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The influence of subinhibitory concentrations of quinupristin/dalfopristin, linezolid or vancomycin on slime production by Staphylococcus epidermidis

Wpływ subinhibicyjnych stężeń chinupristyny/dalfopristyny, linezolidu i wankomycyny na produkcję śluzu u *Staphylococcus epidermidis*

INTRODUCTION

Coagulase-negative staphylococci (CoNS), including *Staphylococcus epidermidis*, represent a part of natural microflora of the human body, colonizing preferably the upper respiratory tract or skin [10]. In recent years, these microorganisms have been generally accepted as important nosocomial pathogens involved mainly in infections associated with indwelling medical devices, so-called polymer-associated staphylococcal infections – PASI [8]. One of the most important factors responsible for biofilm formation is an extracellular polysaccharide substance also called slime (glycocalix), responsible mainly for covering of bacterial cells with biofilm [4,6,12].

A wide range of antibiotics are used for the treatment of staphylococcal infections, like β-lactams, macrolides or lincosamides. Moreover, glycopeptides, oxazolidinones or streptogramins are the drugs of choice in the treatment of infections caused by multidrug resistance strains (MDR) of staphylococci, including methicillin-resistant strains [1].

The aim of this paper was to determine the effect of quinupristin/dalfopristin, linezolid and vancomycin at subinhibitory concentrations on slime production by *S. epidermidis* strains isolated from nasopharynx of patients with non-small cell lung cancer undergoing pulmonary resection.

MATERIAL AND METHODS

Bacterial strains. A collection of 22 isolates of *S. epidermidis* strains with the ability of slime production was included in the present study. The strains were isolated from throat or nasal specimens from patients with non-small cell lung cancer undergoing pulmonary resection. Routine microbiological tests were used for isolation and identification of staphylococci.

Determination of slime production. Strains were inoculated onto nutrient agar containing 5% sucrose and 0.8% Congo red (CRA) and then incubated for 18–24 hours at 35°C. Slime-producing strains grew as black colonies with metallic sheen, whereas non-producing strains – as red colonies, according to criteria of Freeman et al. [7].

Determination of minimal inhibitory concentration (MIC) of quinupristin/dalfopristin, linezolid and vancomycin. MICs of the antibiotics were determined using E-tests. Staphylococcal strains were suspended in 0.9% NaCl at a density of 0.5 McFarland standard and inoculated onto Mueller-Hinton agar (MHA) and CRA. After incubation (18 hours at 35°C), MICs were read as a value where the edge of the inhibition ellipse intersects the strip.

Determination of minimal concentration of quinupristin / dalfopristin, linezolid and vancomycin inhibiting slime production. Staphylococcal strains were suspended in 0.9% NaCl at a density of 0.5 McFarland standard and inoculated onto CRA. After incubation (18 hours at 35°C), minimal slime inhibitory concentration (MSIC) was assessed on the basis of the change of colony colour from black to red where the edge of inhibition ellipse of slime production intersects the strip.

Reproducibility of the results. All the experiments were performed in triplicate. Representative data are presented.

RESULTS

The MIC values of vancomycin, linezolid and quinupristin/dalfopristin for the isolates determined on Mueller-Hinton (MHA) and Congo red agar (CRA) were comparable. Vancomycin inhibited growth of staphylococci at MICs between 1.5–3.0 mg/l on MHA and 1.5–4.0 mg/l on CRA. The MICs for linezolid were detected between 0.38 mg/l and 1.0 mg/l on both of the media. The MICs for quinupristin/dalfopristin were evaluated as following: 0.125–0.25 mg/l on MHA and 0.19–0.25 mg/l on CRA.

