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A preliminary study of Aminoglycoside Modifying Enzymes (AMEs) of Multiple Antibiotic Resistance of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from clinical specimens in Al-Diwaniya/Iraq

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Abstract

Staphylococcus aureus (SA) plays a significant role in numerous serious life-threatening infections that present a major challenge to public health in controlling it, especially those resistant to methicillin (MR) known as (MRSA). These pathogens have resistance to other classes of antimicrobial agents including aminoglycoside molecules which mostly resist it through three types of medically significant enzymes: APH (3')-III, ANT (4')-I and (6')/APH(2"). In this paper, seventy-two MRAS were isolated from different lesions from Al-Diwaniya teaching hospital, and Maternity and Children teaching hospital, during the period from January to July 2018. Disc diffusion method, minimum inhibitory concentration(MIC), and bactericidal (MBC) were carried out to MRAS strains subjected to phenotype and genotype identification as well as to detect AMEs genes. Susceptibility of 29 drugs of MRAS strains was: 100% susceptible to vancomycin and chloramphenicol, but 100% resistant to penicillin, cefoxitin, ceftriaxone, aztreonam, and nitrofurantoin. Thus, a (72.2%) of MRAS were found to be either MDR or XDR including 20 aminoglycoside resistant (AR) strains. Multiple antibiotic resistance (MAR) index of a total of 20(100%)AR strains were recorded high values>0.2 ranged(0.48-0.83) from the maximum MAR index:1. Moreover, MIC and MBC values of vancomycin, for *S. aureus*, ranged from 2 µg/mL to 8 µg/mL. Phenotype resistance of MRSA strains to aminoglycoside molecules was: kanamycin 20(27.8%)tobramycin 18(25%); gentamicin 16(22.2%);amikacin 14(19.4%); and netilmicin 8(11.1%). PCR analysis led to all 100% MRSA caring for the *mecA* gene. Frequency of genes encoding aminoglycosides resistance *aac(6')/aph (2")*;80%, *aph (3')-IIIa*;45%, and *ant (4')-Ia*; 35%. The *aac(6')/aph (2")* and *ant (4')-Ia* genes was the only determinant of resistance in 5 and 1 strains respectively. Correlation between MRSA-AR strains and AMEs genes was 90%. In conclusion, MRSA strains harbouring the *mecA* gene are currently widely distributed in the Al-Diwaniya governorate. Co-production with AMEs may increase the risk of the spreading of multiple drug resistance clinical strains in communities and hospitals.

Keywords: MRSA, MDR, XDR, MAR, *mecA* gene, AME genes.

1. Introduction

Opportunistic *Staphylococcus aureus* (SA) infections are among the significant bacterial infections in the inpatients and outpatients (Goudarzi *et al*., 2019c; Baines *et al*., 2019; Xu *et al*.,2019; Kavusi *et al*., 2019; Elshabrawy *et al*., 2020) and the most serious worldwide especially which show resistance to methicillin (MR) drug abbreviated called MRSA (Peacock *et al*., 2015; Gajdács, 2019; Goudarzi *et al*.,2019e; Hadyeh *et al*.,2019 Navidinia *et al*., 2019). MRSA has been classified within the high resistance priority tiers (WHO, 2017). Gene is responsible for MRSA named *mecA*(Cikman *et al*., 2019). This gene is encoded to important protein in the synthesis of MRSA cell wall it is termed an acronym PBP2′, while abbreviated SCC*mec* refers to the chromosomal elements transfer of this protein (Gajdács, 2019). Therefore, MRSA infections cure is considered a major public health concern (Goudarzi *et al*., 2019d). It causes much mortality of patients because of multiple drug resistance to antimicrobial categories (Watkins *et al*., 2019). This will reduce therapeutic options for infections caused by MRAS strains (ElFeky *et al*., 2019). Aminoglycosides are mostly used in the treatment of infection that caused by staphylococcal bacteria when it combination with glycopeptide and β-lactam drugs (Kavusi *et al*., 2019), while lincosamide, streptogramin B, and macrolide antibiotics are used as alternatives in treating such infections. (Razeghi *et al*., 2019). Thus, currently, MRSA strains possess multiple drug resistance (MDR) including the previously mentioned(Khosravi *et al*., 2017). Development of this resistance is strongly associated with the production of aminoglycoside modifying enzymes (AMEs) which is the majority mechanism to inactivate aminoglycoside molecules(Garneau-Tsodikova and Labby, 2016; Seyedi-Marghaki *et al*., 2019). *Aph (3')-IIIa, ant (4')-Ia* and *aac(6')/aph (2")* genes are encoded to the most prevalent types of AMEs which are aminoglycoside-3'-O-phosphoryltransferase III, aminoglycoside-4'-O-

