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Research Article

CORRELATION BETWEEN ANTIBIOTIC SUSCEPTIBILITY AND SLIME PRODUCTION OF SOME STAPHYLOCOCCI STRAINS

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Abstract

The purpose of this study was to investigate the correlation between antibiotic susceptibility and slime production of some *Staphylococcus aureus* and coagulase negative staphylococci strains. A total of 248 staphylococci (138 *S.aureus* and 110 coagulase negative staphylococci) isolated from various clinical samples were studied. For determination of slime production, congo red agar method were used. Antibiotic susceptibility to vancomycin, linezolid, teicoplanin, quinupristin/dalfopristin, cefoxitin, clindamycin and erythromycin were studied by disk diffusion method, and inducible macrolide-lincosamide-streptogramin B resistance were determined by D-test according to Clinical and Laboratory Standards Institute and tigecycline resistance was determined by United States Food and Drug Administration criteria. All clinical isolates were susceptible to vancomycin, linezolid, tigecycline, teicoplanin and quinupristin/dalfopristin. 69 *S.aureus* and 80 CNS isolates were resistant to cefoxitin, 56 *S.aureus* and 81 CNS isolates were resistant to erythromycin, 20 *S.aureus*, 18 CNS isolates have shown positive D-test result. Slime production has been found to exist 18.14% of the all staphylococci and compared to resistant and CNS strains, slime production was found at high rates in sensitive *S.aureus* strains. As a result, in this study there was not a parallel relationship between antibiotic resistance and slime production observed, conversely slime-negative isolates were found more resistant.

Keywords: Slime production; antibiotic resistance; MLS_B

Introduction

Staphylococci are nosocomial and community acquired infectious agents that cause high rates of morbidity and mortality all over the world (Akçay et al., 2005). Especially pathogen staphylococci are the most common agents of bacteremias due to gram-positive bacteria and responsible for severe infections like skin and soft tissue infections, surgical site infections, gastroenteritis and pneumonia in different tissues (Cercenado et al., 2008; Fridkin et al., 2005). Staphylococci strains have the ability to form slime (biofilm), an extracellular substance which surrounds multiple cell layers and eases bacterial adherence (Götz, 2002). Slime layer protects bacteria from phagocytosis, prevents neutrophil effect and reduces leucocyte activity. It stated in literatures that some slime

produced strains are resistant to antimicrobial therapy than slime negative strains (Boussard et al., 1993; Donlan, 2000). Slime layer fails host immune defense mechanism to reach of bacteria and often resulting in persistent infections. Antimicrobials are prevented from reaching the bacteria surrounded by slime layer and antimicrobial resistance of staphylococcal strains causes important problems in treatment of infections (Gowrishankar et al., 2012; Atshan et al., 2012). Glycopeptide antibiotics such as vancomycin and teicoplanin are still used in therapy for infections caused by methicillin resistant staphylococci. In the last decade isolates with reduced susceptibility and *in vitro* resistance to vancomycin constitute a threat and new antimicrobial alternatives such as linezolid, tigecycline,

quinupristin/dalfopristin are introduced for difficult-to-treat infections (Sancak, 2011). The commonest antibiotic that preferred for the treatment of resistant staphylococcus infections is clindamycin (Hussain, 2000). Clindamycin is an antibiotic that belongs to macrolid-lincosamide-streptogramin B (MLS_B) family (Lowy, 2003). Macrolides, lincosamides and streptogramins are structurally unrelated however they have similar mechanism of action and it may lead to the development of cross-resistance to this antibiotics (Ciraj et al., 2009).

In this study our aim was to investigate the correlation between antibiotic susceptibility and slime production of some *Staphylococcus aureus* (*S.aureus*) and coagulase negative staphylococci (CNS).

