

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 sen-

REZUMAT

Studiul are drept scop evaluarea relației dintre pattern-urile fenotipice de sensibilitate la antibiotice și genele de rezistență și investigarea prevalenței genelor codante pentru rezistența la macrolide, lincosamide, streptogramină, aminoglicozide și tetraciline la izolatele de stafilococ auriu meticilino-rezistent (MRSA). Studiul a inclus un număr de 55 de izolate clinice de MRSA, rezistența lor la antibiotice fiind testată prin metoda difuzimetrică Kirby Bauer, testul microdilutiei în mediu lichid și tehnica PCR multiplex. Rezultatele noastre au arătat că nu există discordanță între testele convenționale de sensibilitate la antibiotice și detectarea genelor prin PCR multiplex.

Prevalența genelor *erm(A)*, *erm(C)*, *tetK*, *tetM*, *aacA-aphD*, *vat(A)*, *vat(B)* și *vat(C)* în izolatele de MRSA a fost de 30,9%, 74,5%, 76,4%, 16,4%, 74,5%, 1,8%, 0% și 5,5%. Tulpinile MRSA au aparținut tipurilor SCCmec II, III, IVa și V. Testarea sensibilității la antibiotice printr-o metodă rapidă și sigură este importantă pentru a stabili tratamentul adecvat. Tehnica PCR multiplex poate să fie folosită în confirmarea rezultatelor obținute prin metoda difuzimetrică sau ca metodă alternativă de diagnostic curent pentru a detecta rapid, sensibil și specific genele de rezistență la antibiotice, purtate de MRSA.

sitive *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].

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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_B) have similar inhibitory effects on bacterial protein synthesis, but are chemically different. The *erm(A)* and *erm(C)* genes are frequently responsible for the MLS_B resistance in staphylococci coding for rRNA methylases, which can be either constitutive or inducible. Clinical *S. aureus* strains carrying *erm(B)* 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. Antibiotic disks (Oxoid) used were ofloxacin (5 μ g), tetracycline (30 μ g), clindamycin (2 μ g), erythromycin (15 μ g), cefotaxime (30 μ g), 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 35°C 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 37°C 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 °C in ambient air. Blunting of the clindamycin zone of inhibition proximal to the erythromycin disk indicated an inducible type (D-shaped zone) of MLS_B 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 TE (Tris 10 mM, EDTA 1 mM, pH 8), DNA concentration was determined using spectrophotometer and the samples were stored at -20°C until use for further DNA analysis.

Multiplex PCR Assay for SCC_{mec} Typing

The primer sets used for SCC_{mec} 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 94°C followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 1.5 min and extension at 72°C for 1.5 min ending with a final extension step at 72°C for 5 min, and followed by a holding step at 4°C. The PCR products (10 µL) were analyzed by electrophoresis on 2% agarose gel. The SCC_{mec} 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, 1000, 677, 1242, 663 and 325 bp for subtypes I, II, III, IVa, IVb, IVc, IVd, IVh and V, respectively. Control strains for different SCC*mec* 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°C for 2 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55 °C for 30 s and extension at 72°C for 30 s ending with a final extension step at 72 °C 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)