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nucleotidyltransferase I and aminoglycoside-6'-N-acetyltransferase/2"-phosphoryltransferaserespectively(Ramirez and Tolmasky,2010; Namvar *et al*., 2017). Medically, in staphylococci these enzymes that are known abbreviation [(APH (3')-III, ANT (4')-I and (6')/APH(2")] are the most frequent AMEs and, which inactivate aminoglycosides of curative importance including respective kanamycin, tobramycin, and gentamicin (Klingenberg *et al*., 2004; Szymanek-Majchrzak, *et al*.,2018a).In different parts of the world including the Middle East, there are several neoteric studies expounding the growing prevalence of AMEs in MRSA strains(Goudarzi *et al*., 2018; Seyedi-Marghaki *et al*., 2019; Kavusi *et al*.,2019; Beigverdi *et al*.,2019). Nevertheless, in Iraq, the resistance problem of antimicrobial drugs is exacerbated by the overuse and misapply of them. There is no systematic national control of AR, scanty data is available to identify this problem, and there is no database of the genes encoding AMEs among gram-positive bacteria especially MRSA strains. So, the study aimed to assess the occurrence of genes encoding clinically important AMEs such as *aph (3')-IIIa*, *ant (4')-Ia*, and *aac(6')/aph (2")*, and to estimate the relationship between MRSA phenotypes of aminoglycosides resistance and the occurrence of genes responsible for this resistance in patients were attending to Al-Diwaniya hospitals.

2. Methodology

2.1. The population of the study, S. aureus, and MRSA identification

For the period January to July 2018, 72 MRSA were isolated from different lesions(wound, abases, throat swab, blood, and urine) from randomly the 72 patients(without data related patients) were attending to Al-Diwaniya teaching hospital, and Maternity and Children teaching hospital which is two mains hospitals in Al-Diwaniya province centre of Iraq. Bacterial isolates were identified depending on the traditional morphological(Gram stain/ Himedia, India) and bacteriological tests (Haemolysis on blood agar, mannitol salt medium / Oxoid, UK, and coagulase production) in microbiology laboratory belong to the Faculty of Science- University of Al-Qadisiyah. The media were incubated at 37˚C for 48 hours according to the method of(Forbes *et al*., 2007). All *S. aureus* isolates were tested for detecting phenotypic MRSA depending on the cefoxitin disc-diffusion method (Kirby-Bauer) following (CLSI, 2019)

2.2. Antibacterial susceptibility testing

Antibacterial sensitivity patterns of the MRSA strains were performed through Bauer *et al*. (1966) and CLSI (2019), on Mueller-Hinton medium (Oxoid, UK) plates. Bacterial inoculum was modified according to the 0.5 McFarland tube. Antibacterial discs were selected carried out based on the (CLSI, 2019). All isolates tested for sensitivity of 10molecules classes were divided into 29 antibacterial agents, which are : penicillin (PEN, 10units), cefoxitin (FOX, 30 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), cefotaxime (CTX,30 µg),amoxicillin/clavulanic acid (AUG, 30 µg), kanamycin (K, 30 µg), netilmicin (NET, 30 µg), amikacin (AK, 30 µg), gentamicin (GM, 10 µg), tobramycin (TOB,10 µg),aztreonam (ATM, 30 µg), ciprofloxacin (CIP, 5 µg), moxifloxacin(MFX, 5µg),ofloxacin (OFX, 5 µg),norfloxacin (NOR, 10 µg),tetracycline (T, 30 µg), doxycycline (DXT, 30 µg), trimethoprim (TM, 5 µg), trimethoprim/sulfamethoxazole (SXT, 25 µg), chloramphenicol (C, 30 µg), nitrofurantoin (NI, 300 µg), vancomycin(VA, 30µg),imipenem (IMP, 10 µg), erythromycin (E,15 µg), rifampin (RA, 5 µg), teicoplanin (TEC, 30µg), clindamycin (CD, 2µg) and oxacillin(OX, 5µg) (Bioanalyse, Turkey and Mast Diagnotics, UK). Furthermore, the MRSA strains were stratified to MDR and XDR based on (Magiorakos *et al*., 2012). A laboratory stock culture of genus *S. aureus* ATCC 25923 was used as a quality control organism to confirm the accuracy of the antibacterial disks. Strain giving intermediate sensitivity was calculated as resistant. MICs and MBCs values were detected (Andrews, 2001) and calculation of the multiple antibiotic resistance (MAR) index of 20 MRSA-AR was conducted based on (Krumpernam, 1983; Riaz *et al*., 2011).

2.3. Isolation of deoxyribonucleic acid

DNA isolation was perfect using a specific procedure of (+)ve bacteria (proteinase K) and according to the manufacturer's instructions of Kit (Geneaid, USA).

