African Journal of Microbiology Research Vol. 6(24), pp. 5259-5265, 28 June, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.791
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Heterogeneity of aminoglycoside resistance genes profile in clinical *Staphylococcus aureus* isolates

Salwa Bdour

Department of Biological Sciences, Faculty of Science, University of Jordan, Amman, Jordan.
E-mail: bsalwa@ju.edu.jo. Tel: 962-6-5355000 Ext. 22333. Fax: 962-6-5348939.

Accepted 25 May, 2012

One hundred clinical *Staphylococcus aureus* including 57 methicillin-resistant (MRSA) and 43 methicllin-sensitive (MSSA) isolates were analyzed for susceptibility to three aminoglycosides and for the presence of genes encoding aminoglycoside modifying enzymes (AMEs). 52% of these isolates were resistant to 1-3 aminoglycosides, which included 65% MRSA and 35% MSSA isolates. The aminoglycoside resistance genes were more frequently identified in MRSA than in MSSA isolates. The most frequent gene was *aac(6')/aph(2")* and it was detected in 45% *S. aureus* isolates which included 52.6% MRSA and 34.8% MSSA isolates. The second prevalent gene was *ant(4',4")* and it was detected in 31% *S. aureus* which included 40.3% MRSA and 18.6% MSSA isolates. 21% of *S. aureus* isolates including 29.8% MRSA and 9.3% MSSA isolates, carried the *aph(3')*III gene. The most frequent combination of genes was *aac(6')/aph(2")* with *ant(4',4")* in 22.8% MRSA and in 16.2% MSSA isolates. The second dominant gene combination was *aac(6')/aph(2")* with *aph(3')III* in 17.5% MRSA and in 6.9% MSSA isolates. The *ant(4',4")* and *aph(3')III* combination existed only in 7% MRSA isolates. The 3 genes coexisted in 5.3% MRSA and in 2.3% MSSA isolates. The concordance between the presence of genes and aminoglycoside resistance phenotype was observed in most MRSA and MSSA isolates. Emerging of isolates harboring these genes must not be ignored because it limits the choices in the number of antibiotics available to clinicians to treat staphylococcal infections in risk patients.

Key words: *Staphylococcus aureus*, aminoglycoside resistance genes, methicillin-resistant, methicillin-sensitive, polymerase chain reaction, Jordan.

INTRODUCTION

Methicillin–resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital and community-acquired infections (Konno et al, 1995; Kluytmans-VandenBergh and Kluytmans, 2006). MRSA isolates may also be resistant to a wide range of antibiotics including aminoglycosides which are often used in combination with either a β-lactam or a glycopeptide, for treatment of serious staphylococcal infections such as bacteremia and endocarditis (Baddour et al., 2005; Cosgrove et al., 2009; Kim et al., 2010). The main mechanism of aminoglycoside resistance is drug inactivation by aminoglycoside modifying enzymes (AMEs) (Nakaminami et al., 2008; Fatholahzadeh et al., 2009) which can be plasmid-borne or chromosomally encoded on transposable elements (Byrne et al., 1990; Chambers, 1997; Ito et al., 1999; Vakulenko and Mobashery, 2003). The bifunctional enzyme, aminoglycoside-6'-N-acetyltransferase/2"-O-phosphoryltransferase [AAC(6')/APH(2")], encoded by the *aac(6')/aph(2")* gene, is the most frequently encountered AME in staphylococcal isolates and mediates resistance to gentamicin, tobramycin, kanamycin, dibekacin, netilmicin, amikacin and isepamicin (Byrne et al., 1990; Vakulenko and Mobashery, 2003). Additional enzyme such as aminoglycoside-4'-O–nucleotidyltransferase I [ANT(4')-I] encoded by *ant(4')-Ia* is known to mediate resistance to neomycin, amikacin, kanamycin and tobramycin in staphylococci (Chambers, 1997; Ito et al., 1999; Vakulenko and Mobashery, 2003). Resistance to

Table 1. Primers and the anticipated sizes of the target regions for the tested genes.