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

^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

*Antibiotic	Methicillin-resistant <i>S. aureus</i> isolates (n = 55)					PCR positive isolates No. (%)	PCR negative isolates No. (%)
	MIC (µg/ml)	Susceptible strains by disk diffusion method Number (%)	Resistant strains by disk diffusion method Number (%)				
AML	≥4	0 (0%)	55 (100%)				
OX	Not detected	0 (0%)	55 (100%)			<i>mecA</i> 55 (100%)	<i>mecA</i> 0 (0.0%)
TE	0.5-128	11 (20%)	44 (80%)			<i>tetK</i> 35 (63.6%) <i>tetM</i> 2 (3.6%) <i>tetK/tetM</i> 7 (12.7%) Total 44 (80%)	<i>tetK</i> 13 (23.6%) <i>tetM</i> 46 (83.6%) Total 11 (20%)
DA	0.125-64			DA 33 (60%) E 47 (85.5%)		<i>erm(A)</i> 6 (10.9%) <i>erm(C)</i> 30 (54.5%) <i>erm(A)/erm(C)</i> 11 (20.0%) Total 47 (85.5%)	<i>erm(A)</i> 38 (69.1%) <i>erm(C)</i> 14 (25.5%) Total 8 (14.5%)
E	0.125-64	DA 14 (25.5%) E 8 (14.5%) E/DA 8 (14.5%)	Inducible type 8 (14.5%) M-type 6 (10.9%) E/DA 47 (85.5%)				
CN	0.5-64	14 (25.5%)	41 (74.5%)			<i>aacA-aphD</i> 41 (74.5%)	<i>aacA-aphD</i> 14 (25.5%)
QD	Not detected	52 (94.5%)	3 (5.5%)			<i>vat(A)</i> 0 (0.0%) <i>vat(B)</i> 0 (0.0%) <i>vat(C)</i> 2 (3.6%) <i>vat(C) + vat(A)</i> 1 (1.8%) Total 3 (5.5%)	<i>vat(A)</i> 54 (98.2%) <i>vat(B)</i> 55 (100%) <i>vat(C)</i> 52 (94.5%) Total 52 (94.5%)
S	Not detected	20 (36.4%)	35 (63.6%)				
OFX	Not detected	39 (70.9%)	16 (29.2%)				
CTX	≥64	0 (0%)	55 (100%)				
CIP	0.5-32	30 (54.5%)	25 (45.5%)				
AMC	≥16/8	0 (0%)	55 (100%)				
VA	Not detected	55 (100%)	0 (0.0%)				

*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.

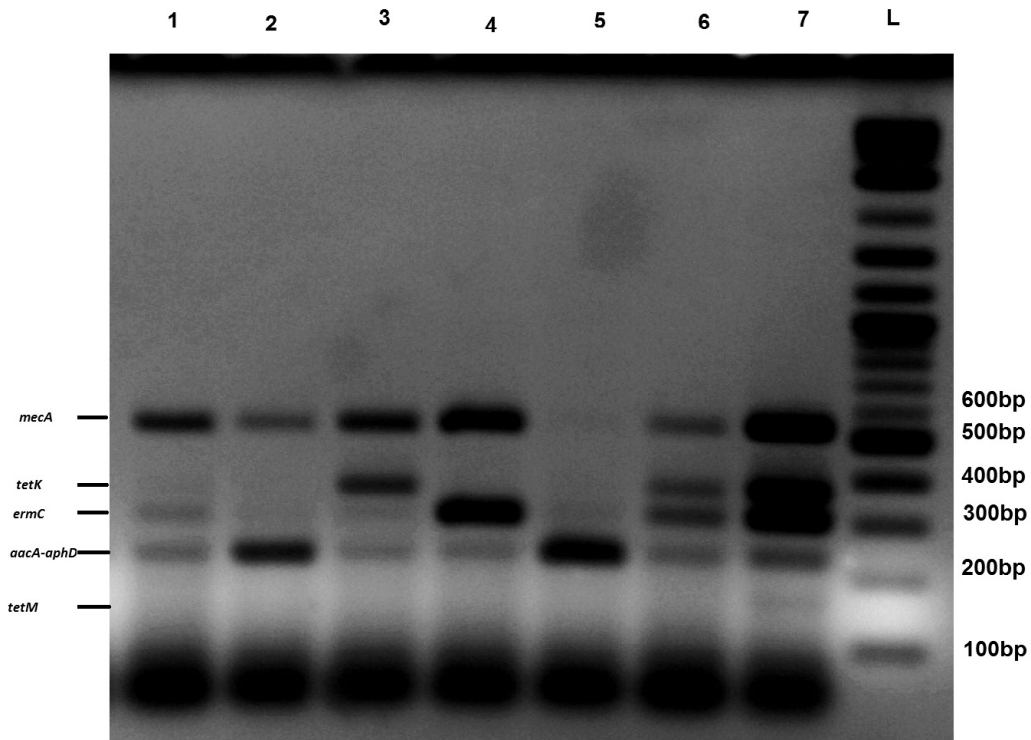


Fig. 1. Multiplex PCR amplifications on template DNA of 7 different *S. aureus* isolates