Materials and Methods

A total of 248 strains, 138 *S.aureus* and 110 coagulase negative staphylococci isolated from various clinical samples from different hospitals of Ankara in Turkey, were included in this study. Antibiotic susceptibilities of isolates to vancomycin (30µg, Bioanalyse-Turkey), linezolid (30µg, Bioanalyse-Turkey), teicoplanin (30µg, Bioanalyse-Turkey), quinupristin/dalfopristin (15µg, Bioanalyse-Turkey), cefoxitin (30µg, Bioanalyse-Turkey), clindamycin (2µg, Bioanalyse-Turkey) and erythromycin (15µg, Bioanalyse-Turkey) were determined by Kirby Bauer disk diffusion method and inducible clindamycin resistance was determined by D test according to Clinical and Laboratory Standards Institute (CLSI) criteria (Wayne, 2012). Tigecycline (15µg, Bioanalyse-Turkey) resistance was determined by United States Food and Drug Administration (FDA) criteria (United States Food and Drug Administration, 2010). Methicillin resistance was determined by cefoxitin disk. Slime production of all isolates were evaluated by the protocol of Freeman et al's congo red agar method (CRA) (Freeman et al., 1989).

For antimicrobial susceptibility test, clinical isolates were cultivated on Mueller-Hinton Agar (MHA, Merck) at 37°C for 24 hours before the assay. The colonies were suspended in Mueller-Hinton Broth (Merck) and the turbidity was compared with the 0.5 McFarland standard. Sterile cotton swab dipped into the bacterial suspension. The inoculum evenly spreaded over the entire surface of the plate prepared by inoculating in three directions. Antimicrobial susceptibility disks of all antibiotics were placed on the surface of the inoculated MHA plates and incubated 18-24 h at 37°C and the zones of growth inhibition around each of the antibiotic disks were measured.

For the detection of MLS_B phenotypes of isolates, an erythromycin disk was placed 15 mm to 26 mm (edge to edge) from a clindamycin disk. After 18-24 h incubation at 35°C, erythromycin resistant and clindamycin susceptible isolates with flattening zone of inhibition between two disks were evaluated positive for inducible clindamycin resistance (D zone positive, iMLS_B phenotype). Erythromycin resistant and clindamycin susceptible isolates with both shape circular zone of inhibition were considered to be negative for inducible clindamycin resistance (D zone negative) but had an efflux phenotype (M/MSB). Isolates that were resistant to both erythromycin and clindamycin were evaluated constitutive phenotype (cMLS_B).

Slime test was prepared by dissolving the following substance in 1 liter of distillate water (brain heart infusion broth, 37 g; sucrose 50 g; agar 10 g; Congo red agar, 0.8 g) and autoclaved at 121°C for 15 minutes. Congo red stain was prepared as a concentrated solution and autoclaved separately, then added to the medium when the agar had cooled to 55°C. Plates were incubated 24h at 37°C. A positive result was indicated by black colonies with a dry crystalline consistency.

Results

In investigated strains there were not reduced susceptibility or resistance to glycopeptides detected. All clinical isolates were susceptible to vancomycin, linezolid, tigecycline, teicoplanin and quinupristin/dalfopristin. Among these 69 of 138 *S.aureus* and 80 of 110 coagulase negative staphylococci isolates were resistant to methicillin. A total of 137 staphylococci (56 *S.aureus* and 81 CNS) isolates were determined erythromycin resistant. However strains were found to be sensitive to all other antibiotics, the correlation between erythromycin resistance and slime formation was evaluated, MLS_B phenotypes of 248 isolates in addition to antibiotic susceptibility and slime production results were shown at Table 1. The presence of iMLS_B was confirmed by D test and 16 of MRSA, 12 of MRCNS, 4 of MSSA, 6 MSCNS isolates have shown positive test results. The rates of cMLS_B, M/MSB phenotype were determined 44.5% (61 strains) and 27.7% (38 strains) in all erythromycin resistant strains. Slime production was detected by congo red agar method and 6 (7.5%) of MRCNS, 12 (17.3%) of MRSA, 24 (34.7%) of MSSA and 3 (10%) of MSCNS were found to be positive. Antibiotic resistance compared with slime positive and negative strains, the expected resistance in positive strains was not observed height. Conversely methicillin resistance of slime-negative staphylococci strains were found to be higher.

