MOLECULAR DETECTION OF NINE ANTIBIOTIC RESISTANCE GENES IN METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES

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ABSTRACT

This study aimed to evaluate the relation between the phenotypic antibiotic susceptibility patterns and the antibiotic resistance genes and to investigate the prevalence of macrolide, lincosamides, streptogramin, aminoglycoside and tetracycline resistance genes among MRSA isolates. A total of 55 clinical MRSA isolates were included in this study, antibiotic resistance was conducted by Kirby-Bauer disk diffusion method, broth microdilution assay and multiplex PCR technique. Our results showed that there was no discordance between conventional susceptibility testing and gene detection by multiplex PCR assay.

The prevalence of erm(A), erm(C), tetK, tetM, aacA-aphD, vat(A), vat(B) and vat(C) gene among MRSA isolates was 30.9%, 74.5%, 76.4%, 16.4%, 74.5%, 1.8%, 0% and 5.5%, respectively. These MRSA strains belonged to SCCmec types II, III, IVa and V. Rapid and reliable method for antibiotic susceptibility is important to determine the appropriate therapy decision. Multiplex PCR can be used for confirmation of the results obtained by disk diffusion method or could be used as an alternative diagnostic method in the routine diagnosis for rapid, sensitive, and specific detection of MRSA associated antibiotic resistance genes.

Keywords: MRSA, antibiotic resistance genes, multiplex PCR assay, macrolide, lincosamides, streptogramin, aminoglycoside, tetracycline.

INTRODUCTION

The resistance to antimicrobial agents is considered one of the major problems worldwide, especially among nosocomial pathogens.

Methicillin resistant S. aureus (MRSA) infections are of special concern due to few therapeutic options, also MRSA increases duration of hospitalization, hospital costs, might lead to significantly increased morbidity and mortality in patients as compared with those associated with methicillin sensitive S. aureus (MSSA) [1]. PCR-based molecular methods are often preferred for determination of antibiotic resistance genes. Therefore, the availability of sensitive and specific methods for the accurate detection of antibiotic resistance in these multi-drug resistant pathogens has become an important tool in clinical diagnosis and permits timely implementation of effective antimicrobial therapy, preventive control strategies, screening of the patient contacts and staff, and appropriate disinfection measures which in turn reduce the costs [2].
Methicillin-resistant *S. aureus* (MRSA) are resistant to all penicillins, including semi synthetic penicillinase-resistant congeners, penems, carbapenems, and cephalosporins. The most important mechanism of resistance to penicillin is caused by the acquisition of an exogenous gene, *mecA*, that encodes an additional β-lactam-resistant penicillin-binding protein (PBP), termed PBP-2’ (or PBP-2a). Another gene involved in penicillin resistance in staphylococci is *blaZ* which encodes β-lactamase [3]. Mechanisms of resistance against aminoglycosides are including reduced uptake, decreased cell permeability, alteration of the ribosomal binding site by mutation expression of rRNA methylases and production of aminoglycoside-modifying enzymes. Enzymatic modification mechanism is the most prevalent in the clinical setting. The *aacA-aphD* gene, which encodes the bi-functional enzyme aminoglycoside-6-N-acetyltransferase/2-O-nucleotidyltransferase, mediates resistance to all clinically used aminoglycosides except streptomycin and neomycin [4]. Macrolides (e.g., erythromycin), lincosamides (e.g., clindamycin), and streptogramin (e.g., quinupristin-dalfopristin) are antimicrobial groups collectively known as MLS agents and used in the treatment of staphylococcal infections. Macrolides, lincosamides and streptogramin B (e.g., Quinupristin) (MLS<sub>B</sub>) have similar inhibitory effects on bacterial protein synthesis, but are chemically different. The *erm*<sup>A</sup> and *erm*<sup>C</sup> genes are frequently responsible for the MLS<sub>B</sub> resistance in staphylococci coding for rRNA methylases, which can be either constitutive or inducible. Clinical *S. aureus* strains carrying *erm*<sup>B</sup> are rather infrequent [5]. Bacteria resist to the macrolide and lincosamide antibiotics through target-site modification (methylation or mutation) that prevents the binding of the antibiotic to its ribosomal target, efflux of the antibiotic, and by drug inactivation [6]. Resistance to streptogramins A (e.g., Dalfopristin) takes place either by active efflux mediated by the *vga* gene or by inactivation via enzymatic acetylation of the antibiotic carried out by streptogramin (virginiamycin) acetyltransferases [7]. Quinupristin-dalfopristin, both components inhibit bacterial protein synthesis by interfering with different targets of 23S RNA in the 50S subunit of the ribosome [8]. For full resistance to the streptogramin combination quinupristin-dalfopristin, acetyltransferase *vat*(A), *vat*(B), and *vat*(C) genes are needed in bacterial strains to carry additional resistance to streptogramin A compounds [5]. Resistance to Quinupristin-dalfopristin is common in animal staphylococci isolates [8]. A large number of tetracycline resistance genes have been identified. The main mechanisms conferring resistance to tetracycline to bacteria are active efflux proteins, ribosomal protection proteins and enzymatic inactivation [9].