- | | |
|---|--|
| 1. Strain no. 8 (<i>mecA</i> ⁺ , <i>tetK</i> ⁺ , <i>erm(C)</i> ⁺ , <i>aacA-aphD</i> ⁺) | 5. Strain no. 44 (<i>mecA</i> ⁺ , <i>erm(C)</i> ⁺ , <i>aacA-aphD</i> ⁺) |
| 2. Strain no. 15 (<i>mecA</i> ⁺ , <i>erm(C)</i> ⁺ , <i>aacA-aphD</i> ⁺) | 6. Strain no. 52 (<i>mecA</i> ⁺ , <i>tetK</i> ⁺ , <i>erm(C)</i> ⁺ , <i>aacA-aphD</i> ⁺) |
| 3. Strain no. 16 (<i>mecA</i> ⁺ , <i>tetK</i> ⁺ , <i>erm(C)</i> ⁺ , <i>aacA-aphD</i> ⁺) | 7. Strain no. 55 (<i>mecA</i> ⁺ , <i>tetK</i> ⁺ , <i>erm(C)</i> ⁺ , <i>aacA-aphD</i> ⁺ , <i>tetM</i> ⁺) |
| 4. Strain no. 22 (<i>mecA</i> ⁺ , <i>erm(C)</i> ⁺ , <i>aacA-aphD</i> ⁺) | L. DNA Ladder 100 bp |

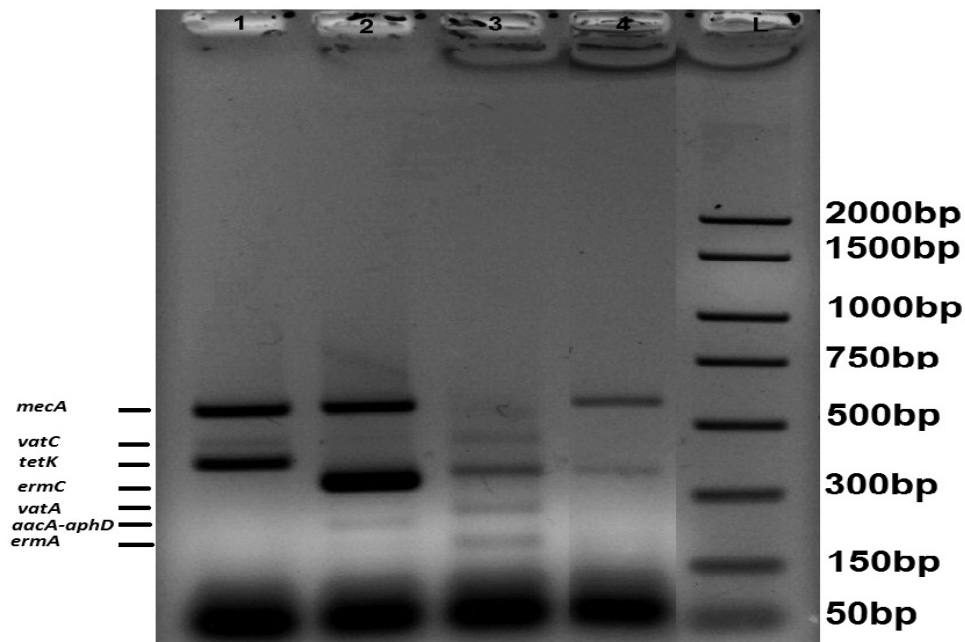


Fig. 2. Multiplex PCR amplifications on template DNA of 4 different *S. aureus* isolates

