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# *Potential pathogenicity of Staphylococcus epidermidis strains colonizing upper respiratory tract in patients with resectable lung cancer*

Coagulase-negative staphylococci (CNS) represent a part of physiological microflora of human beings (2, 3). The most frequent species is *Staphylococcus epidermidis* – a natural inhabitant of the skin and mucous membrane of the upper respiratory tract (15). CNS, for years considered harmless commensals, are now known to be major nosocomial pathogens. Recently, they have been regarded as etiologic agents of hospital-acquired infections, especially related to catheters and other indwelling medical devices – so-called polymer-associated staphylococcal infections (PASI) (6, 12, 14, 15). Several studies have been undertaken to elucidate mechanisms of CNS pathogenicity. The most important factors responsible for their virulence seem to be production of slime (glycocalix), as well as hydrophobicity of cell surface. These factors may favour an ability of bacterial cells to adhere not only to skin or mucous membrane but also to biomaterials (4, 11, 14). Besides, some staphylococcal enzymes are likely to mediate colonization or to allow persistence in the host organism (6, 7, 10).

The aim of this study was to determine potential pathogenicity of *S. epidermidis* strains isolated from mucous membrane of the throat and/or nose in patients with lung cancer, undergoing thoracic surgery. These strains create a potential reservoir of endogenous infections, especially in immunocompromised patients.

## MATERIAL AND METHODS

Patient population. 28 men with non-small cell lung cancer (NSCLC) undergoing pulmonary resection were included in the present study. All patients were hospitalised at the Clinic of Thoracic Surgery, Medical University of Lublin.

Microbiological assay. 110 strains of *S. epidermidis* were isolated from the throat or nasal specimens were taken up from each patient. The routine microbiological methods were used for isolation of staphylococci. All isolates were identified by the biochemical microtests Api STAPH (bioMerieux).

Determination of protease production. Strains were inoculated onto nutrient agar plates containing gelatine and cultured for 5 days at 37°C. Proteolytic activites were determined with sublimate (mercuric chloride), as a clear zone formed around protease producing colonies (7).

Determination of lipase production. Strains were inoculated onto nutrient agar plates containing calcium chloride and Tween 80, and then were cultured for 5 days at 37°C. Lipolytic activity was determined as cloudy zone of precipitate formed around lipase producing colonies (10).

Determination of slime production. Strains were inoculated onto nutrient agar plates containing sucrose, Congo red and cultured for one day at 37°C. Slime production was assessed on the basis of the colour of staphylococcal colonies according to criteria presented by Freeman et al. (13) as following: black colonies with metalic sheen – strains intensively producing slime, dark-pink colonies – strains moderately producing slime, light-pink colonies – strains non-producing slime.

Determination of cell surface properties. The relative cell surface hydrophobicity of staphylococci was determined by using modified ammonium sulfate salt aggregation test (SAT) (5). 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.

## RESULTS

First, we assayed adhesion properties of nasopharyngeal *S. epidermidis* strains isolated from lung cancer patients. It was found that slime (glycocalix) was produced by 91/110 (83%) strains, including 86/110 (78%) isolates strongly producing this extracellular polysaccharide (Fig. 1). As shown in Figure 2, 107/110 (97.3%) strains showed hydrophobic properties of cell surface, among which 48/110 (37.3%) possessed strong hydrophobic properties and 20/110 (18.2%) – very strong hydrophobic properties.

Next, we assay production of extracellular enzymes such, as lipases and proteases by the isolated *S. epidermidis* strains. It was shown that the majority of nasopharyngeal isolates 101/110 (92%) possessed lipolytic activity (Fig. 3). In contrast, only 42/110 (38%) strains were able to produce extracellular proteases (Fig. 4).

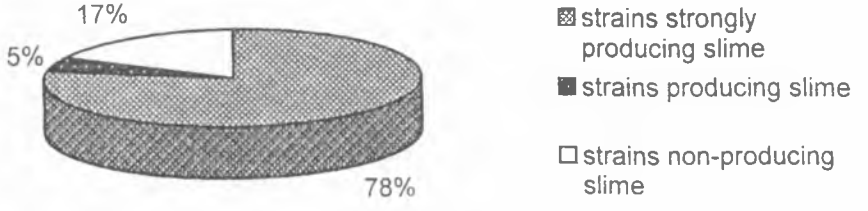


Fig. 1. Slime production by *S. epidermidis* strains colonizing nasopharynx in patients with resectable lung cancer

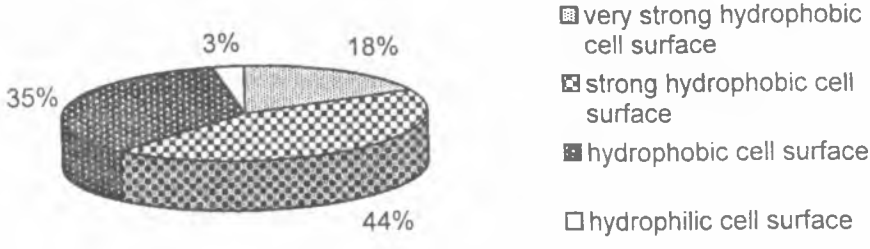


Fig. 2. Properties of cell surface of *S. epidermidis* strains colonizing nasopharynx in patients with resectable lung cancer

