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Effects of Slime Produced by Clinical Isolates of Coagulase-Negative Staphylococci on Activities of Various Antimicrobial Agents

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A novel in vitro semiquantitative method was developed to investigate the influence of staphylococcal slime on the activities of 22 antimicrobial agents. Pefloxacin, teicoplanin, and vancomycin demonstrated remarkable decreases in efficacy: 30, 52, and 63%, respectively. The activity of rifampin was not significantly reduced (0.99%), whereas all other agents tested were modestly affected (<15% decrease). These data could be influential in the treatment of implant-associated infections caused by slime-producing staphylococci.

The use of synthetic materials for temporary or permanent implantation has been accompanied by the emergence of a new challenging entity, namely, implant-associated infection. *Staphylococcus epidermidis* strains with the ability to form biofilms are the predominant pathogens (17, 20, 22, 30). Biofilm consists of multilayered cell clusters embedded in a matrix of extracellular polysaccharide (referred to as slime) (6). It is now well documented that efforts to eradicate biofilm bacteria are often unsuccessful and removal of the infected device is required (3, 6, 12, 29). The mechanism of resistance of biofilm bacteria to antimicrobial agents still remains unclear but appears to depend on both diffusion limitation (11, 16, 18, 19) and altered physiology associated with low growth rates and atypical phenotypes in these cells (1–3, 6, 23). The present study was designed to address the issue of reduced antibiotic penetration through staphylococcal slime due to trapping. For this purpose, a novel in vitro semiquantitative method was developed. To our knowledge, this is the first study to assess the effect of slime on 22 antistaphylococcal agents, by a uniform method.

(Part of this work was presented at the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy [13].)

Twenty-six clinical isolates of *S. epidermidis* were used in the study. Strains were collected at Laiko General Hospital in Athens, Greece, over a 6-month period. The collection consisted of isolates from pus (13 strains), catheter tips (7 strains), blood (3 strains), and cerebrospinal fluid shunts (3 strains). All strains were identified as slime producers (4). Two additional strains of *S. epidermidis*, ATCC 35983 and ATCC 35984 (American Type Culture Collection, Rockville, Md.), were used as slime-producing standard strains for quality control. The antimicrobial agents used and their final concentrations are shown in Table 1.

All strains were cultured on staphylococcus agar no. 110 (Becton Dickinson, Cockeysville, Md.), which promotes slime production (4, 31). Single colonies were inoculated in tryptic soy broth (Gibco BRL, Paisley, United Kingdom), and after a 24-h incubation, adherent bacteria from the biofilm formed on the inner surface of each tube were released by vortexing. Forty microliters of this suspension was inoculated on 13-mm-pore-size sterile cellulose filters (Millipore Corporation, Bedford, Mass.) placed on staphylococcus agar no. 110 plates. An adherent biofilm was visible on the surface of each filter after 24 h of incubation. To remove nonadherent bacteria, each filter was eluted in tryptic soy broth. The remaining slime was collected with a sterile loop, mixed with 40 μl of each antimicrobial solution, and transferred into wells of specially prepared Mueller-Hinton agar (Becton Dickinson) plates preseeded with *Bacillus subtilis* spores (MD32SDR). An equal volume (40 μl) of each antimicrobial solution without slime was placed in an additional well and used as a relevant control. Since the strains were exposed to the same environmental conditions across all experiments, the quantity of slime produced by each strain was considered to be constant (4). The suspensions used in each experiment were of standard volume, and all the wells were filled to the rim. Following a 24-h incubation, inhibition zone diameters were measured. Each experiment was simultaneously done in triplicate, and each time, samples (antibiotic with slime from each strain) and the relevant controls were placed in random places on the plate in order to eliminate the edge effect and other possible gradients across the plates.

In performing the statistical analysis, we calculated the difference between the average of the three measurements for each strain and the average control value and then tested whether these differences were statistically significant by the Wilcoxon signed rank test. In order to summarize the effects of slime on various antibiotics, we calculated the percent difference of each sample (antibiotic with slime from each strain) from the relevant control and took the average across the 26 strains as the percent reduction in the effectiveness of the antibiotic.

The results of the study are summarized in Table 2. Although the literature contains reports that argue against the presence of an antibiotic diffusion barrier in biofilms (7, 24), our results suggest that staphylococcal slime is responsible for a significant decrease in the efficacy of certain antimicrobial agents, whereas its effect is minimal for others.