The strongest ability to inhibit slime production was shown by vancomycin (20 isolates), and to lesser extent – by linezolid (13 isolates) and quinupristin/dalfopristin (7 isolates). The MSIC was determined between 0.5–1.0 mg/l for vancomycin, 0.25–0.75 mg/l for linezolid, 0.047–0.094 mg/l for quinupristin/dalfopristin (Fig. 1). It was also found that vancomycin (0.75 mg/l), linezolid (0.38 mg/l) and quinupristin/dalfopristin (0.064 mg/l) inhibited slime production in 50% of the isolates. The MSIC values inhibiting slime production in 90% of the strains were estimated as follows: 0.75 mg/l, 0.75 mg/l, 0.094 mg/l for vancomycin, linezolid and quinupristin/dalfopristin, respectively.

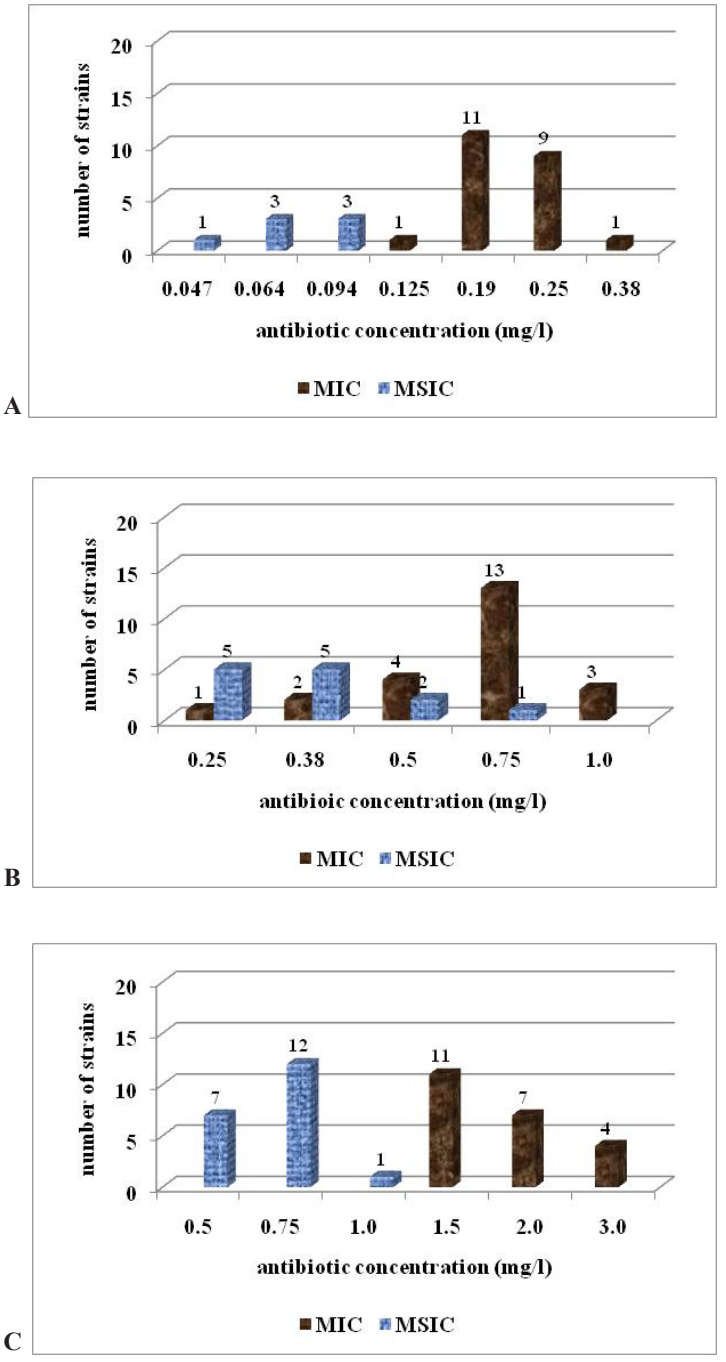

Fig. 1. The minimal inhibitory concentration (MIC) and minimal concentration inhibiting slime production (MSIC) of quinupristin/dalfopristin (A), linezolid (B) and vancomycin (C) for *Staphylococcus epidermidis* strains

DISCUSSION

Production of slime by bacterial species building biofilm structure is the final phase in biofilm maturation process. According to Carpentier and Cerf [12], biofilm is a "community of microbes embedded in an organic polymer matrix, adhering to a surface". According to the above biofilm definition, microbes, slime and surface are three basic ingredients of biofilm. On the other hand, if one of this element does not exist, the structure cannot be built. Moreover, slime is the neccessary factor for the maturation of biofilm structure, which allows for the development of cell-to-cell bridges that, in turn, cement the cells to the surface [4,6,13].