2.4. PCR analysis

PCR assay was done by components that were accumulated in a PCR tube and mixed under sterile conditions on an ice bag. The reaction was performed using a 25 µl mixture including 12.5 µl Go Tag Green Master mix(Promega, USA), 2.5 µl of 10µM each primer (Macrogen, Korea), 5 µl of genomic DNA, and 2.5 µl nuclease-free water. The PCR program was done with a (Biometra, Germany). Universal specific primer sequence listed in table 1, the amplification conditions of each primer of *ant (4')-Ia*, *mecA*, *aph (3')-IIIa*, *aac(6')/aph (2")* genes describe in the same references in table 1. The amplifications were electrophoresed (Biometra, Germany)through 1.5% agarose gel pretreated with ethidium bromide, utilizing a UV imager(Biometra, Germany). The results were documented. Times of electrophoresis were at 75 volts for 90 minutes. Molecular weight DNA markers were used (Ladder 100 bp Promega, USA).

Table 1: Oligonucleotides sequence of primes used to encoding genes of AMEs and PBP2'.

Gene target	Forward primer ('5 to 3')	Reverse primer ('5 to 3')	Amplicon size (bp)	Annealing temperature	Reference
mecA	aaaatcgatggtaaaggttggc	agttctgcagtaccggatttgc	533	55°C	Munger and Kelly, (1973)
ac(6')/aph (2")	gaa gta cgc aga aga ga	aca tgg caa gct cta gga	508	54°C	Choi *et al*.(2003)
aph (3')-IIIa	ggctaaaatgagaatatcaccgg	ctttaaaaaatcatacagctcgcg	526	55°C	Vakulenko *et al*. (2003)
ant (4')-Ia	tggggatgatgttaaggc	gcgttttgacacatccac	670	50°C	Riesen and Perreten. (2009).

2.5. Analysis

$\chi 2$ test was used to determine the significant frequencies of resistance results. P-value < 0.01, Prism 5 (Graphpad Software Inc., San Diego, CA, USA).

3. Results

72 MRSA strains were obtained from patients who were suffering from various infections. The main different lesions of this causative agent were: urine 32 (44.5%), wounds 18(25), abases 13 (18.0%), blood cultures 5 (6.9%), and throat swabs 4 (5.6%). Of these 72.2% were found to be either MDR or XDR, thus a (44/72; 61.1%) of MRSA were found to be MDR while the remaining (8/72;11.1%) were XDR. Whole, 100% strains were sensitive to vancomycin(MICs and MBCs values ranged 2 µg/mL to 8 µg/mL.) and chloramphenicol. Strains exhibited significant frequencies of antibacterial agents resistance (P < 0.01)(Figure 1).The highlight indicates cases considered to be resistant to the respective drugs. Penicillin, cefoxitin, ceftriaxone, aztreonam, and nitrofurantoin showed a resistance of 100% from all the isolates. A high rate of resistance (94.5, 88.9, 83.3, 79.2, 77.8, 73.6, and 61.2)% showed that *S. aureus* to ceftazidime, cefotaxime, trimethoprim, erythromycin, oxacillin, tetracycline, and teicoplanin respectively. The resistance of clindamycin and Amoxicillin/clavulanic acid were 37.5% and 30.6%. Also, the most effective of antibacterial agents were imipenem, rifampin, ofloxacin, doxycycline, moxifloxacin, ciprofloxacin, norfloxacin and trimethoprim/sulfamethoxazole with resistance rates 11.1%, 11.1%, 22.2%, 22.2%, 26.4%,27.8%, 27.8% and 33.3% (Figure 1).