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|---|
| 1. Strain no. 1 (<i>mecA</i> ⁺ , <i>vat(C)</i> ⁺ , <i>tetK</i> ⁺). |
| 2. Strain no. 46 (<i>mecA</i> ⁺ , <i>erm(C)</i> ⁺ , <i>aacA-aphD</i> ⁺). |
| 3. Strain no. 2 (<i>mecA</i> ⁺ , <i>vat(C)</i> ⁺ , <i>erm(C)</i> ⁺ , <i>vat(A)</i> ⁺ , <i>erm(A)</i> ⁺). |
| 4. Strain no. 23 (<i>mecA</i> ⁺ , <i>erm(C)</i> ⁺). |
| L. DNA Ladder 50 bp. |

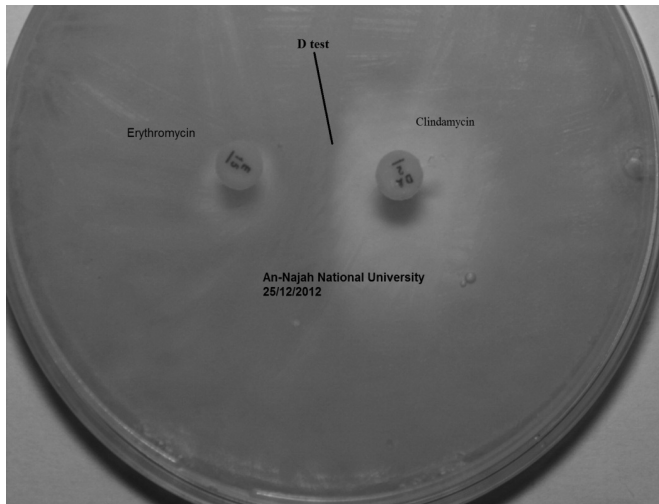


Fig. 3. Erythromycin and clindamycin double-disk diffusion assay (D test) of *S. aureus* isolates from Palestine. D test demonstrating erythromycin disk induction of clindamycin resistance; a blunting of the zone of inhibition around the clindamycin disk is produced that forms a D shape

These MRSA strains belonged to SCC*mec* 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 MLS_B 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

