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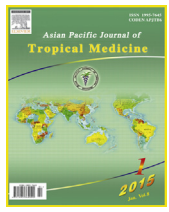

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Antimicrobial resistance and underlying mechanisms in *Staphylococcus aureus* isolates

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ABSTRACT

Objective: To investigate the antimicrobial susceptibility of 97 clinical *Staphylococcus aureus* strains against 14 antimicrobials and corresponding resistance mechanisms.

Methods: The antimicrobial susceptibility of the isolates was determined using a disk diffusion method and antimicrobial resistance genes were screened by polymerase chain reaction. Mutations responsible for ciprofloxacin and rifampicin resistance were investigated by polymerase chain reaction and DNA sequencing.

Results: All isolates were found to be susceptible to vancomycin. Various rates of resistance to penicillin (83.5%), ampicillin (77.3%), erythromycin (63.9%), tetracycline (16.5%), amoxicillin/clavulanic acid (16.5%), ciprofloxacin (15.5%), trimethoprim/sulfamethoxazole (15.5%), oxacillin (13.4%), fusidic acid (12.4%), rifampin (6.2%), clindamycin (6.2%), gentamicin (6.2%) and mupirocin (5.2%) were determined. In addition, different combinations of resistance genes were identified among resistant isolates. Ciprofloxacin resistant isolates had mutations in codon 84 (Ser84Leu) and 106 (Gly106Asp) in the *gyr*A gene. Mutations in *grl*A were mostly related to Ser80Phe substitution. Leu466Ser mutation in the *rpo*B gene was detected in all rifampin resistant isolates. All methicillin resistant *S. aureus* isolates were SCC*mec* type V.

Conclusions: In conclusion, it was determined that the isolates were resistant to different classes of antimicrobials at varying rates and resistance was mediated by different genetic mechanisms. Therefore, continuous monitoring of resistance in *S. aureus* strains is necessary to control their resistance for clinically important antimicrobials.

1. Introduction

Staphylococcus aureus (S. aureus) is one of the most common human pathogens causing different sequelae of infections in both genders and all age groups [1]. The emergence and spread of antimicrobial resistant *S. aureus* isolates, particularly methicillin resistant *S. aureus* (MRSA), constitutes a global challenge for the treatment of infections caused by these bacteria [2]. Infections caused by resistant bacteria extend the duration of stay at the hospital, increase the cost of health care services, and most importantly, lead to a significant increase in both morbidity and mortality rates [3].

S. aureus develops resistance to antimicrobials by different mechanisms. These mechanisms include limiting uptake of the drug, modification of the drug target, enzymatic inactivation of the drug, and active efflux of the drug. Depending on the antimicrobial involved, the bacteria may use one or several of these resistance mechanisms. In particular, the localization of resistance genes on transferable genetic elements such as plasmids and transposons facilitates horizontal transfer of resistance between bacteria [4].

Rapid and accurate determination of the antimicrobial resistance phenotype and resistance mechanisms has great importance, not only for treatment options but also public health risks [5]. In the present study, the susceptibilities of 97 *S. aureus* strains from various clinical specimens in Hatay, Turkey were tested by the disk diffusion method and the underlying molecular mechanisms were investigated.

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2. Materials and methods

2.1. Bacterial isolates

A total of 97 *S. aureus* isolates obtained from various clinical specimens such as wound swabs (63, 64.90%), urine (17, 17.50%), blood cultures (3, 3.10%), sputum (4, 4.12%) and other samples (1, 1.03%) between January and July 2011 at the Microbiology Laboratory of Antakya Public Hospital (Hatay) were used in the study. Antimicrobial susceptibility and molecular analysis of the isolates were performed at Department of Microbiology, Faculty of Veterinary Medicine, Mustafa Kemal University. Isolation and identification of the isolates involved standard biochemical tests such as colony morphology, Gram staining, catalase reaction and tube coagulase test. All isolates were confirmed using species-specific polymerase chain reaction [6].

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates was determined by the disk diffusion method according to Clinical Laboratory Standards Institute guidelines (CLSI) [7]. The following antimicrobial disks were used: vancomycin (VA, 30 μg), penicillin (P, 10 U), ampicillin (AM, 10 μg), erythromycin (E, 15 μg), tetracycline (TE, 30 μg), amoxycillin-clavulanic acid (AMC, 20 μg/10 μg), ciprofloxacin (CIP, 5 μg), trimethoprim-sulfamethoxazole (SXT, 1.25 μg/23.75 μg), oxacillin (OXA, 1 μg), fusidic acid (FA, 10 μg), rifampicin (RA, 5 μg), clindamycin (DA, 2 μg), gentamicin (CN, 10 μg), and mupirocin (MUP, 5 μg). Since there are no standardized CLSI breakpoints for mupirocin and fusidic acid, the results of these antibiotics were interpreted as described previously [8,9]. *S. aureus* ATCC 29213 was also used as a quality control. The isolates resistant to at least three different antimicrobial classes were accepted as multidrug resistant.