Target gene	Primer sequence	Size of the target region (bp)
***rrs* (16S rRNA)**	5'-GGATTAGATACCCTGGTAGTCC-3' 5'-TCG TTGCGGGACTTAACCCAAC -3'	320
aac(6')/aph(2")	5'-CCAAGAGCAATAAGGGCATA-3' 5'-CACTATCATAACCACTACCG -3'	220
aph(3') III	5'-GCCGATGTGGATTGCGAAAA-3' 5'-GCTTGATCCCCAGTAAGTCA -3'	292
ant(4', 4")	5'-GCAAGGACCGACAACATTTC -3' 5'-TGGCACAGATGGTCATAACC -3'	165

neomycin, and kanamycin is also conferred by an aminoglycoside-3'-O-phosphoryltransferase III [APH(3')-III] encoded by *aph(3')-III* (Vakulenko and Mobashery, 2003; Woodford, 2005). The acetylated, phosphorylated or adenylated aminoglycosides do not bind to ribosomes, and thus do not inhibit protein synthesis (Woodford, 2005). AME produced by MRSA isolates can be determined by identifying the corresponding genes (Van De Klundert and Vliegenthart 1993; Schmitz et al., 1999; Hauschild et al., 2008).

Development of multiple aminoglycosides resistant MRSA isolates was reported in different countries around the world (Schmitz et al., 1999; Ida et al., 2001; Choi et al., 2003; Nakaminami et al., 2008; Ardic et al., 2006; Fatholahzadeh et al., 2009; Liakopoulos et al., 2011). Differences in prevalence of these isolates is linked to the antibiotic policies applied in different countries (Schmitz et al., 1999; Mangeney et al., 2002). Infections caused by these isolates are particularly difficult to treat, often associated with high mortality and increased healthcare costs (Klein et al., 2007; Nickerson et al., 2009). There is currently little information on the prevalence and predominant types of AME genes in MRSA isolated in the Middle-East countries where the prevalence of MRSA is high (Ardic et al., 2006; Fatholahzadeh et al., 2009). In Jordan, the prevalence of clinical MRSA is 57% (Al-Zu'bi, et al. 2004) and the aminoglycosides are widely used in the hospitals and community in the absence of national antimicrobial use guidelines (Al-Bakri et al., 2005). Also, reports on the aminoglycoside susceptibility phenotype of MRSA isolates, and the prevalence and distribution of the aminoglycoside resistance genes in these isolates are lacking. Therefore, the aim of the present study was to provide information regarding the prevalence and distribution of *aac(6')/aph(2")*, *ant(4', 4")* and *aph(3')-III* genes encoding the most clinically relevant AMEs in clinical methicillin-resistant and sensitive *S. aureus* isolates. This information is necessary to define a baseline for monitoring possible future increase in the prevalence of resistant strains and for the implementation of an antibiotic use policy in Jordan.

MATERIALS AND METHODS

Bacterial strains

This study included 100 clinical *S. aureus* isolates which were obtained from various clinical specimens submitted to the microbiology laboratory of Jordan University Hospital, Amman, Jordan. These isolates were identified by biochemical tests. Their sensitivity to oxacillin was studied and the *mecA* gene was detected by polymerase chain reaction (PCR) in 57 MRSA isolates (Al-Zu'bi et al., 2004).

Antimicrobial susceptibility test

In vitro susceptibility of 57 MRSA and 43 methicillin-sensitive (MSSA) isolates to the aminoglycosides: gentamicin (Gen), tobramycin (Tob) and kanamycin (K) (Sigma, USA) was tested using the agar dilution method (Woods and Washington., 1995). Isolates with minimum inhibitory concentrations (MICs) of ≤4 µg/ml to gentamicin and tobramycin and MICs of ≤16 µg/ml to kanamycin were considered to be susceptible. Isolates with MICs of ≥16 µg/ml to gentamicin and tobramycin and MICs of ≥64 µg/ml to kanamycin were considered to be resistant.

PCR detection of aminoglycoside resistance genes

The aminoglycoside resistance genes in the cell lysate of the antibiotic resistant *S. aureus* isolates were detected by multiplex PCR (Van De Klundert and Vliegenthart, 1993) using 15 p.mole of each primer shown in Table 1. DNA amplification was carried out in GeneAmp PCR system 9600 (Perkin Elmer, USA) with the following thermal cycling profile: an initial denaturation at 94°C for 3 min followed by 32 cycles of 30 s at 94°C, 45 s at 60°C , 2 min at 72°C and final extension at 72°C for 7 min (Van De Klundert and Vliegenthart, 1993). The PCR products were detected in 3% agarose gel and band size was assessed by direct comparison with 50 bp DNA ladder (Invitrogen life technologies, UK). *S. aureus* CECT 976 [possessing *aaphA3* gene] kindly provided by the Spanish Type Culture Collection] and the *mecA*-positive *S. aureus*

Table 2. Susceptibility of the clinical *S. aureus* isolates to the tested aminoglycosides by the agar dilution method.