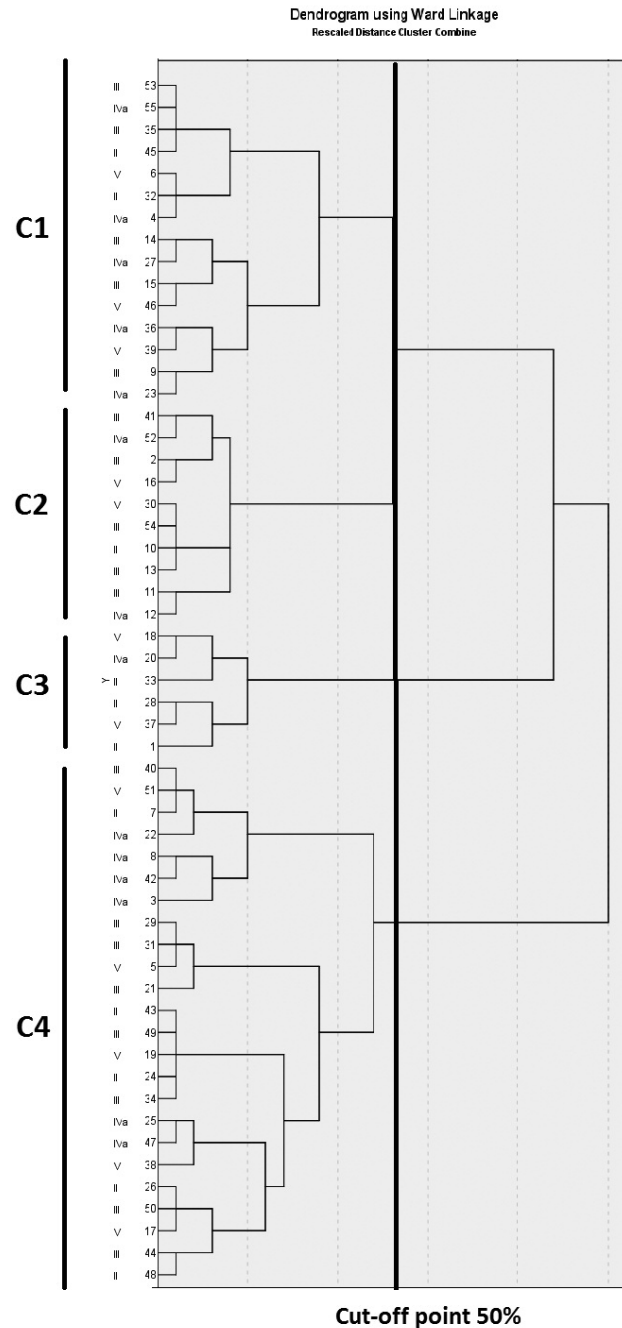


Fig. 4. Dendrogram showing antibiotic resistance profile using Ward's Method/ Squared Euclidean Distance by SPSS software version 20

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

1. **Sydnor ER, Perl TM.** Hospital epidemiology and infection control in acute-care settings. *Clin Microbiol Rev* 2011. **24**: 141-173.
2. **Pillai MM, Latha R, Sarkar G.** Detection of Methicillin resistance in *Staphylococcus aureus* by polymerase chain reaction and conventional methods: A Comparative Study. *J Lab Physicians* 2012. **4**: 83-88.
3. **Malachowa N, DeLeo FR.** Mobile genetic elements of *Staphylococcus aureus*. *Cell Mol Life Sci* 2010. **67**: 3057-3071.
4. **van Den Braak N, van Belkum A, Kreft D, te Witt R, Verbrugh HA, Endtz HP.** The prevalence and clonal expansion of high-level gentamicin-resistant enterococci isolated from blood cultures in a Dutch university hospital. *J Antimicrob Chemother.* 1999. **44**: 795-798.
5. **Strommenger B, Kettlitz C, Werner G, Witte W.** Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. *J Clin Microbiol* 2003. **41**: 4089-4094.
6. **Leclercq R.** Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin Infect Dis* 2002. **34**: 482-492.
7. **Seoane A, García Lobo JM.** Identification of a streptogramin A acetyltransferase gene in the chromosome of *Yersinia enterocolitica*. *Antimicrob Agents Chemother* 2000. **44**: 905-909.
8. **Hershberger E, Donabedian S, Konstantinou K, Zervos MJ.** Quinupristin-dalfopristin resistance in gram-positive bacteria: mechanism of resistance and epidemiology. *Clin Infect Dis* 2004. **38**: 92-98.
9. **Michalova E, Novotna P, Schlegelova J.** Tetracyclines in veterinary medicine and bacterial resistance to them. *Vet Med Czech* 2004. **49**: 79-100.
10. **Clinical and Laboratory Standards Institute (CLSI).** Performance standards for antimicrobial susceptibility testing; informational supplement, 21st ed. CLSI document 2011. M100-S21. Wayne, PA: CLSI, USA;
11. **Adwan G, Adwan K, Jarrar N, Salama Y, Barakat A.** Prevalence of *seg*, *seh* and *sei* genes among clinical and nasal *Staphylococcus aureus* isolates. *Br Microbiol Res J* 2013. **3**: 139-149.
12. **Ghaznavi-Rad E, Nor Shamsudin M, Sekawi Z, van Belkum A, Neela V.** A simplified multiplex PCR assay for fast and easy discrimination of globally distributed staphylococcal cassette chromosome *mec* types in methicillin-resistant *Staphylococcus aureus*. *J Med Microbiol* 2010. **59**: 1135-1139.
13. **Adaleti R, Nakipoglu Y, Karahan ZC, Tasdemir C, Kaya F.** Comparison of polymerase chain reaction and conventional methods in detecting methicillin-resistant *Staphylococcus aureus*. *J Infect Dev Ctries* 2008. **2**: 46-50.
14. **Mohanasoundaram KM, Lalitha MK.** Comparison of phenotypic versus genotypic methods in the detection of methicillin resistance in *Staphylococcus aureus*. *Indian J Med Res* 2008. **127**: 78-84
15. **Martineau F, Picard FJ, Grenier L, Roy PH, Ouellette M, Bergeron MG.** Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in staphylococci isolated from patients infected after cardiac surgery. *J Antimicrob Chemother* 2000. **46**: 527-534.
16. **Sabet NS, Subramaniam G, Navaratnam P, Sekaran SD.** Detection of *mecA* and *ermA* genes and simultaneous identification of *Staphylococcus aureus* using triplex real-time PCR from Malaysian *S. aureus* strain collections. *Int J Antimicrob Agents* 2007. **29**: 582-585.
17. **Martineau F, Picard FJ, Lansac N, Ménard C, Roy PH, Ouellette M, Bergeron MG.** Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* 2000. **44**: 231-238.
18. **Gao J, Ferreri M, Liu XQ, Chen LB, Su JL, Han B.** Development of multiplex polymerase chain reaction assay for rapid detection of *Staphylococcus aureus* and selected antibiotic resistance genes in bovine mastitic milk samples. *J Vet Diagn Invest* 2011. **23**: 894-901.
19. **Zmantar T, Chaieb K, Ben Abdallah F, Ben Kahla-Nakbi A, Ben Hassen A, Mahdouani K, Bakhrouf A.** Multiplex PCR detection of the antibiotic resistance genes in *Staphylococcus aureus* strains isolated from auricular infections. *Folia Microbiol (Praha)*. 2008. **53**: 357-362.
20. **Amghalia E, Nagi AA, Mariana NS, Radu S, Rosli R, Neela V, Rahim RA.** Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in *Staphylococcus aureus* isolated from Malaysian Hospitals. *Res J Biol Sci* 2009. **4**: 444-448.
21. **Duran N, Ozer B, Duran GG, Onlen Y, Demir C.** Antibiotic resistance genes & susceptibility patterns in staphylococci. *Indian J Med Res* 2012. **135**: 389-396.
22. **Gomaa NIM, Younes S.** Multiplex PCR assay for the simultaneous species identification and detection of

