagulase-negative staphylococci and to exclude *S. aureus*. The automated BBL Crystal gram-positive ID System (Becton, Dickinson) was used for identification of isolates on the species level. The resistance to a panel of antimicrobials was tested by the disk diffusion method according to

CLSI standards [21, 22]. The following enzyme activities were studied: alkaline phosphatase (PHA), beta-glucosidase (LAC), arginine dehydrolase (ARG), urease (URE), maltosidase (PAM), N-acetylglutamic acid hydrolase (FPY), N-acetylglucosaminehydrolase (FGA). The haemolytic activity of microorganisms was tested on plates with 5% sheep or 5% human blood agar.

The CSI reference strain *S. epidermidis* ATCC 12228 was used as the control.

Results

Cultures

A total of 437 cultures from clinical samples were isolated, from them 380 (87%) were gram-positive microorganisms, and 57 (13%) gram-negative. So, the leading nosocomial flora occurred to be gram-positive.

The analysis of gram-positive microorganisms revealed the prevalence of staphylococci, both coagulase-positive and coagulase-negative staphylococci (CNS) (Table 1).

Table 1. Isolated gram-positive microorganisms

Microorganisms	Number of cultures (abs., % of gram-positive) (n = 380)
S. aureus	168 cultures – 44.2%
CNS spp.	146 cultures – 38.4%
Enterococcus	34 cultures – 9.0%
Others	32 cultures – 8.4%

From coagulase-negative staphylococci, 91.3% belonged to the novobiocin sensitive group, the other 8.7% were novobiocin resistant.

The results of species identification of the isolated CNS are presented in Table 2.

Table 2. Species identification of isolated CNS

Species	Number of cultures (abs., %) (n = 104)
S. epidermidis	53 (51.0%)
S. haemolyticus	27 (26.0%)
S. hominis	4 (3.8%)
S. capitis	4 (3.8%)
S. warneri	4 (3.8%)
S. simulans	3 (2.9%)
S. saprophyticus	7 (6.8%)
S. cohnii	2 (1.9%)

S. epidermidis was the most frequently isolated species. The leading role of this species is confirmed by many researchers [1, 9, 18]. *S. epidermidis* was followed by *S. haemolyticus*.

The remaining species were distributed among *S. hominis*, *S. capitis*, *S. warneri*, *S. simulans*, *S. saprophyticus* (Table 3).

The species ratios of CNS isolated at our hospital during the last 5 years were rather stable.

Table 3. Isolated CNS species during 2005-2009

Species	Year 2009	Year 2008	Year 2007	Year 2006	Year 2005
S. epidermidis	51.0%	56.0%	56.0%	55.0%	64.0%
S. haemolyticus	26.0%	14.2%	14.2%	14.1%	11.2%
S. hominis	3.8%	10.2%	12.2%	7.9%	8.4%
S. capitis	3.8%	4.1%	2.1%	6.7%	10.5%
S. warneri	3.8%	2.9%	0.7%	5.6%	3.5%
S. simulans	2.9%	2.9%	3.5%	2.2%	0.7%
S. saprophyticus	6.7%	5.0%	5.7%	9.0%	2.8%

Enzymatic activity

Staphylococci are known for their pronounced phenotypical variability [8, 16, 18]. The heterogenous gene expression is typically observed in nosocomial microbial strains due to the adaptation to the changing environment. So, we performed a comparative assessment of the enzymatic activity of nosocomial and commensal strains of *S. epidermidis*. Two groups of enzymes were studied in respect to variable activities: alkaline phosphatase (PHA), beta-glucosidase (LAC), arginine dehydrolase (ARG), and in respect to stable activities: urease, (URE), maltosidase (PAM), N-acetylglutamic acid hydrolase (FPY), N-acetylglucosaminehydrolase (FGA). The results are presented in Table 4.

Table 4. Enzymatic activity of commensal and nosocomial *S. epidermidis* strains

Enzymes	Commensal strains (n = 83)		Nosocomial strains (n = 30)	
	Positive reaction	Negative reaction	Positive reaction	Negative reaction
Alkaline phosphatase (PHO)	40 (48.0%)	43 (52.0%)	15 (50.0%)	15 (50.0%)
Urease (URE)	77 (93.0%)	6 (7.0%)	23 (77.0%)	7 (23.0%)
Arginine dehydrolase (ARG)	80 (96.4%)	3 (3.6%)	30 (100%)	0 (0%)
Maltosidase (PAM)	18 (22.0%)	65 (78.0%)	6 (20.0%)	24 (80.0%)
N-acetylglycosamine hydrolase (FGA)	0 (0%)	83 (100%)	0 (0%)	30 (100%)
L-piroglutamic acid hydrolase (FPY)	0 (0%)	83 (100%)	0 (0%)	30 (100%)
ß-glucosidase (LAC)	78 (94.0%)	5 (6.0%)	28 (93.0%)	2 (7.0%)

It can be seen from Table 4 that differences in enzyme production between commensal isolates and nosocomial isolates of *S. epidermidis* were observed only in the case of urea utilization. Other enzymatic activities did not differ significantly.

Haemolytic activity

The results of the investigation of the haemolytic activity of different strains of *S. epidermidis* were as follows. 71 (85.5%) of the 83 commensal cultures produced hemolysins in human blood agar, while in the nosocomial group, the activity was lower, i.e. 63.3%. In sheep blood agar, the difference was opposite and more significant, i.e. only 16.7% of 54 commensal strains demonstrated the haemolytic activity, but from nosocomial strains, 52.4% occurred to be haemolytic.

Slime production

According to our previously published data, slime production in nosocomial *S. epidermidis* strains was more active than in commensal strains, i.e. 33.6% and 10.2% respectively [23]. The trypan blue test was used in these experiments. For confirmation of these results, at the present moment, the work is continued applying genotypical methods – detection of *ica*A and *aap* genes.

Discussion

The study of the pathogenicity and virulence factors of coagulase-negative staphylococci was carried out by several groups of researchers. The difference was not found in production of exoenzymes – lecithinase, lipase, DNAse, TNA-se and phenol-soluble modulins in virulent and non-virulent strains [9, 15, 19]. So, a reliable phenotypical test as a marker for the detection of the aggressivity of clinical strains of *S. epidermidis* is not yet found.

We propose the following method for the detection of the virulence of the isolated *S. epidermidis* strain in clinical practice: at first phenotypical features must be evaluated, then urease-negative organisms with the haemolytic activity in sheep blood agar as more perspective must be studied for biofilm formation. The problem is that many hospital diagnostic laboratories frustrate the precise recognition of the diverse microbial nature of CNS infections. We suggest that rapid automated biochemical identification of CNS, together with conventional methods, is necessary prior to genotypical studies.

Conclusions

1. Rapid automated biochemical identification of coagulase-negative staphylococci is the first necessary step for the detection of their virulence.
2. Urease activity in nosocomial strains is lowered in comparison with the case of commensal strains.
3. The haemolytic activity of nosocomial strains in sheep blood agar is more than three times higher than in commensal strains. In human blood agar, differences were not found.

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