Molecular detection techniques continue to increase in utility in clinical routine screening of *S. aureus* isolates to determine antimicrobial susceptibility patterns. Data concerning the prevalence and mechanisms of some antibiotics resistance have not been reported previously in the MRSA isolates from Palestine. The aim of this study was to evaluate the relation between the phenotypic antibiotic susceptibility patterns and the antibiotic resistance genes and to investigate the prevalence of macrolide, lincosamides, streptogramin, aminoglycoside and tetracycline resistance genes among MRSA isolates using multiplex PCR technique. Genes included in this study were *mecA, aacA-aphD, erm*(A), *erm*(C), *tetK, tetM, vat*(A), *vat*(B) and *vat*(C).

**MATERIALS AND METHODS**

Sample Collection and Identification

A total of 55 MRSA isolates were included in this study, collected from different clinical samples, obtained from three different health centers in northern Palestine during 2011-2012. All strains were isolated from clinical samples: 12 pus samples, 16 urine samples, 10 blood samples, 8 wound samples, 5 diabetic foot samples, 4 semen samples. The patients were 31 females and 24 males, their ages ranged between 5 and 70. Replicate isolates from the same patient were excluded. Identification of *S. aureus* was confirmed on the basis of Gram staining, catalase test, culture properties on Mannitol salt agar, detection of hemolysis on blood agar, and coagulase test.

Antimicrobial Susceptibility Testing by Disk Diffusion Method

The *S. aureus* strains were tested for antibiotic resistance using the Kirby-Bauer disk diffusion method. Antimicrobial disks (Oxoid) used were ofloxacin (5 µg), tetracycline (30 µg), clindamycin (2 µg), erythromycin (15 µg), cefotaxime (30 µg), genta-
micin (10 µg), quinupristin-dalfopristin (15 µg), streptomycin (10 µg), amoxicillin-clavulanic acid (20/10 µg), vancomycin (30 µg), ciprofloxacin (5 µg) and ampicillin (10 µg). Zones of inhibition were determined in accordance with procedures of the Clinical and Laboratory Standard Institute standard guidelines [10].

**Oxacillin Disk Diffusion Test**

Oxacillin disk susceptibility testing was performed on all isolates of *S. aureus* using the Kirby-Bauer disk diffusion method. Oxacillin disks (1 µg, Oxoid) were used. Zones of inhibition were determined in accordance with procedures of the CLSI standard guidelines [10]. According to CLSI, *S. aureus* isolates were considered susceptible to oxacillin if inhibition zones were ≥13 mm after incubation on 2% NaCl Mueller Hinton agar at 35ºC for 18-24 h. Oxacillin resistant control strains from our department collection and Oxacillin susceptible reference strain (*S. aureus* ATCC 25923) were used in this study.

**Determination of Minimum Inhibitory Concentrations (MIC)**

All isolates were tested by the broth micro-dilution assay as described in the CLSI standard guidelines [10]. The antibiotic panel included ampicillin, tetracycline, clindamycin, erythromycin, amoxicillin-clavulanic acid, ciprofloxacin, cefotaxime and gentamicin. Two-fold serial dilutions of antibiotics were made in Mueller-Hinton broth to achieve a concentration range from 0.0625 to 128 µg/ml. The MIC was defined as the lowest concentration of antibiotics that produced no growth after incubation at 37ºC for 24 h.