*S. aureus	Number	ᵡNo. (%) of isolates resistant to			ǂAminoglycoside susceptibility profile	ᵡNo. (%) aminoglycoside resistant isolates
		Gentamicin	Tobramycin	Kanamycin		
MRSA	57	30 (52.6)	35 (61.4)	31 (54.4)	Gen^R, Tob^R, K^R	26 (45.6)
					Gen^R, Tob^R, K^S	2 (3.5)
					Gen^R, Tob^S, K^R	1 (1.7)
					Gen^R, Tob^S, K^I	1 (1.7)
					Gen^S, Tob^R, K^R	4 (7)
					Gen^S, Tob^R, K^S	3 (5.3)
Total						37(65)
MSSA	43	14 (32.5)	14 (32.5)	13 (30.2)	Gen^R, Tob^R, K^R	13 (30.2)
					Gen^R, Tob^S, K^I	1 (2.3)
					Gen^I, Tob^R, K^I	1 (2.3)
Total						15 (35)
Overall total	100	44 (44)	49 (49)	44 (44)		52 (52)

* MRSA: Methicillin Resistant *S. aureus*; MSSA: Methicillin Sensitive *S. aureus.* ǂ Abbreviations: Gen, gentamicin; Tob, tobramycin; K, kanamycin. R, resistant; I, intermediate; S, sensitive to the antibiotic. ᵡThe percentage is based on the number of MRSA (57) or MSSA (43) isolates.

ATCC 43300 [possessing *aac* (*6'*)/*aph*(*2''*) and *ant*(*4',4''*) genes] strains were used as positive PCR controls throughout this study.

Statistical analysis

The Z-test was used to compare the proportion of aminoglycoside resistance rate in MRSA and MSSA isolates. $P < 0.05$ was considered statistically significant (Johnson and Bhattacharyya, 1996).

RESULTS

Susceptibility to the aminoglycosides

The MICs of 100 *S. aureus* isolates to the tested aminoglycosides ranged from 0.25 to >256 µg/ml. A total of 48 (48%) isolates which included 20/57 (35%) MRSA and 28/43 (65%) MSSA isolates were sensitive to the three aminoglycosides. Fifty two (52%) *S. aureus* isolates were resistant to 1-3 aminoglycosides and included 37/57 (65%) MRSA and 15/43 (35%) MSSA isolates. Of the 100 *S. aureus* isolates, 44 (44%), 49 (49%) and 44 (44%) were resistant to gentamicin, tobramycin and kanamycin, respectively (Table 2). The aminoglycoside resistance rate in MRSA was significantly ($P < 0.05$) higher than that in MSSA isolates (Table 2). One MRSA isolate was intermediate resistant to kanamycin (MIC= 32 µg/ml). Two MSSA isolates were intermediate resistant to kanamycin and one was intermediate resistant to gentamicin (MIC = 8 µg/ml).

Multi-resistance to three aminoglycosides (Gen^R, Tob^R, K^R) was observed in 26/57 (45.6%) MRSA isolates and was significantly ($P <0.05$) higher than that observed in 13/43 (30.2%) MSSA isolates (Table 2). Multi-resistance to two aminoglycosides (Gen^R, Tob^R; Gen^R, K^R; and Tob^R, K^R) was detected in 7/57 (12.3%) MRSA and 0% MSSA isolates. Four (7%) MRSA and two (4.6%) MSSA isolates demonstrated resistance to one of the aminoglycosides tested (Table 2).

PCR detection of aminoglycoside resistance genes

The aminoglycoside resistant *S. aureus* isolates were screened by PCR for the presence of three genes encoding the most clinically relevant AMEs. The PCR positive isolates produced the expected 220, 292 and 165 bp PCR products (Van De Klundert and Vliegenthart, 1993) for *aac(6')/aph(2")*, *aph(3')III* and *ant(4',4")* genes, respectively. The prevalence of the three AME genes is shown in Table 3 and it was significantly ($P < 0.05$) higher in MRSA than in MSSA isolates. The most frequently gene was *aac(6')/aph(2")* and it was detected in 45 (45%) *S. aureus* isolates which included 30/57 (52.6%) MRSA and 15/43 (34.8%) MSSA isolates. The concordance between the presence of *aac(6')/aph(2")* gene and aminoglycoside resistance phenotype was observed in most MRSA and all MSSA isolates (Table 3).

The second prevalent AME gene was *ant(4',4")* and it was detected in 31 (31%) *S. aureus* isolates which included 23/57 (40.3%) MRSA and 8/43 (18.6%) MSSA isolates (Table 3). This gene was detected in 65.7% (23/35) Tob^R-MRSA and in 57% (8/14) Tob^R-MSSA isolates. Also, it was detected in 64.5% (20/31) K^R-MRSA and in 53.8% (7/13) K^R-MSSA isolates. On the other hand, 21(21%) *S. aureus* isolates including 17/57 (29.8%) MRSA and 4/43 (9.3%) MSSA isolates, carried the *aph(3')III* gene. It was detected in 51.6% (16/31)

Table 3. The prevalence of the AME genes in the clinical *S. aureus* isolates.

