Articolo originale

Original article

Assessment of methicillin and clindamycin resistance patterns in *Staphylococcus aureus* isolated from a tertiary hospital in Turkey

Determinazione dei profili di resistenza a meticillina e clindamicina in isolati di Staphylococcus aureus in un ospedale terziario in Turchia

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INTRODUCTION

Staphylococcus aureus is one of the most significant causes of hospital-acquired and community-acquired infections all over the world. Methicillin resistance in staphylococci is an increasing problem throughout the world. Methicillin-resistant *S. aureus*, briefly referred as MRSA, are resistant to β-lactam group of antibiotics and recently gained resistance to many other antibiotics and also to glycopeptides. *In vitro* susceptibility to clindamycin, trimethoprim-sulfamethoxazole, erythromycin, quinolones and tetracyclines has frequently been reported worldwide. Macrolides (e.g. erythromycin, azithromycin, spiramycin), lincosamides (e.g. clindamycin, lincomycin), and group B streptogramins (e.g. quinupristin) are antibiotic groups collectively named macrolide-lincosamide-streptogramin B (MLS_B) group. While MLS_B were commonly used agents for treatment of staphylococcal infections, due to their widespread use the frequency of MLS_B resistance in staphylococci isolates has gradually increased (1-5). Members of MLS_B group have chemically distinct structure but they demonstrate their effects by inhibiting protein synthesis of bacteria. Genes which are responsible from resistance to one member of the MLS_B group of antibiotics may confer cross-resistance to the other members of group (6). The most common mechanism of acquired resistance to this group of antibiotics is modification of a target site by ribosomal RNA methylation. Other common mechanisms are enzymatic inactivation and active efflux pump. Target site modification is mediated by the presence of erythromycin resistance methylase (erm) genes. Erythromycin resistance methylase (erm) genes encode methylases. Methylases cause conformational modification of 23S rRNA and lead to reduced binding of MLS_B group of antibiotics to the target site in the 50S ribosomal subunit (6). Phenotypic expression of MLS_B resistance in *S. aureus* can be either inducible (following exposure to a macrolide) that is referred to briefly as $iMLS_B$ or constitutive (always produced) that is referred to briefly as $cMLS_B$. *S. aureus* with $cMLS_B$ are observed both erythromycin-resistant and clindamycin-resistant as *in vitro*. On the other hand, *S. aureus* with $iMLS_B$ are observed erythromycin-resistant but appear to be clindamycin-sensitive as *in vitro*. $cMLS_B$ can be

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detected by routine antimicrobial susceptibility testing. However, *S. aureus* with $iMLS_B$ are not differentiated by using routine antimicrobial susceptibility testing, but can be distinguished by clindamycin-erythromycin disk approximation test (D-test) and demonstration of resistance genes by molecular methods.

Clindamycin is a preferred agent for treatment of skin, soft tissues and bone infections caused by MRSA. This antimicrobial agent displays good efficacy against staphylococci infections and demonstrate good penetration to skin, soft tissues and bone (6). Clindamycin-resistant *S. aureus* can be detected as clindamycin-sensitive by routine antimicrobial susceptibility testing and these wrong results lead to treatment failure in patients with staphylococci infection.

In this study, we aimed to determine the methicillin susceptibility and the incidence of MLS_B resistance in *S. aureus* from various clinical specimens in a tertiary hospital during a period of four years.

MATERIALS AND METHODS

Study design

A total of 254 *S. aureus* isolates obtained from various clinical specimens at the clinical Microbiology laboratory of Dicle University Hospital in the period comprised between January 2008 and December 2011 were included in this study. Duplicate isolates from the same patient were not included in this study. In total, 254 *S. aureus* isolates were obtained from clinical specimens, comprising 199 (78.3%) inpatients and 55 (21.7%) outpatients. Of the 254 *S. aureus* isolates, 108 (42.5%) were isolated from wound swabs, 56 (22.1%) from cerebrospinal fluids, 42 (16.5%) from joint fluids, 32 (12.6%) from tissue biopsy specimens, 10 (3.9%) from abscess materials (pus) and six (2.4%) from sputum. Definitions of inpatient infections were set according to the Centers for Disease Control and Prevention (CDC), which defines a hospital-acquired infection as a localized or systemic condition that results from adverse reaction to the presence of an infectious agent not present or incubating at the time of admission to the hospital.