2.3. Oxacillin disk diffusion test

The oxacillin susceptibility test was performed according to CLSI [7] recommendations using a 1 μg oxacillin disk. *S. aureus* ATCC 25923 (susceptible) and *S. aureus* ATCC 43300 (resistant) were used as control strains.

2.4. Determination of antimicrobial resistance genes and mutations

Bacterial DNA samples were prepared according to the method as previously described [10]. Antimicrobial resistance genes for macrolide (*erm*A, *erm*B, *erm*C, *msr*A, *mph*C) [11–13], lincosamide (*lnu*A) [14], aminoglycoside (*aac(6')-aph(2'')*, *aph(3')-IIIa*, *ant(4)-Ia*) [15], tetracycline (*tet*K, *tet*M) [16], mupirocin (*ileS*-2) [17] and fusidic acid (*fus*B, *fus*C) [18] were researched as previously reported.

In order to determine the mutations, *grl*A (469 bp), *gyr*A (398 bp) and *rpo*B (1052 bp) genes were amplified, and the nucleotide sequences of the amplified products were subsequently determined commercially (Macrogen, Netherlands). Mutations were determined by comparison with the published sequences (for *grlA* gene of *S. aureus* D67074 and D67075, for *gyr*A gene of *S. aureus* D10489, for *rpo*B gene of *S. aureus* CAA45512) [19–22].

2.5. SCCmec typing

SCC*mec* types of *mec*A positive isolates were determined using the method and primers described by Kondo *et al.* [23]. SCC*mec* type assignment of the isolates was carried out according to *ccr* and *mec* gene complexes.

3. Results

3.1. Antimicrobial susceptibility testing

Of 97 *S. aureus* isolates, 9 (9.3%) were susceptible to all the antimicrobials tested. None of the isolates were resistant to vancomycin. Various rates of resistance were observed to penicillin (83.5%), ampicillin (77.3%), erythromycin (63.9%), amoxicillin/clavulanic acid (16.5%), tetracycline (16.5%), ciprofloxacin (15.5%) and trimethoprim/sulfamethoxazole (15.5%), followed by oxacillin (13.4%), fusidic acid (12.4%), rifampin (6.2%), clindamycin (6.2%), gentamicin (6.2%) and mupirocin (5.2%). Multidrug resistant was detected among 28 (28.7%) isolates and multidrug resistant to 8, 7, 6, 5, 4 and 3 antimicrobials was detected in 3 (10.7%), 1 (3.6%), 3 (10.7%), 6 (21.4%), 7 (25.0%) and 8 (28.6%) isolates, respectively. Resistance phenotypes determined among *S. aureus* isolates are given in Table 1.

Table 1
Resistance phenotypes determined among *S. aureus* isolates.

Phenotype	No of isolates
OXA, P, AMP, AMC, MUP, CIP, FA, SXT, TE, CN, E	1
OXA, P, AMP, AMC, RA, CIP, FA, SXT, DA, TE, E	1
OXA, P, AMP, AMC, MUP, FA, SXT, DA, CN, E	1
OXA, P, AMP, AMC, CIP, SXT, TE, CN, E	1
OXA, P, AMP, AMC, RA, CIP, FA, SXT, E	1
P, AMP, AMC, RA, CIP, DA, TE, E	1
P, AMP, AMC, MUP, CIP, DA, TE	1
P, MUP, CIP, SXT, DA, TE, CN, E	1
P, AMP, AMC, RA, CIP, TE, E	1
P, AMP, AMC, CIP, DA, TE, E	1
OXA, P, AMP, FA, TE, E	1
OXA, P, AMC, FA, SXT, E	1
P, AMP, AMC, CIP, TE, E	1
P, AMP, AMC, CIP, TE, E	1
P, AMP, MUP, FA, SXT, E	1
OXA, P, AMP, SXT, CN, E	1
P, AMP, AMC, CIP, E	2
FA, SXT, TE, CN, E	1
P, AMP, CIP, TE, E	1
OXA, P, RA, SXT, E	1
OXA, P, AMP, E	1
P, AMP, AMC, E	1
P, AMP, AMC, E	1
P, AMP, E, TE	2
P, AMP, FA, E	2
P, AMP, FA	1
P, AMP, CIP	1
P, AMP,TE	1
OXA, P, E	1
P, SXT, E	1
P, AMP, E	28
P, AMP	20
FA, TE	1
SXT, E	2
E	3
Pan-susceptible	9

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