S. aureus	*No. (%) of isolates harboring the AME genes		
	aac(6')/aph(2'')	***ant(4', 4'')***	***aph(3') III***
MRSA (n = 57)	30 (52.6)	23 (40.3)	17 (29.8)
Gen^R	30	0	0
Tob^R	28	23	0
K^R	27	20	16
MSSA (n = 43)	15 (34.8)	8 (18.6)	4 (9.3)
Gen^R	14	0	0
Tob^R	14	8	0
K^R	13	7	4
ǂ Total No. (%)	45 (45)	31 (31)	21 (21)

AME, aminoglycoside modifying enzyme; R, resistant; Gen, gentamicin; Tob, tobramycin; K, kanamycin.* The percentage is based on the number of MRSA (57) or MSSA (43) isolates; ǂ Total number of MRSA and MSSA isolates harboring each AME gene and percentage per 100 *S. aureus* isolates.

K^R-MRSA and 30.7 % (4/13) K^R-MSSA isolates (Table 3).

AME gene combinations in the multi-resistant *S. aureus* isolates

Table 4 shows the distribution of the AME genes in *S. aureus* isolates with different aminoglycoside susceptibility phenotype. A total of 41% of the *S. aureus* isolates including 30/57 (52.6%) MRSA and 11/43 (25.5%) MSSA isolates harbored the *aac(6')/aph(2'')* gene in combination with either *ant(4',4'')* and/or *aph(3')III* or carried *ant(4',4'')* in combination with *aph(3')III* only. The most frequent combination of AME genes was *aac(6')/aph(2'')* with *ant(4',4'')*. The prevalence of this combination in 13/57 (22.8%) MRSA isolates was significantly higher (P = 0.0088) than that in 7/43 (16.2%) MSSA isolates. The second dominant gene combination was *aac(6')/aph(2'')* with *aph(3')III*. The prevalence of this combination in 10/57 (17.5%) MRSA isolates was not significantly higher (P = 0.1212) than that in 3/43 (6.9%) MSSA isolates. The *ant(4',4'')* and *aph(3')III* combination existed only in 4/57 (7%) MRSA.

Four AME gene profiles (i to iv) were detected in the multi-resistant MRSA and MSSA isolates with Gen^R, Tob^R, K^R_phenotype (Table 4). The *aac(6')/aph(2'')* and *ant(4',4'')* gene combination was predominant in the four profiles and occurred in 13/26 (50%) Gen^R, Tob^R, K^R-MRSA and in 6/13 (46%) Gen^R, Tob^R, K^R-MSSA isolates. The second dominant AME gene profile in this phenotype included the *aac(6')/aph(2'')* and *aph(3')III* combination in 9/26 (34.6%) Gen^R, Tob^R, K^R-MRSA and in 3/13 (23%) MSSA isolates. The 3 AME genes coexisted in 3/26 (11.5%) multi-resistant Gen^R, Tob^R, K^R-MRSA and in 1/13 (7.7%) Gen^R, Tob^R, K^R-MSSA isolates. These isolates were highly resistant to the tested aminoglycosides and most have MIC ≥128 µg/ml.

Two AME gene profiles (i and ii) were detected in two MRSA isolates with Gen^R, Tob^R, K^S_phenotype (Table 4). The *aac(6')/aph(2'')* and *aph(3')III* combination was detected in one isolate only. However, *aph(3')III* and *ant(4',4'')* combination was detected only in 4 multi-resistant (Gen^S, Tob^R, K^R) MRSA isolates. The *aac(6')/aph(2'')* and *ant(4',4'')* combination was detected in one MSSA with Gen^I, Tob^R, K^I phenotype (Table 4). On the other hand, one AME gene was detected in the remaining multi-resistant *S. aureus* isolates (Table 4).