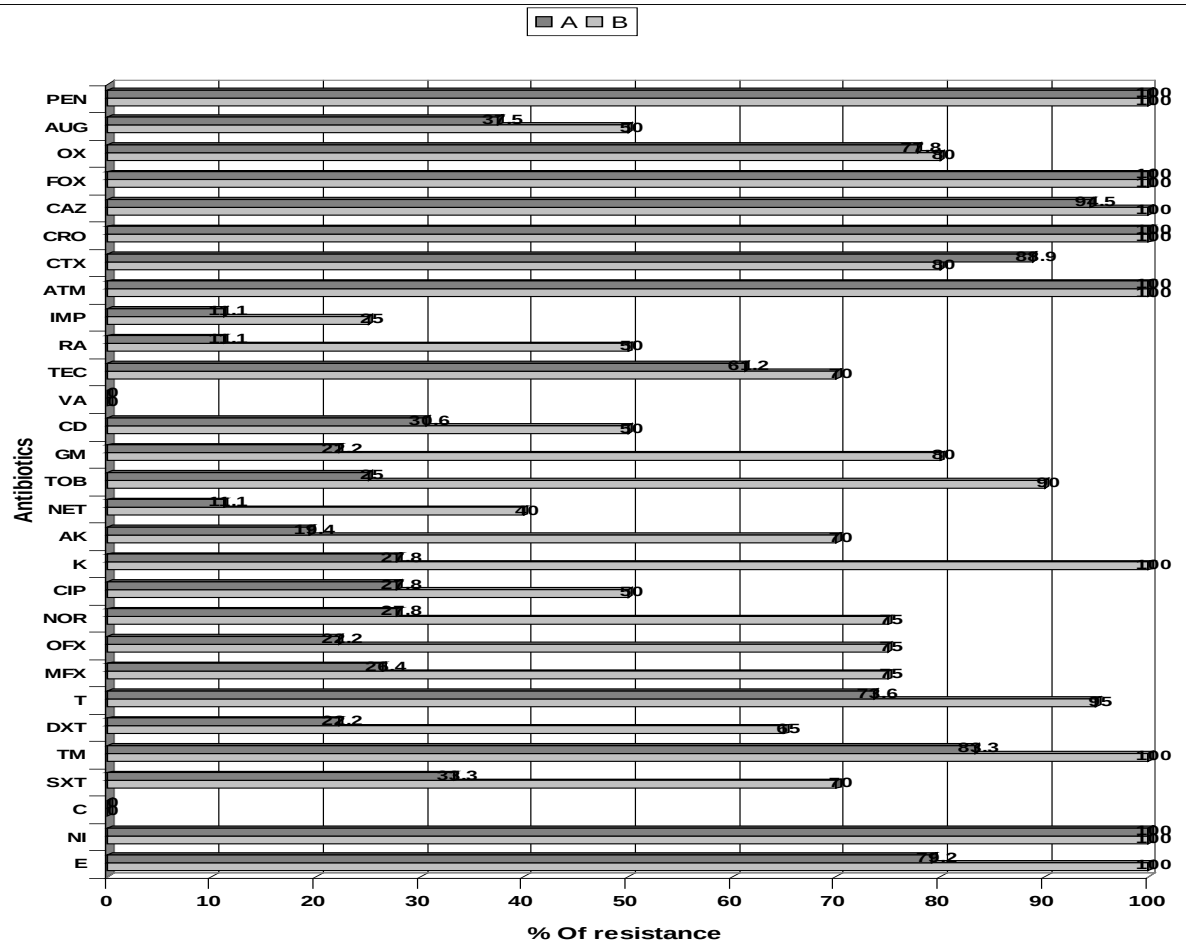

Figure 1. Comparison between the rates of resistance for 72 strains of MRSA(A) and 20 strains of MRSA which show resistance to aminoglycosides(B).

PEN, penicillin; AUG, amoxicillin-clavulanic acid; OX, oxacillin;FOX, cefoxitin;CAZ, ceftazidime; CRO, ceftriaxone; CTX, cefotaxime;ATM, aztreonam;IMP, imipenem;RA, rifampin; TEC teicoplanin; VA, vancomycin; CD, clindamycin; GM,gentamicin; TOB, tobramycin;NET, netilmicin; AK, amikacin; K, kanamycin; CIP, ciprofloxacin;NOR, norfloxacin; OFX, ofloxacin;MFX,moxifloxacin; T, tetracycline;DXT,doxycycline;TM,trimethoprim;SXT,trimethoprim/sulfamethoxazole;C,chloramphenicol;NI,nitrofurantoin; E, erythromycin.

The aminoglycosides resistance rate among the tested *S. aureus* strains ranged from 27.8%-11.1%. The present study showed that netilmicin was the most potent aminoglycoside; its overall potency over the isolated *S. aureus* was 11.1%, while amikacin, gentamicin, tobramycin, and kanamycin were 19.4%, 22.2%, 25%, and 27.8% respectively. The full (100%) dissemination of the *mecA* gene in MRSA strains is shown in (Figure2).

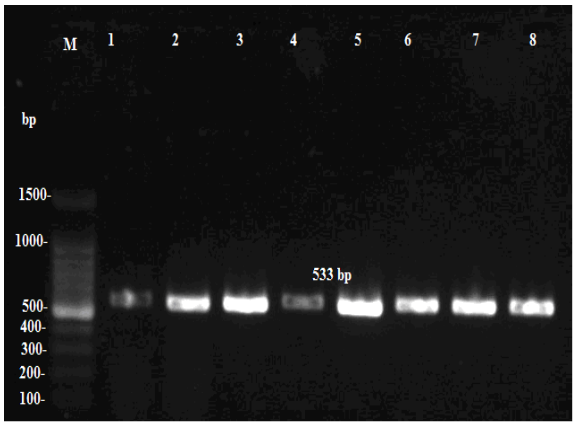

Figure 2. Image of electrophoresis gel of *S. aureus*. Lane M, PCR ladder (100-1500 bp), amplified products of the *mecA* gene (533 bp).Lanes (1-8) positive results.

Out of 20, MRSA was AR eighteen (90%) carrying at minimum 1 of genes encoded AR. The most common of genes encoded AMEs were *aac(6')/aph (2")*;80%, *aph (3')-IIIa*;45%, and *ant (4')-Ia*; 35% (Figure 3,4 and 5 respectively).