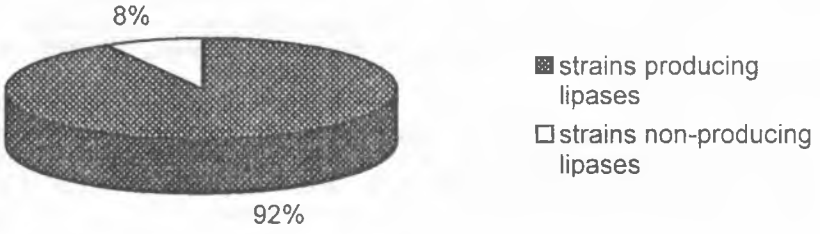


Fig. 3. Lipases production by *S. epidermidis* strains colonizing nasopahrynx in patients with resectable lung cancer

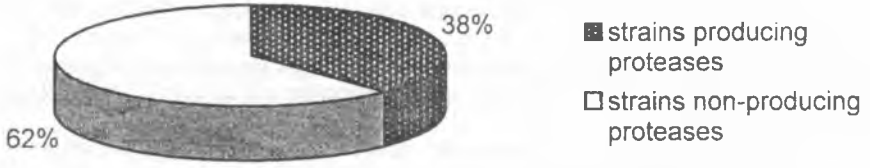


Fig. 4. Protease production by *S. epidermidis* strains colonizing nasopharynx in patients with resectable lung cancer

## DISCUSSION

In the 1980's the coagulase-negative staphylococci (CNS), particularly *S. epidermidis*, became the fifth most common cause of nosocomial infections (12), and the important agent of infections associated with biomaterials – PASI (14). The pathogenesis of such infections involves an initial step of the contact between the colonizing microorganisms and the biomaterials with subsequent biofilm formation. This structure is composed of the attached cells surrounded by slime (glycocalix), a viscous extracellular glycoconjugate (1). It is noteworthy that biofilm protects bacterial cells against defense mechanisms of immune system (e. g., phagocytosis, opsonization by antibodies) and activity of antimicrobial agents. Besides, it is also a potential source for systemic infections as a result of release of bacterial cells from biofilm and their circulation in bloodstream. Patients with cancer, especially undergoing chemotherapy and/or surgery are predisposed to PASI (12, 14). Data obtained in this paper indicate that the majority of *S. epidermidis* strains isolated from nasopharynx of lung cancer patients have à priori potential ability to form a biofilm by non-specific interactions (cell surface hydrophobicity) or due to the presence of specific extracellular matrix-reactive adhesins. This is an important observation in the light of the literature data showing that the source of the CNS species contaminating various indwelling devices is usually species of endogenous microflora from skin or mucosal membranes of the patients (15). Indeed, Korona-Głowniak et al. (8, 9) showed that CNS, most probably of nasopharyngeal origin, are the most frequently isolated strains from fluid of pleural drains in patients with resectable lung cancer after thoracic surgery.

Another factor responsible for pathogenesis of CNS is their ability to produce extracellular enzymes, including proteases and lipases. Bacterial invasion of tissues is often facilitated by the possession of proteolytic enzymes, while the ability of lipase production, by hydrolysis of lipids, may allow staphylococci to colonize skin (6). Our data indicate that the majority of nasopharyngeal isolates of *S. epidermidis* showed lipolytic activity, which can be also regarded as important colonization factor of mucosal mebranes of the upper respiratory tract by staphylococci. However,

strains of *S. epidermidis* isolated from nasopharynx possessed a limited ability to invade tissues and their damage is linked to protease production.

The mechanisms of CNS pathogenicity are still poorly recognized. Data obtained in this paper indicate that adhesion properties of staphylococci responsible for bacterial adherence to mucous membranes of the upper respiratory tract also appear to play a crucial role in infections associated with indwelling medical devices. Therefore, a possibility to limit slime production and/or to change interfere with cell surface hydrophobicity and should be considered as an alternative, valid method of prophylaxis and treatment of PASI.

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## SUMMARY

In the present study were included 110 strains of *S. epidermidis* colonizing nosopharynx in patients with non-small cell lung cancer undergoing thoracic surgery. There were tested their adhesive properties – hydrophobicity of cell surface and slime production but also an ability to produce extracellular enzymes (protease, lipase). The majority of strains showed the ability to

produce slime and possessed hydrophobic cell surface. These data indicate that *S. epidermidis* strains colonizing nosopharynx had potential ability to adhere and to form biofilm structure.

Potencjalne właściwości chorobotwórcze szczepów *S. epidermidis* kolonizujących górne drogi oddechowe

Badaniem objęto 110 szczepów *S. epidermidis* izolowanych z nosogardzieli pacjentów z rozpoznaniem niedrobnokomórkowego raka płuca, poddawanych zabiegom torakochirurgicznym. Określano potencjalne właściwości adhezyjne gronkowców, oceniając produkcję śluzu i właściwości powierzchni komórki oraz zdolność do wytwarzania enzymów zewnątrzkomórkowych (proteazy, lipazy). Wykazano, iż większość badanych szczepów charakteryzowała się hydrofobową powierzchnią komórki oraz znaczną zdolnością produkcji śluzu. Dane te dowodzą, że szczepy *S. epidermidis* kolonizujące nosogardziel posiadały potencjalne zdolności do adhezji i tworzenia struktury biofilmu.