DISCUSSION

Development of aminoglycoside resistance MRSA strains (Schmitz et al., 1999; Ida et al., 2001; Choi et al., 2003; Ardic et al., 2006; Nakaminami et al., 2008; Fatholahzadeh et al., 2009; Liakopoulos et al., 2011) has become a global threat to effective health care delivery (Klein et al., 2007; Nickerson et al., 2009). In the present study, 52% of *S. aureus* isolates were resistant to 1-3 aminoglycosides which is higher than that in some European countries including Poland (38.1%) (Hauschild et al., 2008) and Greece (48.2%) (Liakopoulos et al., 2011). The higher prevalence may be due to the misuse of antibiotics in Jordan (Al-Bakri et al., 2005). A total of 44, 49 and 44% of the Jordanian isolates were resistant to gentamicin, tobramycin and kanamycin, respectively (Table 2), which is within the range reported in Europe and Korea for gentamicin (6.3 to 66%), tobramycin (12.9 to 71%) and kanamycin (48.2 to 97.8%) (Liakopoulos et al., 2011; Schmitz et al., 1999; Choi et al., 2003; Hauschild et al., 2008). The aminoglycoside resistance rate in MRSA was almost double that in MSSA isolates (Table 2) and it was due to the presence of 1-3 AME

Table 4. Aminoglycoside resistance genes in the clinical *S. aureus* isolates with different susceptibility phenotypes.

S. aureus	* Aminoglycoside susceptibility phenotype		‡ Aminoglycoside resistance genes			† Number (%) of isolates
			aac(6')/aph(2'')	*ant(4',4'')*	*aph(3')III*	
	Gen^S, Tob^S, K^S		ND	ND	ND	20 (35)
	Gen^R, Tob^R, K^R					
	Gene profile	(i)	+	+	–	13 (22.8)
		(ii)	+	–	+	9 (15.8)
		(iii)	+	+	+	3 (5.3)
		(iv)	+	–	–	1 (1.7)
MRSA	Gen^R, Tob^R, K^S					
	Gene profile	(i)	+	–	–	1 (1.7)
		(ii)	+	–	+	1 (1.7)
	Gen^R, Tob^S, K^R		+	–	–	1 (1.7)
	Gen^R, Tob^S, K^I		+	–	–	1 (1.7)
	Gen^S, Tob^R, K^R		–	+	+	4 (7)
	Gen^S, Tob^R, K^S		–	+	–	3 (5.3)
	Total		30	23	17	57
	Gen^S, Tob^S, K^S		ND	ND	ND	28 (65)
	Gen^R, Tob^R, K^R					
	Gene profile	(i)	+	+	–	6 (14)
		(ii)	+	–	+	3 (7)
		(iii)	+	+	+	1 (2.3)
MSSA		(iv)	+	–	–	3 (7)
	Gen^R, Tob^S, K^I		+	–	–	1 (2.3)
	Gen^I, Tob^R, K^I		+	+	–	1 (2.3)
	Total		15	8	4	43
Overall total			45 (45)	31 (31)	21 (21)	100 (100)

genes. The *aac(6')/aph(2'')* gene was the most dominant gene as reported in Europe, Korea, Japan and Middle East countries (Schmitz et al., 1999; Hauschild et al., 2008; Choi et al., 2003; Nakaminami et al., 2008; Ardic et al., 2006; Fatholahzadeh et al., 2009) and in MSSA isolates in Korea (Choi et al., 2003). Detection of this gene in both MRSA and MSSA isolates in Jordan (Tables 3 and 4) could be explained by the fact that it exists as a transposable genetic element (Tn4001) which is carried on different types of plasmids (Byrne et al., 1990; Udou, 2004). The presumed horizontal transfer of this gene among MRSA and MSSA isolates in Jordan mediates resistance to gentamicin, tobramycin and kanamycin (Tables 3 and 4).

The second prevalent AME gene was *ant(4',4'')* and it was detected in 31% of *S. aureus* isolates (Tables 3 and 4). Similarly, it was the second dominant gene detected in 26.7 to 48% of *S. aureus* isolates in Europe and Korea (Choi et al., 2003; Schmitz et al., 1999; Hauschild et al., 2008). In contrast, this gene was the least frequent AME gene (26%) among the Iranian MRSA isolates (Fatholahzadeh et al., 2009) and the most prevalent gene (84.5%) in MRSA isolated in Japan (Ida et al., 2001). In the present study, the prevalence of this gene in MRSA (40.3%) was 2 folds that in MSSA (18.6%) isolates (Tables 3) which is presumed to be due to the integration of pUB110 containing the *ant* (*4', 4''*) gene in the *mec* element downstream of *mecA* gene (Ito et al., 1999; Chambers, 1997). However, *mec* elements lacking this plasmid have been described (Oliveira et al., 2000) which could explain the absence of this gene in our remaining tobramycin and kanamycin-resistant MRSA isolates and all MRSA isolates in Turkey (Ardic et al., 2006). In Jordan, the concordance between tobramycin and kanamycin resistance and the presence of this gene in MRSA isolates and in MSSA isolates (Table 3) was higher than that in the Korean isolates (45%) (Choi et al. 2003) and lower than that in the Polish isolates (100%),

(Hauschild et al., 2008).