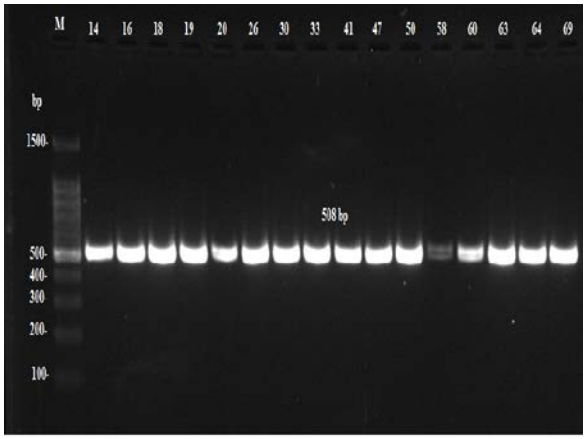

Figure 3. Image of electrophoresis gel of *S. aureus*. Lane M, PCR ladder (100-1500 bp), amplified products of the *ac(6')/aph (2")*gene (508 bp).Lanes (14,16,18,19,20,26,30,33,41,47,50,58, 60,63,64,69) positive results.

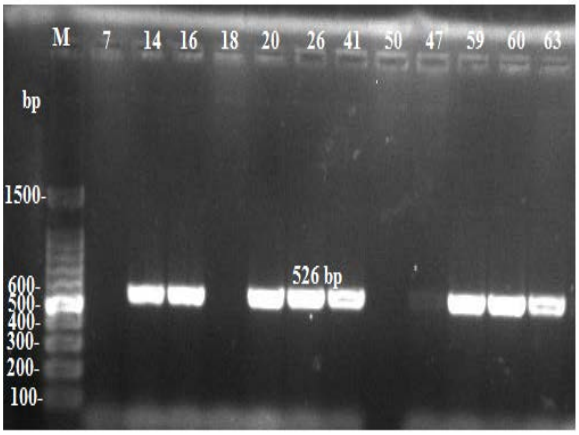

Figure 4. Image of electrophoresis gel of *S. aureus*. Lane M, PCR ladder (100-1500 bp), amplified products of the *aph (3')-IIIa*gene (526bp).Lanes (14,16, 20,26,41,47,59, 60,63) positive results, lanes (7,18,50) negative results.

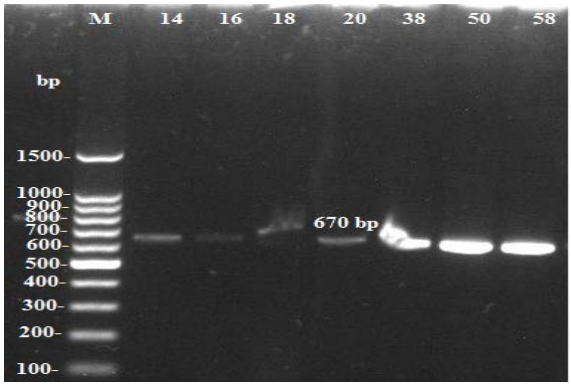

Figure 5. Image of electrophoresis gel of *S. aureus*. Lane M, PCR ladder (100-1500 bp), amplified products of the *ant (4')-Ia* gene (670 bp).Lanes (14, 16,18, 20,26,38,50,58) positive results.

Interestingly, it is observed that all(100%) strains in which positive results of AR genes were resistant to at least three aminoglycoside molecules. The highest resistance of 20 AR isolates compares with 72 MRSA isolates against all drugs, especially aminoglycoside as mentioned in figure 1. 100% of MRSA-AR were either MDR(12/20; 60%) or XDR(8/20; 40%). The dissemination, frequency of co-occurrence genes encoding AMEs, and relationship within phenotypic AR among MRSA harboring *mecA* gene are listed in Table (2).

Table 2: Phenotypic and molecular analysis of aminoglycosides resistance patterns possessed 20 MRSA strains harboring the *mecA* gene.

No.	Phenotypic	No. (%)	Genotypic	No. (%)	Profile type
G1	NET, AK, GM, TOB, K	6(30)	*aac(6')/aph (2")*	6(33.3)	3G2,2G3,1G4
G2	AK, GM, TOB, K	6(30)	*aph (3')-IIIa*	0	-
G3	NET, GM, TOB, K	2(10)	*ant (4')-Ia*	1(5.6)	G6
G4	GM, TOB, K	2(10)	*aac(6')/aph (2")+ aph (3')-IIIa*	5(27.8)	3G1,2G2
G5	AK TOB, K,	1(5)	*aac(6')/aph (2") + ant (4')-Ia*	2(11.1)	1G2,1G4
G6	TOB, K	1(5)	*aph (3')-IIIa + ant (4')-Ia*	1(5.6)	G5
G7	K	2(10)	*aac(6')/aph (2")+ aph (3')-IIIa+ ant (4')-Ia*	3(16.7)	3G1
	Total	20(100)		18(100)	

The most frequent 11/18(61.1%) of MRSA strains which comprise AMEs genes were as combinations or simultaneously. Correlation between the AR patterns and their presence of plasmid-mediated AR genes among MRSA isolates tested are shown in (Table 2 and 3). The results showed that there is 100% compatibility between the presence of aac(6')/aph (2") and resistance to gentamicin. It was discovered in all strains resistant to gentamicin (Table 3). Lastly, only 2 strains of MRSA were resistant to kanamycin did not give any result with AMEs genes.