Vancomycin is the drug of choice for the treatment of infections caused by methicillin-resistant staphylococci (14). However, our findings as well as data from previous reports (7, 9–11, 28, 31) suggest that the glycopeptides may not be the optimal antimicrobial agents for the treatment of foreign-body infections. A possible explanation could be the entrapment of vancomycin and teicoplanin by the extracellular mucopolysaccharide because of their high molecular weights (MWs) (1,450 and 1,600 to 1,900, respectively). These agents have higher

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TABLE 1. Concentration of each antimicrobial agent in the 40-μl solution used to fill the wells

Antimicrobial agent	Concn (μg)
Cloxacillin	20
Amoxicillin-clavulanic acid	5/2.5[a]
Imipenem	5
Cefpirome	5
Erythromycin	20
Roxithromycin	40
Clindamycin	20
Rifampin	20
Fusidic acid	20
Trimethoprim-sulfamethoxazole	20/400[a]
Doxycycline	40
Gentamicin	20
Tobramycin	20
Netilmicin	20
Amikacin	40
Isepamicin	40
Ofloxacin	20
Ciprofloxacin	20
Pefloxacin	20
Daptomycin	20
Teicoplanin	20
Vancomycin	40

[a] Ratio of first to second drug.

TABLE 2. Average inhibition zone diameters of the 26 samples, control values, and reductions in effectiveness of the antimicrobial agents in the presence of slime

Antimicrobial agent	Avg zone diam (mm) ± SD	Control[a]	*P* value[c]	Reduction in effectiveness (%)[b]
Cloxacillin	12.0 ± 0.7	12.5	<0.01	3.7
Amoxicillin-clavulanic acid	29.7 ± 1.5	31.4	<0.001	5.3
Imipenem	42.8 ± 1.1	46	<0.001	7.0
Cefpirome	30.2 ± 0.8	34	<0.001	11
Erythromycin	25.4 ± 0.6	26	<0.001	2.3
Roxithromycin	25.8 ± 0.4	26	<0.05	0.94
Clindamycin	28.6 ± 1.0	29	<0.05	1.4
Rifampin	30.7 ± 0.8	31	NS[d]	0.99
Fusidic acid	23.9 ± 1.0	25	<0.001	4.4
Trimethoprim-sulfamethoxazole	31.4 ± 0.5	33	<0.001	4.8
Doxycycline	26.5 ± 0.9	29.5	<0.001	10
Gentamicin	24.7 ± 1.2	27.4	<0.001	9.8
Tobramycin	23.5 ± 0.8	28	<0.001	16
Netilmicin	22.2 ± 1.5	25	<0.001	11
Amikacin	21.9 ± 1.0	25	<0.001	12
Isepamicin	24.7 ± 1.3	27.5	<0.001	10
Ofloxacin	24.1 ± 0.8	27.7	<0.001	13
Ciprofloxacin	31.7 ± 0.6	35	<0.001	9.5
Pefloxacin	16.7 ± 0.7	24	<0.001	30
Daptomycin	20.9 ± 0.9	23	<0.001	9.1
Teicoplanin	14.5 ± 0.5	30	<0.001	52
Vancomycin	10.9 ± 3.7	29.5	<0.001	63

[a] Control value, inhibition zone diameter (millimeters) produced by each antimicrobial agent alone.
[b] Reduction in effectiveness (percent) was calculated as the average of the percent difference of each sample (antibiotic plus slime from each strain) from the control.
[c] *P* values were calculated by the Wilcoxon signed rank test.
[d] NS denotes a *P* value not statistically significant at the 95% level.

MWs than most of the agents used in this study (range of MWs, 360 to 830). However, this explanation should exclude daptomycin, which also shares a rather high MW (1,619) but was shown to be less influenced by staphylococcal slime. Our efforts to correlate the hydrophobicities or the charges of the antibiotic molecules tested with the reductions in effectiveness produced by slime were unsuccessful, although these physicochemical properties could partly explain the reduced permeabilities of certain antibiotics through biofilms (12, 19).

We have shown that the presence of slime did not influence the activity of rifampin and had minimal influence on the activities of clindamycin and the macrolides. There is evidence to suggest that slime itself interferes with local host defenses and creates conditions of local immunosuppression (15, 26). Therefore, bacteriostatic agents such as the macrolides may not be the agents of choice for the treatment of these infections. Rifampin is a bactericidal agent with a high level of intrinsic activity against staphylococci and could be active against methicillin-resistant staphylococci (14). It also remains very potent in the presence of slime, as confirmed by other related in vitro and in vivo studies (8, 9, 21, 28, 31), suggesting that it would be an indispensable agent in combination regimens for the treatment of prosthetic-device infections. In view of the data presented in Table 2, we could conclude that among the tested agents the β-lactams, trimethoprim-sulfamethoxazole, the aminoglycosides, and the quinolones, with the exception of pefloxacin, would be potent alternatives in combination regimens for the treatment of prosthetic-device infections.

Recently, several publications have focused on the in vitro use of electric fields to enhance the penetration of antibiotics through microbial biofilms (5, 25, 27). Until this approach finds its application in clinical practice, the successful treatment of device-related infections will depend on the prudent use of antibiotics and surgery. The findings of this in vitro study may be influential in the appropriate use of antibiotics for the treatment of implant-associated infections caused by slime-producing staphylococci. This is of the utmost importance, especially for debilitated patients who cannot undergo surgical removal of the infected foreign body.

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