The third prevalent AME gene was the *aph(3')III* gene. Detection of this gene among MRSA and MSSA isolates could be due to its existence on a transposable genetic element (Tn5405) (Derbise et al., 1996) which can be disseminated among the isolates. This gene was also the third frequently encountered AME gene in MRSA and MSSA isolates of Korea (Choi et al., 2003), Japan (Ida et al., 2001) and European countries (Schmitz et al., 1999; Hauschild et al., 2008). In contrast, this gene was the second prevalent AME gene in Iranian MRSA (71%) isolates (Fatholahzadeh et al., 2009), while all Turkish MRSA isolates were negative for this gene (Ardic et al., 2006). The concordance between kanamycin resistance (K^R) and the presence of this gene was 51.6% in K^R-MRSA and 30.7% in K^R-MSSA isolates (Table 3) compared to 45% in the Korean (Choi et al., 2003) and 100% in the Iranian MRSA (Fatholahzadeh et al., 2009) isolates.

A total of 41% of the *S. aureus* isolates carried the *aac(6')/aph(2")* gene in combination with either *ant(4',4")* and/or *aph(3')III* or carried *ant(4',4")* in combination with *aph(3')III* only (Table 4). In Korea, 50% of the *S. aureus* isolates carried the *aac(6')/aph(2")* gene in combination with either *ant(4',4")* and/or *aph(3')III* (Choi *et al*., 2003). In the present study, the most frequent combination of genes was *aac(6')/aph(2")* with *ant(4', 4"*) (Table 4) which is similar to that detected in Korea (Choi et al., 2003). The coexistence of these genes could be due to the fact that the *ant(4', 4")* gene is also carried next to the *aac(6')/aph(2")* gene on some plasmids (Byrne et al., 1990). The second dominant AME gene combination was *aac(6')/aph(2")* with *aph(3')III* (Table 4) which could be due to harboring the transposons Tn4001 (Byrne et al., 1990) and Tn5405 (Debrise et al., 1996). The *ant(4',4")* and *aph(3')III* combination coexisted only in 4/57 (7%) MRSA which could harbor the integrated copy of pUB110 carrying *ant(4',4")* (Ito et al., 1999; Chambers, 1997) and Tn405 carrying *aph(3')III* (Debrise *et al*., 1996). The 3 genes coexisted only in 3/57 (5.3%) MRSA and in 1/43 (2.3%) MSSA isolates which were highly resistant to the 3 tested aminoglycosides.

There is a concordance between the AME gene combinations and the phenotypic multi-resistance of *S. aureus* isolates to 2 and 3 aminoglycosides (Tables 2 and 4). Multi-resistance to three aminoglycosides (Gen^R, Tob^R, K^R) was observed in 26/57 (45.6%) MRSA and in 13/43 (30.2%) MSSA isolates (Table 2) with four heterogenous AME gene profiles (i to iv) (Table 4). In these profiles, the *aac(6')/aph(2")* and *ant(4',4")* gene combination was predominant followed by *aac(6')/aph(2")* and *aph(3')III* combination. In contrast, the *aac(6')/aph(2")* and *aph(3')III* combination was dominant over the *aac(6')/aph(2")* and *ant(4',4")* gene combination in Iran (Fatholahzadeh et al., 2009). However, multi-resistance to two aminoglycosides was detected only in 7/57 (12.3%) MRSA isolates (Tables 2 and 4) harboring the *aac(6')/aph(2")* gene only, the *aph(3')III* and *ant(4',4")* combination, and the *aac(6")/aph(2")* and *aph(3')III* combination.

Four (7%) MRSA and two (4.6%) MSSA isolates demonstrated resistance to one of the aminoglycosides tested and most harbored the *ant(4', 4"*) gene (Table 4). The finding of at least one AME gene is important in terms of showing the possibility of spreading of other AME genes to the aminoglycoside sensitive MRSA (35%) and MSSA (65%) isolates (Table 4). The speed of resistance development in these isolates could be linked to the absence of national antimicrobial use guidelines and regulations which control the community use of antibacterial drugs in Jordan. These drugs are dispensed with and without a prescription, either via self-medication or pharmacist recommendation (Al-Bakri et al., 2005).