The subinhibitory concentrations of antibiotics are often present during the antimicrobial treatment of infections. It was found that some antibiotics, e.g. aminoglycosides, tetracyclines or macrolides, at subinhibitory concentrations, not effective in bactericidal or bacteriostatic activity, can be responsible for modification of the architecture of the external cell surface and some bacterial functions, like the ability of adhesion [3,11,14]. It was found that dicloxacillin and quinolones were responsible for the reduction of biofilm formation by *S. epidermidis* strains [2,13]. The mechanism of this phenomenon is difficult to assess; for example it was found that dicloxacillin changed hydrophobic bacterial cell surface to hydrophilic, leading to disturbance of adhesion [2]. The data obtained in this paper suggest that quinupristin/dalfopristin, linezolid and vancomycin at subinhibitory concentrations showed some potential ability to inhibit slime production by *S. epidermidis* strains. Probably, this phenomenon may be as the result of suppressing steps of the synthesis of monosaccharides or destroying hexose-containig polysaccharides by antibiotics affecting protein synthesis (e.g. linezolid, chinupristin/dalfopristin) [5]; the mechanism of vancomycin inhibitory activity against slime production is not known yet.

CONCLUSIONS

It is assumed that vancomycin, linezolid and quinupristin/dalfopristin may be considered as factors directly interferring in slime production at subinhibitory concentrations and thereby indirectly in biofilm formation of *S. epidermidis* strains on biomaterials; the most active agent inhibiting the slime production appears to be vancomycin.

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SUMMARY

One of the most important factors involved in biofilm formation of *Staphylococcus epidermidis* is the ability of slime production, creating a system for trapping nutrients and protecting bacterial cells with the biofilm against activity of antibiotics and immune system. The aim of this study was to assess the influence of some antibiotics on ability of slime production by *S. epidermidis*. We found that quinupristin/dalfopristin, linezolid and vancomycin inhibited slime production by S. epidermidis at subinhibitory concentrations in the following order – vancomycin < linezolid < quinupristin/dalfopristin. Our data suggest that these antibiotics, especially vancomycin, may be considered as factors interferring directly in slime production and thereby indirectly in biofilm formation of S. epidermidis.

Key words: *Staphylococcus epidermidis*, slime production, quinupristin/dalforpistin, linezolid, vancomycin, subinhibitory concentration

STRESZCZENIE

Jednym z głównych czynników odpowiedzialnych za zdolność do produkcji biofilmu szczepów *Staphylococcus epidermidis* jest wytwarzanie zewnątrzkomórkowego śluzu. Stanowi on swoistą barierę ochronną przed aktywnością antybiotyków oraz układu immunologicznego gospodarza, ale również jest źródłem składników odżywczych dla komórek tworzących tę strukturę. Celem pracy była ocena wpływu wybranych antybiotyków na zdolność do produkcji śluzu u *S. epidermidis*. Stwierdzono, że najsilniej wankomycyna, a w następnej kolejności linezolid i chinupristyna/dalfopristyna hamowały produkcję śluzu w stężeniach subinhibicyjnych. Uzyskane dane sugerują,

że te antybiotyki, szczególnie wankomycyna, mogą hamować produkcję biofilmu, prawdopodobnie bezpośrednio poprzez inhibicję wytwarzania zewnątrzkomórkowego śluzu.

Słowa kluczowe: *Staphylococcus epidermidis*, produkcja śluzu, chinupristyna/dalfopristyna, linezolid, wankomycyna, stężenia subinhibicyjne