- antibiotic resistance genes in Staphylococci isolates. *Egypt J Med Microbiol* 2006. **15**: 763-772.
23. **Schmitz, F.J., R. Sadurski, A. Kray, M. Boos, R. Geisel, K. Kohrer, J. Verhoef, Fluit Ad C.** Prevalence of macrolide resistance genes in *Staphylococcus aureus* and *Enterococcus faecium* isolates from 24 European university hospitals. *J Antimicrob Chemother* 2000. **45**: 891-894.
 24. **Lim JA, Kwon AR, Kim SK, Chong Y, Lee K, Choi EC.** Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in a Korean hospital. *J Antimicrob Chemother* 2002. **49**: 489-495.
 25. **Lim K T, Hanifah Y A, Yusof M, Thong K L.** *ermA*, *ermC*, *tetM* and *tetK* are essential for erythromycin and tetracycline resistance among methicillin-resistant *Staphylococcus aureus* strains isolated from a tertiary hospital in Malaysia. *Indian J Med Microbiol* 2012. **30**: 203-207.
 26. **Ozyurt, M., B. Sareyyupoglu, and N. Ardic.** Erythromycin and tetracycline resistance genes were investigated in methicillin resistant staphylococci isolates by multiplex-PCR. TMC Congress Abstr 2004. XXX1: 262.
 27. **Aktas Z, Aridogan A, Kayacan CB, Aydin D.** Resistance to macrolide, lincosamide and streptogramin antibiotics in staphylococci isolated in Istanbul, Turkey. *J Microbiol* 2007. **45**: 286-290.
 28. **Westh H, Hougaard DM, Vuust J, Rosdahl VT.** *erm* genes in erythromycin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci. *APMIS*. 1995. **103**: 225-232.
 29. **Heidari M, Momtaz H, Madani M.** Detection of the antibiotic resistance genes in *Staphylococcus aureus* isolated from human infections and bovine mastitis. *Afr J Microbiol Res* 2011, **5**: 5132-5136.
 30. **Werner G, Cuny C, Schmitz FJ, Witte W.** Methicillin-resistant, quinupristin-dalfopristin-resistant *Staphylococcus aureus* with reduced sensitivity to glycopeptides. *J Clin Microbiol* 2001. **39**: 3586-3590.