Table 1. Correlation between erythromycin resistance and slime production of *S.aureus* and coagulase negative staphylococcus strains

MLSB phenotypes	MRCNS	MRCNS slime positive strains	MRSA	MRSA slime positive strains	MSSA	MSSA slime positive strains	MSCNS	MSCNS slime positive strains	Total n
D zone (iMLSB)	12	-	16	1	4	-	6	-	38
Constitutive phenotype (cMLSB)	38	-	14	-	2	-	7	-	61
Eflux pump (M/MSB)	16	3	11	1	8	1	3	-	38
Sensitive to erythromycin	14	3	28	10	55	23	14	3	111
Total n	80	6	69	12	69	24	30	3	

(**MRCNS**: Methicillin resistant coagulase negative staphylococcus, **MRSA**: Methicillin resistant *S.aureus*, **MSSA**: Methicillin susceptible *S.aureus*, **MSCNS**: Methicillin susceptible coagulase negative staphylococcus **n**: Number of clinical isolates.)

Discussion

Because of developing resistance to various antibiotics, resistant staphylococci infections are becoming difficult to treat. *S.aureus* and coagulase negative staphylococci are known as causing nosocomial and community acquired infections worldwide (Maltezou and Giamarellou, 2006).

Pathogenicity of this staphylococci infections might be related to a virulence factor known as slime which permits these microorganisms to adhere to surfaces (Vogel et al., 2000) and to hamper antibiotics to access microorganisms by inhibiting the diffusion (Kloss and Bannerman, 1994).

In this present study, we determined the antibiotic susceptibility of 248 staphylococci isolates. All strains were susceptible to vancomycin, linezolid, tigecycline, teicoplanin and quinupristin/dalfopristin and 149 isolates were resistant to methicillin, 137 isolates were resistant to erythromycin. The methicillin resistance in staphylococci is an important clinical problem because of the development of resistance to other antimicrobial agents and isolates with reduced susceptibility (Baragundi Mahesh et al., 2013). While investigating a suitable alternative antimicrobial for the treatment of resistant staphylococcal infections, clindamycin is the most preferred (Hussain et al., 2000) which belongs to the macrolide, lincosamide and streptogramin B (MLS_B) family of antibiotics. Expression of MLS_B resistance can be either constitutive or inducible. (Tang et al., 2012). Slime production has been found

to exist 18% of the all staphylococci isolates. In various studies, slime production of CNS strains has been reported to be correlated with increasing resistance to antibiotics (Boussard et al., 1993). Boynukara et al. (2007) determined that 60% of CNS were found to be positive for slime production. Aral et al. (2004) found that 40% of 30 CNS strains isolated from maxillary and ethmoid sinuses had slime production. Arciola et al. (2002) reported that 65 (57.5 %) of the 113 clinical CNS isolates were slime-producer strains and Stepanovic et al. (2001) reported that out of 107 (88.4 %) slime-producing CNS strains, 26 (24.3 %) were strongly positive. Also similar studies (Kogan et al., 2006; Foka et al., 2006) have shown that the rates of slime-producing CNS strains from various clinical samples can be found vary percentages, but in our study compared to resistant and CNS strains, slime production was found at high rates in sensitive *S.aureus* strains.

The findings of our study showed that slime formation wasn't more prominent in CNS strains than *S.aureus* strains isolated from various clinical samples. Slime formation have been detected in methicillin-erythromycin susceptible *S.aureus* strains most (34.7%). Slime production rates of methicillin-resistant strains, MRSA and MRCNS were 17.3% and 7.5% respectively. Kart-Ya ar et al. (2011) were observed similar results with us and reported that methicillin and antimicrobial resistance were significantly higher in slime negative strains, slime production rate was higher at *S.aureus* strains.

Conclusion

Slime production rates of clinical staphylococci strains were consistent with literatures. It was thought that the reason for this rate is higher in *S. aureus* isolates depends on the relationship between slime production and pathogenicity but in this study, there was not a parallel relationship between antibiotic resistance and slime production observed, conversely slime-negative isolates were found more resistant. As a result, we believe that the ability of slime production alone is not enough for developing antimicrobial resistance but at the end of the formation of biofilm layer, bacteria gain resistance to antibiotics.

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