Identification of S. aureus isolates and antibiotic susceptibility test

S. aureus isolates were identified by conventional methods such as colony morphology onto culture medium, Gram staining, catalase activity, slide and tube coagulase tests, DNase test and BD Phoenix™ 100 (Becton Dickinson, MD, USA) fully automated microbiology system. Methicillin susceptibility of strains was investigated by measuring the inhibition zone diameter (as mm) using Kirby-Bauer's disk diffusion method. Cefoxitin (30 µg) and oxacillin (1 µg) impregnated disks (Oxoid Ltd., Basingstoke, UK) were used for the investigation of methicillin susceptibility. Vancomycin susceptibility was tested by determining the minimal inhibitory concentration (MIC as µg/mL) value using E-test method. For Kirby-Bauer's disk diffusion method and E-test method, a 0.5 McFarland turbidity standard equivalent suspension of microorganisms was inoculated onto Mueller-Hinton agar (Merck KGaA, Darmstadt, Germany) plates as described in the Clinical and Laboratory Standards Institute (CLSI) recommendations (7). For methicillin susceptibility accordance with CLSI guidelines, inhibition zone diameter for oxacillin of ≥13 mm was considered as sensitive, 11-12 mm as intermediate, ≤10 mm as resistant; inhibition zone diameter for cefoxitin of ≥22 mm was considered as sensitive, ≤21 mm as resistant. According to the MIC interpretive standard of CLSI, MIC value for vancomycin of ≤2 µg/mL was considered as sensitive, 4-8 µg/mL as intermediate, ≥16 µg/mL as resistant. All isolates were also examined for susceptibility to clindamycin (2 µg) and erithromycin (15 µg) (Oxoid Ltd., Basingstoke, UK). For antibiotic susceptibility accordance with CLSI breakpoints, inhibition zone diameter for clindamycin of ≥21 mm was considered as sensitive, 15-20 mm as intermediate, ≤14 mm as resistant; inhibition zone diameter for erythromycin of ≥23 mm was considered as sensitive, 14-22 mm as intermediate, ≤13 mm as resistant. Isolates appearing as clindamycin-sensitive and erythromycin-resistant were tested for inducible resistance by the D-test method. For D-test method a 0.5 McFarland turbidity standard equivalent suspension of microorganisms was inoculated onto Mueller-Hinton agar plate according to the CLSI guidelines. Clindamycin and erithromycin disks were placed 25 mm apart from center to center onto Mueller-Hinton agar plates. Plates were examined after 18-24 hours aerobically incubation at 35±2°C. Interpretation of the inhibition zone diameters was as follows: if an isolate was erythromycin-resistance and clindamycin-sensitive, with a D-shaped inhibition zone around the clindamycin disk, it was considered to be

positive for inducible resistance (D-test positive). If the isolate was both erythromycin-sensitive and clindamycin-sensitive, with both zones of inhibition showing a circular shape, the isolate was considered to be negative for inducible resistance (D-test negative), but to have an active efflux pump. If the isolate was both erythromycin-resistant and clindamycin-resistant, the isolate was considered to have the macrolide-lincosamide-streptogramin B constitutive phenotype. Quality control was performed with *S. aureus* ATCC 25923 strain.

Statistical analysis

Data of this study were analyzed by Epi Info™ 7-Community Edition (Centers for Diseases Control and Prevention, Atlanta, GA, USA) statistical package program. Statistical evaluation of difference between inpatients and outpatients strains for D-test positivity was performed with the Chi-square test. Statistical evaluation of difference between MSSA and MRSA strains for constitutive resistance, and for D-test positivity was performed with the *Fisher's exact test*. The P value of <0.05 was selected for statistical significance.

RESULTS

In the present study among a total of 254 *S. aureus* isolates, including 139 (54.7%) MSSA and

Table 1 - Distribution according to the D-test positivity of MSSA and MRSA isolates.

Property	*D-test positive n (%)*	*D-test negative n (%)*	*P*
MSSA (n = 139) MRSA (n = 115)	8 (5.8) 2 (1.7)	131 (94.2) 113 (98.3)	0.118
Total	10 (3.9)	244 (96.1)	
MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus*.			

Table 2 - Distribution according to the constitutive resistance of MSSA and MRSA isolates.

Property	*CR positive n (%)*	*CR negative n (%)*	*P*
MSSA (n = 139) MRSA (n = 115)	- 100 (87)	139 (100) 15 (13)	<0.001
Total	100 (39.4)	154 (60.6)	
MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus*; CR: constitutive resistance.			

Table 3 - Distribution of inducible clindamycin-resistance *S. aureus* isolates according to the type of specimen, clinics, patient's status and methicillin susceptibility.