High values>0.2 ranged(0.48-0.83) from the maximum MAR index(1) were documented in all 20(100%)MRSA-AR strains which summarize in figure 6.

Moreover, the relationship between phenotypic resistance indicators and AR genes among MRSA strains was mentioned in Table(4)

Table 3: Relatedness between phenotypic and the main molecular description of aminoglycosides resistance patterns in a total of 18 MRSA strains harboring AMEs genes.

Genotypic description of AMEs	No. (%)	(%) Of phenotypic expression AMEs				
		NET	AK	GM	TOB	K
aac(6')/aph (2")	16(80)	50	75	100	100	100
aph (3')-IIIa	9(45)	66.7	100	88.9	100	100
ant (4')-Ia	7(35)	42.8	71.4	71.4	100	100

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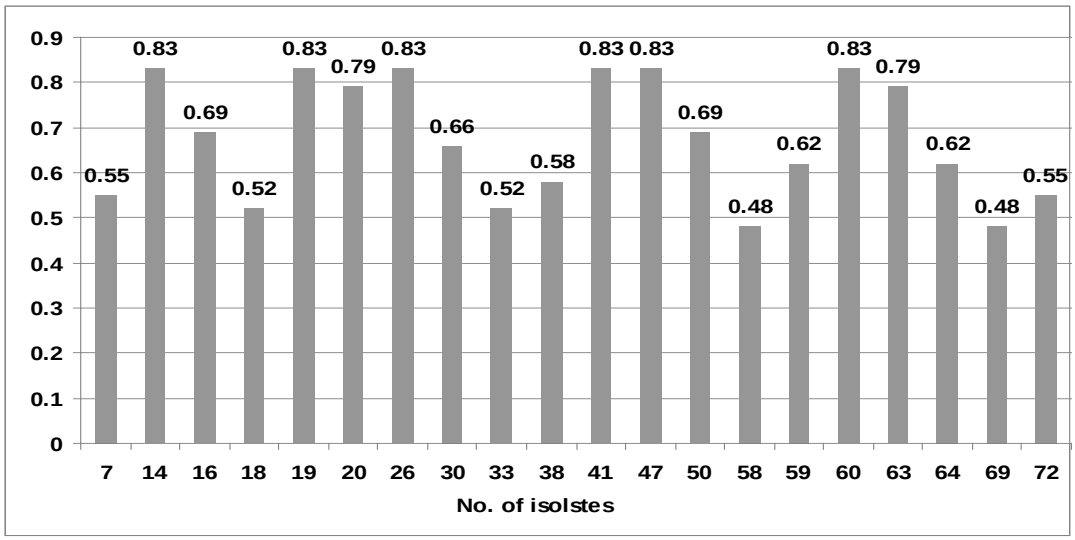

Figure 6. MAR indexes of 20 aminoglycosides resistance MRSA isolates.

Table 4: Dissemination of multiple resistance of drug patterns among MRSA-AR genes.

No. of strain	*mecA* gene	Phenotypic profile		AMEs genes profile		
		MDR / XDR	MAR index	*aac(6')/aph (2")*	*aph (3')-IIIa*	*ant (4')-Ia*
S7	+	MDR	0.55	-	-	-
S14	+	XDR	0.83	+	+	+
S16	+	MDR	0.69	+	+	+
S18	+	MDR	0.52	+	-	+
S19	+	XDR	0.83	+	-	-
S20	+	XDR	0.79	+	+	+
S26	+	XDR	0.83	+	+	-
S30	+	MDR	0.66	+	-	-
S33	+	MDR	0.52	+	-	-
S38	+	MDR	0.58	-	-	+
S41	+	XDR	0.83	+	+	-
S47	+	XDR	0.83	+	+	-
S50	+	MDR	0.69	+	-	+
S58	+	MDR	0.48	+	-	-
S59	+	MDR	0.62	-	+	+
S60	+	XDR	0.83	+	+	
S63	+	XDR	0.79	+	+	
S64	+	MDR	0.62	+	-	
S69	+	MDR	0.48	+	-	
S72	+	MDR	0.55	-	-	-
Total	20			16	9	7

4. Discussion

Antimicrobial stewardship is important to prevent the spread and expansion of MDR strains and to overcome the development of increased resistance to antibiotics in general and aminoglycoside in particular, and continued national surveillance programs are crucial. Aminoglycosides are broad-spectrum bactericidal antibiotics of high potency that have been traditionally used for the treatment of serious and of life-threatening Gram-negative and some Gram-positive infections (Zacharczuk *et al*., 2011; Becker and Cooper, 2013; Garneau-Tsodikova and Labby, 2016). In different parts of the world including in Iraq, aminoglycosides are used for treating severe infections caused by Gram-Positive bacteria. As a result, multiple resistance determinants to these antimicrobial agents have emerged in various pathogenic microbes including MRSA. This organism is a major public health concern representing about 60% of *S. aureus* isolated from hospitalized patients in countries such as the USA and Brazil in the last years(Dos Reis *et al*., 2020).