**Erythromycin-Clindamycin Double Disk Diffusion Test**

The double-disk diffusion test was performed as described previously [6]. Briefly, erythromycin (15 µg) and clindamycin (2 µg) disks (Oxoid) were applied 20 mm apart on Mueller-Hinton agar. Plates were incubated overnight at 35 ºC in ambient air. Blunting of the clindamycin zone of inhibition proximal to the erythromycin disk indicated an inducible type (D-shaped zone) of MLSB resistance, while resistance to both erythromycin and clindamycin indicated a constitutive type. Lack of a D-shaped zone in erythromycin resistant and clindamycin-susceptible isolates was interpreted as efflux phenotype or M-type resistance.

**Antibiotic Resistance Clustering Analysis**

The associations of the resistant patterns among all the tested isolates were analyzed and computed using the software SPSS version 20. Meanwhile, Ward’s Method/Squared Euclidean Distance were employed to construct a dendrogram for clustering analysis.

**DNA Extraction**

*S. aureus* genome was prepared for PCR according to the method described previously [11]. Briefly, cells were scraped off an overnight nutrient agar plate with a sterile loop, washed twice in 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 7.5]), then the pellet was resuspended in 0.5 ml of sterile distilled H2O, and boiled for 10-15 min. The cells were then incubated on ice for 10 min. The debris was pelleted by centrifugation at 11,500 X g for 5 min. DNA was extracted from the supernatant using phenol-chloroform method, then DNA was precipitated using 96% cold ethanol. The nucleic acid pellet was washed with 70% cold ethanol, dried and then resuspended in 300 μl of TE (Tris 10 mM, EDTA 1 mM, pH 8), DNA concentration was determined using spectrophotometer and the samples were stored at -20ºC until use for further DNA analysis.

**Multiplex PCR Assay for SCCmec Typing**

The primer sets used for SCCmec typing were described previously [12]. Each PCR reaction mix (50 μL) contained 1X PCR buffer, 1.5 U Taq DNA polymerase, 3 mM MgCl2, 0.2 µM of each primer, 0.2 mM dNTPs and 150-200 ng DNA template. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 5 min at 94ºC followed by 30 cycles of denaturation at 94ºC for 30 s, annealing at 50ºC for 1.5 min and extension at 72ºC for 1.5 min ending with a final extension step at 72ºC for 5 min, and followed by a holding step at 4ºC. The PCR products (10 µL) were analyzed by electrophoresis on 2% agarose gel. The SCCmec type was determined on the basis of the band pattern obtained. Each individual PCR yielded the fragments of expected
size: i.e. 613, 287, 243, 776, 1242, 663 and 325 bp for subtypes I, II, III, IVa, IVb, IVc, IVd, IVh and V, respectively. Control strains for different SCCmec types from our department collection were used in this study.

**Multiplex PCR for the Detection of Selected Antibiotic Resistance Genes**

The PCR primers used to detect 9 antibiotic resistant genes in a multiplex PCR approach are listed in Table 1 [5]. Multiplex PCR amplifications were carried out in a 50 μl volume contained approximately 100 ng of template DNA, 1X PCR buffer, 4 U Taq DNA polymerase, 0.2 μM of each of the 18 primers, 4 mM MgCl2, 0.2 mM dNTPs. The DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf), after initial denaturation step at 94 ºC for 2 min, followed by 30 cycles of denaturation at 94 ºC for 30 s, annealing at 55 ºC for 30 s and extension at 72 ºC for 30 s ending with a final extension step at 72 ºC for 4 min. Amplification products (15 μl) were analyzed on a 2% agarose gel.