No.	*Specimen*	*Clinic*	*Patient's status*	*Methicillin*
1	joint fluid	oncology	outpatient	S
2	joint fluid	orthopedic surgery	outpatient	S
3	cerebrospinal fluid	endocrinology	outpatient	S
4	cerebrospinal fluid	chest diseases	outpatient	S
5	tissue biopsy specimen	pediatric infectious diseases	inpatient	R
6	tissue biopsy specimen	orthopedic surgery	inpatient	R
7	sputum	adult infectious diseases	inpatient	S
8	sputum	chest diseases	outpatient	S
9	abscess material (pus)	orthopedic surgery	outpatient	S
10	abscess material (pus)	orthopedic surgery	outpatient	S
S: Sensitive; R: Resistance.				

115 (45.3%) MRSA, 10 (3.9%) isolates were found to be clindamycin-sensitive and erythromycin-resistant by the disk diffusion method. All the clindamycin-sensitive and erythromycin-resistant isolates were D-test positive (inducible clindamycin-resistant phenotype). Among 10 inducible clindamycin-resistant isolates, eight isolates were methicillin-sensitive and two isolates were methicillin-resistant (Table 1). There was no statistically significant difference between MSSA and MRSA isolates for D-test positivity (P=0.118). In addition, among inducible clindamycin-resistant isolates, five isolates were obtained from inpatients and five isolates were obtained from outpatients. There was no statistically significant difference between inpatients and outpatients for D-test positivity (P=0.067). Constitutive clindamycin resistance was detected in 100 (39.4%) of MRSA isolates, and it was not observed in MSSA isolates (Table 2). The difference between MSSA and MRSA isolates for constitutive clindamycin resistance was found to be statistically significant (P <0.001). Distribution of inducible clindamycin-resistant isolates according to the type of specimen, clinics, patient's status and methicillin susceptibility is showed in Table 3. All of *S. aureus* isolates were found as susceptible to vancomycin.

DISCUSSION

S. aureus is one of the major microorganisms that cause bacteraemia and/or sepsis and nosocomial infections. The antimicrobial resistance is an increasing problem in *S. aureus* infections all over the world. In recent years the emergence of MRSA isolates led to difficulties for the treatment of infections caused by this microorganism. Currently, 90-95% of clinical staphylococci isolates are resistant to penicillin throughout the world (8-11). MRSA prevalence ranges from 12% to 62.5% in West Asian countries. In European countries, MRSA prevalence varies from 0.6% to 45%. In the Mediterranean region, MRSA prevalence is reported as more than 50%. MRSA prevalence was reported between 33% and 71.3% in several studies from Turkey (5). In our study, methicillin resistance of *S. aureus* isolates was found to be >50%. The differences in the prevalence of MRSA in different countries indicate that local antimicrobial susceptibility testing has a significant role for empiric therapy. Our study showed that beta-lactam antibiotics are inefficient agents for *S. aureus* isolates in our hospital and it is necessary to use different antibiotics for treatment of *S. aureus* infections. In the light of our high methicillin resistance rate, we can say that infection control measures should be increased in our hospital. The hospital staffs should be trained about hand hygiene, some revisions should be made on the sterilization and disinfection strategies, and notification of the results of antibiotic susceptibility testing should be restricted by microbiology laboratory.

Clindamycin is a well tolerated agent that is used for the treatment of skin, soft tissues, bone and intra-abdominal infections caused by *S. aureus*. This agent is an alternative choice for patients resistant or allergic to beta-lactam antibiotics. However the number of constitutive resistant isolates has increased today. On the other hand emergence of inducible clindamycin resistance led to failures in treatment of infections caused by *S. aureus* (5). The constitutive and inducible clindamycin-resistant phenotype predominated in *S. aureus* isolates varies according to countries in worldwide (17-22).

In a study from Indian, carried on 379 *S. aureus* and 472 coagulase-negative staphylococci isolates, constitutive resistance was demonstrated in 47% and inducible clindamycin resistance in 23.5% of isolates (18). In a study from Spain, Merino-Diaz L. et al. detected inducible clindamycin resistance rates as 5.2% in *S. aureus* and constitutive resistance rates as 1.7% in *S. aureus* (19). In a Turkish study, constitutive clindamycin resistance was found in 64.6% of MRSA, and in 11.8% of MSSA isolates. Among these isolates, inducible clindamycin resistance was detected in 90% of *S. aureus* (20). In another study from Turkey, 19.4% of *S. aureus* isolates showed inducible clindamycin resistance and 76.5% of erythromycin-resistant, clindamycin-sensitive isolates showed inducible clindamycin resistance (21).