72 MRSA strains were obtained from patients who were suffering from various infections. The main different lesions of this causative agent were: urine 32 (44.5%), wounds 18(25), abases 13 (18.0%), blood cultures 5 (6.9%), and throat swabs 4 (5.6%). Of these 72.2% found to be either MDR or XDR, thus a (44/72; 61.1%) of MRSA were found to be MDR while the remaining (8/72;11.1%) were XDR. The full (100%) dissemination of the *mecA* gene in MRSA strains are in (Figure2 and Table 4).This finding matches with other reports in West Bank-Palestine and Sri Lanka that found all 112 and 94 *S. aureus* (100%) isolated from different lesions as MRSA strains were caring *mecA*gene (Hadyeh *et al*., 2019; McTavish *et al*., 2019) respectively. This agrees with Goudarzi *et al*. (2019b) in Iran as 78.6% (66/84) of MRSA strains were found to be MDR. The vast majority our results were matched with another report of MRSA strains isolated from the holy shrine in Najaf, Iraq which found that most strains(100%) were resistant to penicillin, ceftriaxone, ceftazidime, (72.7%) to erythromycin, and the most susceptible(100%) to vancomycin, chloramphenicol,(72.3%) to gentamicin and 8/11(72.7%) of MRSA strains were found to be MDR(Al-Mohana *et al*., 2012). Based on the above results, it can be said that vancomycin is considered the best choice of treatment MRSA infections in various parts the world (Szymanek-Majchrzak *et al*., 2018b) including Iraq, despite some resistance cases that have been observed in this area (Al-Jumaily *et al*., 2012; ElFeky *et al*.,2019) and the world (Szymanek-Majchrzak *et al*., 2018b). Also, these results were close to other results of a study documented in Sulaimani city, Iraq concerning the resistance MRSA of a β-lactams drug (Al-Jumaily *et al*., 2012). Moreover, resistance of β-lactam, vancomycin, and gentamicin was documented in MRSA strains which were isolated from West Bank-Palestine (Hadyeh *et al*., 2019). Due to the widespread and indiscriminate use of antibiotics in treatment, a major problem has emerged as the multiple resistance of these drugs from different bacterial species, especially *S. aureus*. This maybe via the biofilms formation which increases from the pathological ability(Gomes *et al*., 2019).Interestingly, in this investigation there was a low level of AR among MRSA strains compared with other studies in Iraq and other parts of the world, which may be explained by a decrease in the number of MRSA strains from the various regions of central Iraq or by low- level description of this drug in the treatment infections of this pathogen. For more than half a century, aminoglycoside has been mainly used against gram-negative and some gram-positive bacterial infections (Garneau-Tsodikova and Labby, 2016), and this reinforces the second reason. The first cause may be close to reality and corresponds to an antibiotic sensitivity analysis performed in the Al-Diwaniya governorate (unpublished) which documented that approximately 30.5% (7/23) of MRSA strains were gentamicin resistant (Al-Mayahi, 2018). Thus, ElFeky *et al*. (2019)who found that 63%(63/100)of MRSA strains were resistant to gentamicin. The resistance pattern in the Al-Diwaniya governorate is somewhat harmonious with other investigations in the Najaf governorate centre Iraq, which showed that MRSA strains were 27.7% (15/54) resistant to gentamicin(Al-Mohana *et al*., 2012). Aminoglycoside molecules still have significant effects alone or in combination with other molecules in treating infections causing staphylococci, despite the emergence of resistance to them in different parts of the world (Kavusi *et al*., 2019). In a previous study, Goudarzi *et al*. (2019e) found that MRSA strains and AMEs production have been developed rapid resistance to a wide range of drugs including tetracyclines, and this agreed with our study at a rate of resistance (73.6%). High values>0.2 ranged(0.48-0.83) from the maximum MAR index Baines *et al*. (2018) were documented in all 20(100%)MRSA-AR strains. A MAR index resistance to >0.20 antibiotics indicates that bacteria originate from an environment where antibiotics are freely available, leading to a high potential for misuse and a 'high-risk' source of contamination (Krumpernam, 1983; Riaz *et al*., 2011). There is scanty information regarding the level of antibiotics resistant to MRSA strains associated with multiple infections in Iraq, thus possibly posing a public health challenge for physicians. Consequently, this study determined the MAR index of these isolates. However, the elevation of MAR index values was observed in our investigation (Figure 5and Table 4). All MRSA-AG strains had MAR index of >0.20, confirming that there was widespread use of antibiotics and high selective pressure in the Al-Diwaniya population. The MAR indices obtained in the present study is a probable signal that a very major ratio of the MRSA strains was displayed to numerous antimicrobial drugs. The high MAR identified in the present research warns us that any use of antibiotics in treatment should be preceded by an accurate diagnosis of the causative agents, followed by an antimicrobial sensitivity test. Such a thing will not only contribute to the effective use of these drugs but also will control the prevalence of resistant isolates of antibiotics in Iraqi hospitals and communities.