**RESULTS**

For 55 MRSA isolates, susceptibility results determined by the disk diffusion method were compared with the results of the multiplex PCR assay for the simultaneous detection of antibiotic resistance genes (Table 2). Our results showed that there was no discordance between conventional susceptibility testing (disk diffusion method) and multiplex PCR assay for the 55 MRSA strains. All strains resistant to erythromycin/clindamycin harbored at least one of the erythromycin resistance genes erm(C) and/or erm(A). The prevalence of erm(A), erm(C) and both erm(A)/erm(C) genes among MRSA isolates was 10.9% (6/55), 54.5% (30/55) and 20.0% (11/55), respectively. Constitutive resistance was predominant among MRSA. It was shown that among the 47 erythromycin/clindamycin resistant MRSA strains, 70.2% (33/47) exhibited the constitutive type, 17.0% (8/47) the inducible type, and 12.8% (6/47) the M-phenotypes. The prevalence of tetK, tetM and both tetK/tetM among MRSA isolates was 63.6% (35/55), 3.6% (2/55) and 12.7% (7/55), respectively. Three isolates 5.4% (3/55) were resistant to quinupristin-dalfopristin, two (3.6%) of them carried the vat(C) gene alone and one (1.8%) isolate carried both vat(A)/vat(C) genes. Data are presented in Table 2 and Fig. 1, 2 and 3.

In MRSA strains using disk diffusion method, antibiotics resistance rates were 100% for ampicillin, amoxicillin-clavulanic acid, cefotaxime. While other antibiotics have resistance ranged from 5.4% to 85.5%. Data about antibiotic resistance by both disk diffusion method and broth microdilution assay are presented in Table 2.

**Table 1. Features of the primers used in this study [5] (Strommenger et al., 2003)**

<table>
<thead>
<tr>
<th>Primer pair</th>
<th>Target gene</th>
<th>Resistance phenotype a</th>
<th>Sequence (5’ → 3’)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA 1 mecA 2</td>
<td>mecA</td>
<td>P, OX</td>
<td>AAA ATC GAT GGT AAA GGT TGG C AGT TCT GCA GTA CCG GAT TTG C</td>
<td>532</td>
</tr>
<tr>
<td>aacA-aphD 1 aacA-aphD 2</td>
<td>aacA-aphD</td>
<td>CN</td>
<td>TAA TCC AAG AGC AAT AAG GGC GCC ACA TCA TAA CCA CTA</td>
<td>227</td>
</tr>
<tr>
<td>ermA 1 ermA 2</td>
<td>erm(A)</td>
<td>E, DA</td>
<td>AAG CGG TAA ACC CCT CTG A TTC GCA AAT CCC TTC TCA AC</td>
<td>190</td>
</tr>
<tr>
<td>ermC 1 ermC 2</td>
<td>erm(C)</td>
<td>E, DA</td>
<td>AAT CGT CAA TTC CTG CAT GT TAA TCG TGG AAT ACG GGT TTG</td>
<td>299</td>
</tr>
<tr>
<td>tetK 1 tetK 2</td>
<td>tetK</td>
<td>TE</td>
<td>GTA GCG ACA ATA GGT AAT AGT GTA GTG ACA ATA AAC CTC CTA</td>
<td>360</td>
</tr>
<tr>
<td>tetM 1 tetM 2</td>
<td>tetM</td>
<td>TE</td>
<td>AGT GGA GGC ATT ACA GAA CAT ATG TCC TGG CTT GTC TA</td>
<td>158</td>
</tr>
<tr>
<td>vatA 1 vatA 2</td>
<td>vat(A)</td>
<td>QD</td>
<td>TGG TCC CCG AAC AAC ATT TAT TCC ACC GAC AAT AGA ATA GGG</td>
<td>268</td>
</tr>
<tr>
<td>vatB 1 vatB 2</td>
<td>vat(B)</td>
<td>QD</td>
<td>GCT GCG AAT TCA GTT GTT ACA CTG ACC AAT CCC ACC ATT TTA</td>
<td>136</td>
</tr>
<tr>
<td>vatC 1 vatC 2</td>
<td>vat(C)</td>
<td>QD</td>
<td>AAG GCC CCA ATC CAG AAG AA TCA ACG TCC TTT GTC ACA ACC</td>
<td>467</td>
</tr>
</tbody>
</table>

a P, penicillin; OX, oxacillin; CN, gentamicin; E, erythromycin; DA, clindamycin; TE, tetracycline; QD, quinupristin-dalfopristin
Table 2: Resistance patterns of *S. aureus* by the disk diffusion method, MIC values for some antibiotics and rates of antibiotic resistance genes detected by multiplex PCR method