In another study from Turkey in 2012, the inducible clindamycin resistance was investigated by D-test among 306 staphylococci isolates. Fifty-three (17.3%) out of 177 *S. aureus* isolates were MRSA and 124 (40.5%) were MSSA. Among the coagulase-negative staphylococci (CoNS), 121 (39.6%) were methicillin-sensitive and 8 (2.6%) were methicillin-resistant. Ninety-nine (36.4%) out of 306 clinical isolates showed erythromycin resistance; among the erythromycin-resistant isolates, 46 (46.5%) were CoNS of which 8 (15.1%) were methicillin-resis-

tant CoNS and 38 (31.4%) methicillin-sensitive CoNS. Twenty (6.5% of total isolates) out of 31 erythromycin-resistant isolates belonged to $iMLS_B$ phenotype and 11 isolates belonged to $cMLS_B$ phenotype [22].

In the present study constitutive clindamycin resistance prevalence was higher (39.4%, n=100) but inducible clindamycin resistance prevalence was lower (3.9%, n = 10) in our hospital isolates.

This study showed that constitutive clindamycin resistance was the major mechanism in our isolates. Constitutive resistance was the most frequent cause of clindamycin resistance in many studies from the world (12-14, 23). On the other hand, 100% of erythromycin-resistant, clindamycin-sensitive isolates have inducible clindamycin-resistant phenotype (D-test positive). Our data also showed that MLS_B resistance was a predominant mechanism in erythromycin-resistant, clindamycin-sensitive isolates of our hospital. Our study revealed that clindamycin was not an effective agent for staphylococcus isolates in our hospital. In addition, as the automated systems may not detect inducible clindamycin resistance, clindamycin-sensitive and erythromycin-resistant isolates should be tested for inducible resistance by the D-test method.

In conclusion, this study indicated that clindamycin was not a suitable antibiotic for *S. aureus* infections in our hospital and that the presence of inducible clindamycin-resistant phenotype isolates may lead to clindamycin treatment failure in patient with *S. aureus* infection.

Keywords: methicillin, clindamycin, *Staphylococcus aureus.*

SUMMARY

In order to detect the methicillin susceptibility and determine the prevalence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* strains, a total of 254 *S. aureus* isolates, including 139 (54.7%) methicillin-sensitive and 115 (45.3%) methicillin-resistant, were tested for clindamycin and erythromycin by Kirby-Bauer's disk diffusion method. The disk diffusion induction test or D-test was performed on erythromycin-resistant and clindamycin-sensitive strains using the disk diffusion method. The erythromycin-resistant and clindamycin-sensitive strains with a D-shaped zone around the clindamycin disk were considered positive for inducible clindamycin resistance. If a strain was found to be both erythromycin-resistant and clindamycin-resistant, it was considered to have constitutive clindamycin resistance. Overall, ten (3.9%) strains, including two methicillin-resistant *S. aureus* and eight methicillin-sensitive *S. aureus*, were found to be erythromycin-resistant and clindamycin-sensitive according to the disk diffusion method and all of these strains showed inducible clindamycin resistance by the D-test method. Constitutive clindamycin resistance was detected in 100 of all MRSA strains.

In conclusion, the presence of inducible clindamycin-resistant strains may lead to clindamycin treatment failure in patients with *S. aureus* infection. The D-test method should be used for antimicrobial susceptibility testing of clindamycin.

RIASSUNTO

Al fine di rilevare la sensibilità alla meticillina e determinare la prevalenza della resistenza costitutiva e inducibile a clindamicina in Staphylococcus aureus, *254 ceppi di* S. aureus, *inclusi 139 (54,7%) meticillino-sensibili (MSSA) e 115 (45,3%) meticillino-resistenti (MRSA), sono stati testati nei confronti di clindamicina ed eritromicina mediante metodo di Kirby-Bauer. Il test di induzione mediante disco-diffusione o D-test è stato effettuato su isolati eritromicina-resistenti e clindamicina-sensibili ricorrendo al metodo di diffusione con dischetto. Gli isolati eritromicina-resistenti e clindamicina-sensibili il cui alone di inibizione intorno al dischetto di clindamicina era a forma di D sono stati considerati positivi per la resistenza inducibile a clindamicina. Il riscontro di resistenza contemporanea a eritromicina e clindamicina è stato considerato come indice di resistenza costitutiva a clindamicina. Complessivamente, l'antibiogramma con il metodo di Kirby-Bauer ha consentito di rilevare dieci (3,9%) isolati eritromicina-resistenti e clindamicina-sensibili, inclusi due MRSA e otto MSSA, che hanno tutti evidenziato una resistenza inducibile a clindamicina mediante il metodo del D-test. Tale resistenza costitutiva a clindamicina è stata riscontrata in 100 di tutti gli isolati MRSA.*

In conclusione, la presenza di isolati caratterizzati da resistenza inducibile a clindamicina può determinare un fallimento del trattamento con clindamicina in pazienti con infezione da S. aureus. *Sarebbe dunque auspicabile che il metodo D-test venisse utilizzato per la determinazione della sensibilità a clindamicina.*

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