Out of 20, MRSA was AR eighteen (90%)carrying at minimum 1 of genes encoded AR. The most genes encoded AMEs common were namely *aac(6')/aph (2")*;80%, *aph (3')-IIIa*;45%, and *ant (4')-Ia*; 35% (Tables 2 and 4). Many reports from Iran have reported that the *aac(6')/aph (2")* gene was the most frequent AMEs gene followed by *aph (3')-IIIa* gene and *ant (4')-Ia* gene in MRSA isolates(Fatholahzadeh *et al*., 2009; Emaneini *et*

al., 2013; Mohammadi *et al*., 2014; Khosravi *et al*., 2017; Khoramrooz *et al*., 2017; Seyedi-Marghaki *et al*.,2019; Goudarzi *et al*.,2019d), from Turkey(Ardic *et al*., 2006), from Australia (Baines *et al*.,2019), from India(Perumal *et al*., 2016) and Europe(Vanhoof *et al*., 1994). However, the prevalence of MRSA strains containing *aac(6')/aph (2")* gene in Al-Diwaniya (80%), is similar with Goudarzi *et al*. (2019a), Szymanek-Majchrzak, *et al*. (2018a), Baines *et al*. (2019), Kavusi *et al*. (2019), Mohammadi *et al*. (2014) and Mahdiyoun *et al*. (2016)in Iran(80%), Europe(80.5%), Australian clade (79.7%), Iran(78.3%), (77.8%) and (77%) respectively, and is less compared to similar studies in Iran (97.22%)(Khoramrooz *et al*., 2017) and Asian-Australian clade (93.2%)(Baines *et al*.,2019). The results showed there is 100% compatibility between the presence of *aac(6')/aph (2")* and resistance to gentamicin (Table 3). It was discovered in all strains resistant to gentamicin. This concordance matches with previous researches(Choi *et al*., 2003; Yadegar *et al*., 2009). The present investigation shows that *aph (3 ') - IIIa* gene was the second dominant gene (45%), and was in agreement with the reports from, Australia(45.0%)(Baines *et al*.,2019), Europe (44%) (Młynarczyk *et al*., 2010), Iran(46.3%) (Goudarzi *et al*.,2019e) and(46.7%)(Goudarzi *et al*.,2019c), while Seyedi-Marghaki *et al*. (2019) and Khoramrooz *et al*. (2017) in Iranian work documented rate of this gene in (19% and 61.1%) of MRSA strains. Dissemination of *ant(4')-I* was detected as 35% (7/20) (Tables 2 and 4). This was similar with other reports from Australia 34.1% (Baines *et al*.,2019), Iran 38.6%(Goudarzi *et al*.,2018), and less than the ratio mentioned in a big Japanese report (84.5%) (Ida *et al*., 2001) and Europe (55.3%)(Szymanek-Majchrzak, *et al*.,2018b). The presence of the *aac(6')/aph (2"),aph (3')-IIIa* and *ant (4')-Ia* genes was sufficient to express the resistance phenotype(100%) to GM/TOB/K, AK/TOB/K and TOB/K respectively (Table 3). AAC(6')/APH(2") enzyme grant resistance to aminoglycosides molecules including GM/TOB/K, APH (3')-IIIa grant resistance AK/TOB/K, and ANT (4')-Ia enzyme grant resistance to TOB/K (Vakulenko and Mobashery, 2003). The relatedness between phenotypic and genotypic AR with MRSA were 27.8% and 25%. This relationship was reported in other studies such as (Yadegar *et al*., 2009; Mohammadi *et al*., 2014; Khosravi *et al*., 2017), where the last study recorded a high correlation (72.7%).

5. Conclusion

In conclusion, this is the first paper that provided baseline prevalence data on the presence of AMEs genes in MRSA strains containing the *mecA* gene in Al-Diwaniya governorate in the centre of Iraq which reached alarming tiers; thus, Aminoglycosides should be used carefully by physicians. The execution of a local and global monitoring system to observe antibiotic resistance, particular aminoglycosides, and growing consciousness of AMEs genes among physicians are necessary for guiding empirical therapy-specific measures against a specific pathogen

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