<table>
<thead>
<tr>
<th><em>Antibiotic</em></th>
<th><strong>MIC (µg/ml)</strong></th>
<th>Susceptible strains by disk diffusion method Number (%)</th>
<th>Resistant strains by disk diffusion method Number (%)</th>
<th>PCR positive isolates No. (%)</th>
<th>PCR negative isolates No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>≥4</td>
<td>0 (0%)</td>
<td>55 (100%)</td>
<td><em>mecA</em> 55 (100%)</td>
<td><em>mecA</em> 0 (0.0%)</td>
</tr>
<tr>
<td>OX</td>
<td>Not detected</td>
<td>0 (0%)</td>
<td>55 (100%)</td>
<td><em>tetK</em> 35 (63.6%)</td>
<td><em>tetK</em> 13 (23.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>tetM</em> 2 (3.6%)</td>
<td><em>tetM</em> 46 (83.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>tetK/tetM</em> 7 (12.7%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total 44 (80%)</td>
<td>Total 11 (20%)</td>
</tr>
<tr>
<td>TE</td>
<td>0.5-128</td>
<td>11 (20%)</td>
<td>44 (80%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>0.125-64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>erm(A)</em> 6 (10.9%)</td>
<td><em>erm(A)</em> 38 (69.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>erm(C)</em> 30 (54.5%)</td>
<td><em>erm(C)</em> 14 (25.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>erm(A)/erm(C)</em> 11 (20.0%)</td>
<td>Total 8 (14.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total 47 (85.5%)</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.125-64</td>
<td>DA 14 (25.5%)</td>
<td>E 47 (85.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E 8 (14.5%)</td>
<td>Inducible type 8 (14.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E/DA 8 (14.5%)</td>
<td>M-type 6 (10.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E/DA 47 (85.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>0.5-64</td>
<td>14 (25.5%)</td>
<td>41 (74.5%)</td>
<td><em>aacA-aphD</em> 41 (74.5%)</td>
<td><em>aacA-aphD</em> 14 (25.5%)</td>
</tr>
<tr>
<td>QD</td>
<td>Not detected</td>
<td>52 (94.5%)</td>
<td>3 (5.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>Not detected</td>
<td>20 (36.4%)</td>
<td>35 (63.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OFX</td>
<td>Not detected</td>
<td>39 (70.9%)</td>
<td>16 (29.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX</td>
<td>≥64</td>
<td>0 (0%)</td>
<td>55 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIP</td>
<td>0.5-32</td>
<td>30 (54.5%)</td>
<td>25 (45.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMC</td>
<td>≥16/8</td>
<td>0 (0%)</td>
<td>55 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VA</td>
<td>Not detected</td>
<td>55 (100%)</td>
<td>0 (0.0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*AML: Ampicillin; OX: Oxacillin; TE: Tetracycline; DA: Clindamycin; E: Erythromycin; CN: Gentamicin; AMC: Amoxicillin-clavulanic acid; QD: Quinupristin-Dalfopristin; S: Streptomycin; OFX: Ofloxacin; CTX: Cefotaxime; CIP: Ciprofloxacin; VA: Vancomycin.
Fig. 1. Multiplex PCR amplifications on template DNA of 7 different *S. aureus* isolates

5. Strain no. 44 (*mecA*+, *erm*(C)+, *aacA*-aph*D*+)
L. DNA Ladder 100 bp

Fig. 2. Multiplex PCR amplifications on template DNA of 4 different *S. aureus* isolates

1. Strain no. 1 (*mecA*+, vat(C)+, *tetK*+).
L. DNA Ladder 50 bp.
These MRSA strains belonged to SCCmec types II, III, IVa and V. A dendrogram obtained from the antibiotic resistance clustering analysis is used to compare the resistant patterns among MRSA isolated from different clinical samples. All MRSA isolates which were from various clinical samples differentiated into four discriminatory clusters at a 50% similarity level, namely, C1 to C4 (Fig. 4).