In conclusion, a high prevalence (52%) of aminoglycoside resistance was determined in the clinical *S. aureus* due to the presence of 1 to 3 AME genes. Emerging of MRSA isolates with heterogenous AME gene profiles, especially those which harbor the 3 types of AME genes, must not be ignored because it limits the choices in the number of aminoglycosides available to clinicians to treat staphylococcal infections in risk patients with either a β-lactam or a glycopeptide. Irrational use of antibiotics is likely behind the selection of these isolates in Jordan. There is a need to force regulations to control the use of antibiotics which could lead to parallel changes in resistance patterns and may favor the emergence of aminoglycosides susceptible-MRSA strains as reported in other countries (Mangeney et al., 2002). Therefore, periodic surveillance of aminoglycoside resistance and of the corresponding genes is recommended.

ACKNOWLEDGEMENTS

We gratefully thank Mr Ahmed Abu-Mizer and Mr Ahmed Abu-Jafal for their technical assistance. The research was made possible by a grant from the Deanship of Scientific Research, University of Jordan.

REFERENCES

Al-Bakri AG, Bustanji Y, Yousef M (2005). Community consumption of antibacterial drugs within the Jordanian population: sources, patterns and appropriateness. Int. J. Antimicrob. Agents, 26: 389-395.

Al-Zu'bi E, Bdour S, Shehabi AA (2004). Antibiotic resistance patterns of mecA-positive *Staphylococcus aureus* isolates from clinical specimens and nasal carriage. Microbial Drug Resistance, 10: 321-324.

Ardic N, Sareyyupoglu B, Ozyurt M, Haznedaroglu T, Ilga U (2006). Investigation of aminoglycoside modifying enzyme genes in methicillin-resistant staphylococci. Microbiol. Res., 161: 49-54.

Baddour LM, Wilson WR, Bayer AS, Fowler VG Jr, Bolger AF, Levison ME, Ferrieri P, Gerber MA, Tani LY, Gewitz MH, Tong DC, Steckelberg JM, Baltimore RS, Shulman ST, Burns JC, Falace DA, Newburger JW, Pallasch TJ, Takahashi M, Taubert KA (2005). Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a statement for healthcare professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the

Young, and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association: endorsed by the Infectious Diseases Society of America. Circulation, 11: e394-434.

Byrne ME, Gillespie MT, Skurray RA (1990). Molecular analysis of a gentamicin resistance transposon like element on plasmids isolated from North American *Staphylococcus aureus* strains. Antimicrob. Agents Chemother., 34: 2106-2113.

Chambers HF (1997). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin. Microbiol. Rev., 10: 781-791.

Choi SM, Kim SH, Kim HJ, Lee DG, Choi JH, Yoo JH, Kang JH, Shin WS, Kang MW (2003). Multiplex PCR for the Detection of Genes Encoding Aminoglycoside Modifying Enzymes and Methicillin Resistance among *Staphylococcus* species. J. Korean Med. Sci., 18: 631-636.

Cosgrove SE, Vigliani GA, Campion M, Fowler VG, Corey G, Abrutyn E, Levine DP, Rupp E, Chambers HF, Karchmer AW, Boucher HW (2009). Initial low-dose gentamicin for *S. aureus* bacteremia and endocarditis is nephrotoxic. Clin. Infect. Dis., 48: 713-721.

Derbise A, Dyke KGH, El Solh N (1996). Characterization of a *Staphylococcus aureus* transposon, Tn*5405,* Located within Tn*5404* and carrying the aminoglycoside resistance genes, *aphA-3* and *aadE*. Plasmid, 35: 174-188.

Fatholahzadeh B, Emaneini M, Feizabadi MM, Sedaghat H, Aligholi M, Taherikalani M, Jabalameli F (2009). Characterisation of genes encoding aminoglycoside-modifying enzymes among meticillin-resistant *Staphylococcus aureus* isolated from two hospitals in Tehran, Iran. Int. J. Antimicrob. Agents, 33: 264-265.

Hauschild T, Sacha P, Wieczorek P, Zalewska M, Kaczyñska K, Tryniszewska E (2008). Aminoglycosides resistance in clinical isolates of *Staphylococcus aureus* from a University Hospital in Bialystok, Poland. Folia Histochem. Cytobiol., 46: 225-228.

Ida T, Okamoto R, Shimauchi C, Okubo T, Kuga A, Inoue M (2001). Identification of aminoglycoside-modifying enzymes by susceptibility testing: Epidemiology of methicillin-resistant *Staphylococcus aureus* in Japan. J. Clin. Microbiol., 39: 3115-3121.

Ito T, Katayama Y, Hiramatsu K (1999). Cloning and nucleotide sequence determination of the entire *mec* DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. Antimicrob. Agents Chemother., 43: 1449-1458.