**DISCUSSION**

Results of antibiotic susceptibility by disk diffusion method were compared with gene analysis results in *S. aureus* isolates. The phenotypic expression of antibiotic resistant genes has been reported to be affected by different conditions such as the incubation temperature and time, test agent, inoculum size, pH, medium inoculated and ionic strength of NaCl [13]. The most commonly conventional methods used to detect antibiotic resistant *S. aureus* in the laboratory are disk diffusion and broth dilution method. The mecA gene may be heterogeneously expressed and, therefore, all methicillin-resistant staphylococcal strains may not be detectable with phenotypical methods and require at least 24 h for evaluation of the results. However, the detection of antibiotic resistance genes such as mecA gene by PCR techniques is considered the gold standard method [14]. The present results showed perfect correlation and no discordance between genotypic and phenotypic analysis, these results were consistent with previous studies which showed a perfect correlation between the results obtained by the multiplex PCR-based assays and the phenotypic antibiotic resistance determination [5,15,16]. A good correlation between phenotypic antibiotic susceptibility patterns and genotypic analysis by PCR was also reported [15,17,18]. Our results were in contrast to other studies which showed that the phenotypic antibiotic susceptibility patterns were not similar or no correlation to those obtained by genotyping done by multiplex PCR [19-21]. In other studies, the correlation between multiplex PCR-based assays and conventional susceptibility test showed a variation between antibiotics [22]. The critical parameters for success of a multiplex PCR-based assays for the detection of multidrug resistant bacteria as MRSA are cost, reliability and practical, fast, accuracy and sensitivity and results were obtained within 4 hours [14].

In this study, the majority of the MRSA isolates carried *erm*(C) gene. These results were in contrast to a previous report which showed that *erm*(A) gene is mostly spread in methicillin-resistant strains [5, 6, 23-25]. However, our results were consistent with previous reports which showed that *erm*(C) gene was the most spread in MRSA isolates [26, 27]. A high incidence of *S. aureus* carrying *erm*(C) has also been reported in Denmark [28]. It is also found in previous reports that, the prevalence of *erm*(A) gene is higher than *erm*(C) gene among clinical *S. aureus* isolates [15, 17, 21, 22]. Clinical isolates that are constitutively resistant to MLSB antibiotics are widespread, particularly among the MRSA strains [6]. Inducible resistance is common in our clinical isolates and D-test should be performed on all *S. aureus* isolates showing clindamycin-erythromycin discordance on disk diffusion test. In present study, the majority of the MRSA isolates carried tetK gene. These results were in contrast to previous reports which showed that tetM gene is mostly spread in clinical *S. aureus* or MRSA strains [5, 21, 25]. Our results were consistent with a recent study which reported the prevalence of tetK gene higher than tetM gene among clinical *S. aureus* isolates as well as among strains isolated from bovine mastitis [29]. Resistance to quinupristin-dalfopristin is rare in
human staphylococci isolates. Our results showed higher prevalence of resistance to quinupristin-dalfopristin than previous report [30]. A total of 35/3052 (1.1%) isolates of *S. aureus* exhibiting insensitivity to quinupristin-dalfopristin originated from the strain culture collection of the European SENTRY study. Quinupristin-dalfopristin-insensitive isolates are usually MRSA strains, and most of them are already resistant to other classes of antibiotics [30]. In general, variation in the prevalence of genes between these studies might be attributable to different factors such as the geographical difference, type of clinical sample, number of samples, specificity and sensitivity of methodology and types of strains (MRSA or MSSA).

Our study confirms the usefulness of multiplex PCR assay for the detection of antibiotic resistance genes associated with *S. aureus* infections. The multiplex PCR assay offers a rapid, simple, and accurate identification of antibiotic resistance profiles and could be used in clinical diagnosis as well as for the surveillance of the spread of antibiotic
resistance determinants in epidemiological studies. Classical methods and molecular approaches especially PCR based techniques were more effective when used together and could provide more accurate and reliable information. Laboratory methods used to detect multidrug resistant bacteria such as MRSA should have high sensitivity and specificity.

REFERENCES

22. Gomaar NIM, Younes S. Multiplex PCR assay for the simultaneous species identification and detection of