Johnson RA, Bhattacharyya GK (1996). Statistics Principles and Methods. 3rd Edition. John Wiley and Sons, Inc. New York.

Kim SW, Lee DG, Choi SM, Park C, Kwon JC, Kim SH, Park SH, Choi JH, Yoo JH, Shin WS (2010). Once-Daily Gentamicin Administration for Community-Associated Methicillin Resistant *Staphylococcus aureus* in an in vitro Pharmacodynamic Model: Preliminary Reports for the Advantages for Optimizing Pharmacodynamic Index. Yonsei Med. J., 51: 722-727.

Klein E, Smith DL, Laxminarayan R (2007). Hospitalizations and Deaths Caused by Methicillin-Resistant *Staphylococcus aureus*, United States, 1999-2005. Emerging Infect. Dis., 13: 1840-1846.

Kluytmans-Vanden Bergh MFQ, Kluytmans JAJW (2006). Community-acquired methicillin-resistant *Staphylococcus aureus*: current perspectives. Clin. Microbiol. Infect., 12: 9-15.

Konno M (1995). Nosocomial infections caused by methicillin–resistant *Staphylococcus aureus* in Japan. J. Infect. Chemother., 1: 30-39.

Liakopoulos A, Foka A, Vourli S, Zerva L, Tsiapara F, Protonotariou E, Dailiana Z, Economou M, Papoutsidou E, Koutsia-Carouzou C, Anastassiou ED, Diza E, Zintzaras E, Spiliopoulou I, Petinaki E (2011). Aminoglycoside-resistant staphylococci in Greece: prevalence and resistance mechanisms. Eur. J. Clin. Microbiol. Infect. Dis., 30: 701-705.

Mangeney N, Drollee K, Cloitre V, Bordes M, Faubert E (2002). Comparative pulsed-field gel electrophoresis typing of gentamicin-resistant and -susceptible methicillin-resistant *Staphylococcus aureus* strains isolated in France between 1991 and 1998. Changes in antibiotic susceptibility. J. Hosp. Infect., 51: 262-268.

Nakaminami H, Noguchi N, Ikeda M, Hasui M, Sato M, Yamamoto S, Yoshida T, Asano T, Senoue M, Sasatsu M (2008). Molecular epidemiology and antimicrobial susceptibilities of 273 exfoliative toxin-encoding gene-positive *Staphylococcus aureus* isolates from patients with impetigo in Japan. J. Med. Microbiol., 57: 1251-1258.

Nickerson EK, Hongsuwan M, Limmathurotsakul D, Wuthiekanun V, Shah KR, Srisomang P, Mahavanakul W, Wacharaprechasgul T, Fowler VG, West TE, Teerawatanasuk N, Becher H, White NJ, Chierakul W, Day NP, Peacock SJ (2009). *Staphylococcus aureus* bacteraemia in a tropical setting: patient outcome and impact of antibiotic resistance. PLoS ONE, 4: e4308.

Oliveira DC, Wu SW, de Lencastre H (2000). Genetic organization of the downstream region of the *mecA* element in methicillin-resistant *Staphylococcus aureus* carrying different polymorphisms of this region. Antimicrob. Agents Chemother., 44: 1906-1910.

Schmitz F, Fluit AC, Gondolf M, Beyrau R, Lindenlauf E, Verhoef J, Heinz HP, Jones ME (1999). The prevalence of aminoglycoside resistance and corresponding genes in clinical isolates of staphylococci from 19 European hospitals. J. Antimicrob. Chemother., 43: 253-259.

Vakulenko SB, Mobashery S (2003). Versatility of Aminoglycosides and Prospects for Their Future. Clin. Microbiol. Rev., 16: 430-450.

Van De Klundert J, Vliegenthart J (1993). PCR detection of genes coding for aminoglycoside-modifying enzymes. In: Persing et al (editors) Diagnostic Molecular Microbiology: Principles and Applications. AMS press, Washington, D.C., pp. 547-552.

Udou T (2004). Dissemination of nosocomial multiple-aminoglycoside-resistant *Staphylococcus aureus* caused by horizontal transfer of the resistance determinant (*aacA/aphD*) and clonal spread of resistant strains. Am. J. Infect. Control, 32: 215-219.

Woods LG, Washington JA (1995). Antimicrobial susceptibility tests: Dilution and disk diffusion methods. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (editors), Manual of Clinical Microbiology, 6th ed. AMS press, Washington, D.C., pp. 1327-1341.

Woodford N (2005). Biological counterstrike: antibiotic resistance mechanisms of Gram-positive cocci. Clin. Microbiol. Infect., 11: 2-21.