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ORIGINAL ARTICLE

Aminoglycoside Resistance Pattern among Hospital Acquired and Community Acquired Methicillin-Resistant *Staphylococcus aureus*

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Key words:
Staphylococcus aureus, Aminoglycosides, MRSA

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ABSTRACT

Background*: MRSA strains are now resistant many antibiotic groups, such as tetracyclines, aminoglycosides, and lincosamides, and become difficult to treat.* ***Aminoglycosides*** *are valuable antibiotics used for treatment of a variety of staphylococcal infections.* ***Objectives:*** *The aim of this study is to detect aminoglycoside resistance in various hospital acquired and community MRSA strain and to identify the genetic basis of this resistance.* ***Methodology:*** *MRSA strains were isolated and identified manually and VITEK 2 system, antibiotic susceptibility of the isolates was tested by VITEK 2 system and the MIC of various aminoglycosides was measured by E test. Conventional PCR was used to detect the genes responsible for aminoglycoside resistance among the isolates.* ***Results:*** *(33.3%) of CA- MRSA isolates were resistant to Amikacin, (20.8%) to Kanamicin and (37.5%) to Gentamicin, while (65.8%) of HA-MRSA strains were resistant to Amikacin, (73.7%) to Kanamycin and (71.1%) to Gentamicin. the aac (6')-Ie/aph (2") gene was found in 58.3 % of the strains of CAIs and in 68.4% of the strains of HAIs. There is no significant difference between HAIs and CAIs harboring aac (6')-Ie/aph (2") gene (p value 0.419). While aph (3)-IIIa gene was found in 45.8% of the strains of CAIs and in 44.7% of the strains of HAIs. Conclusion: There was a non-significant difference between HAIs and CAIs harboring aph (3)-IIIa gene (p value 0.933). It is important to control development of aminoglycoside-resistance in MRSA strains and to monitor the potential developing of new aminoglycoside resistant genes.*

INTRODUCTION

Staphylococcus aureus is one of the most important causative agents in both HAIs and CAIs. This bacterium can use various types of infection, including sepsis, pneumonia, wound sepsis, endocarditis, catheter-related infections, and UTIs [1]. The main cause of the success of *S. aureus* as a human pathogen is its flexibility. As part of its adaptation in the antibiotic era, *S. aureus* has been able to acquire resistance to almost all antibiotics. Resistance to penicillin was described in 1942; only 1 year after the incredible drug was presented. In the mid-1940s the mechanism of penicillin resistance due to an inducible beta-lactamase was discovered [2].

Methicillin-resistant *Staphylococcus aureus* (MRSA) formed an important portion of the isolates, from about 43 to 58 % according to the hospital ward or the infections type [3]. Many countries informed a percentage of MRSA above 20 % and, seldom, up to 80 %. So, second line antibiotics are mandatory for the treatment or the prophylaxis of *S. aureus* infections worldwide. MRSA infections are linked with an increase in mortality and in duration of hospital stay[4].

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are resistant to beta-lactams, including penicillins and cephalosporins [5]. MRSA isolates have a penicillin-binding protein (PBP2a). It has a lower affinity to beta-lactam antibiotics than PBP2, which is the main receptor of methicillin [6].

Health care-associated MRSA (HA-MRSA) strains are those isolated from patients after hospital admission by 2 or more days or with the MRSA risk factors, which includes: history of recent hospital-admission, surgical operation, renal dialysis, or residence in a long-term care facility within 1 year before the MRSA- isolation date or presence of a permanent catheter or percutaneous medical device at the time of culture or previous isolation of MRSA [7].

The increase in the occurrence of HA-MRSA through the world has been dramatic. In the United States, the percentage of MRSA among *S. aureus* isolates from the hospitalized patients was 2.4% in 1975, which rose to 51.6% (ICU patients) and 42% (non-ICU inpatients) by 1998–2003 [8].

Community-acquired MRSA clones were documented in Europe, United States, Latin America, and Asia. These clones often affected young people without healthcare contact, producing purulent skin infections or pneumonia[9]. CA-MRSA differs from HA-MRSA in that it is not one of the major clonal groups of epidemic MRSA and is susceptible to most antibiotics

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other than b-lactams. In contrast, nosocomial MRSA is generally multidrug-resistant [10].

Aminoglycosides are one of the classes of antibiotics that play an important role in the treatment of staphylococcal infections. These are often used in combination with either beta-lactam or glycopeptides to abtain synergism, especially for the treatment of severe staphylococcal infections. However, today MRSA have acquired resistance to a many antibiotics including aminoglycosides [11].

The three main mechanisms of resistance to aminoglycosides; changes in the position of the ribosomal binding site for the drug, decreased permeability to the drug, and inactivation of the drug by enzymes. Acquisition of aminoglycoside modifying enzymes (AMEs) is an important mechanism of resistance in staphylococcal species. These enzymes are classified in three different categories: aminoglycoside acetyl transferases (AACs), aminoglycoside phosphotransferases (APHs), and aminoglycoside nucleotidyl transferases (ANTs), on the basis of modifying effects. Three enzymes, AAC (6')/APH (2"), APH (3)-III, and ANT (4), are encoded by *aa c (6')-Ie/aph (2")*, *aph (3)-IIIa*, and *ant (4)-Ia genes*, respectively [11].

In MRSA, the commonest are 2-domain, bifunctional AAC (6´)-Ie/ APH(2´´)-Ia acetyltransferase and phosphotransferase, ANT(4´)-Ia nucleotidyl transferase, and APH(3´´)-IIIa phosphotransferase, encoded by the next genes: aacAaphD, aadD, and aph(3´´)-IIIa. These genes are transferred on mobile genetic elements (transposons, plasmids) through diverse mechanisms of horizontal transfer, and so the resistance conditioned by AMEs is an acquired and strongly different trait [11].

The aim of the current study is to isolate methicillin-resistant staphylococcal isolates causing different hospital acquired and community acquired infections, to test their susceptibility to different antibiotic groups, to compare the pattern of aminoglycoside resistance among HA and CA strains, and to detect the most common genes encoding for aminoglycoside resistance in such strains.

METHODOLOGY

Study groups:

This study was conducted in the Department of Medical Microbiology and Immunology during the period from December 2018 to December 2019; patients included in the study were two groups:

Group 1: Patients presented by hospital acquired infections suspected to be caused by *S. aureus* like chest infections, surgical site infections and urinary tract infections.

Group 2: Patients presented by community acquired infections like skin and soft tissue infections, osteomyelitis, and respiratory infections, urinary tract infections presented at different out patients Clinics of Sohag University Hospitals.

Informed oral consent was taken from the patients included in the study, and the study was approved from the local ethical committee of the faculty.

Sampling:

Samples were collected under complete aseptic conditions by sterile cotton swab for pus samples and dry sterile well-closed plastic cups for urine, sputum and endotracheal aspirate samples. Pus samples were enriched with nutrient broth for 24 hours at 37^{o}c. Urine samples were vortexed and plated-out on culture media by 10 µl calibrated loops for bacterial counts.

Samples were inoculated on nutrient agar and manitol salt agar, catalase and coagulase (slide and tube method) tests were performed on isolated staphylococcal colonies. Coagulase positive colonies were further identified as *Staphylococcus aureus* by VITEK 2 system *(BioMerieux, France).*

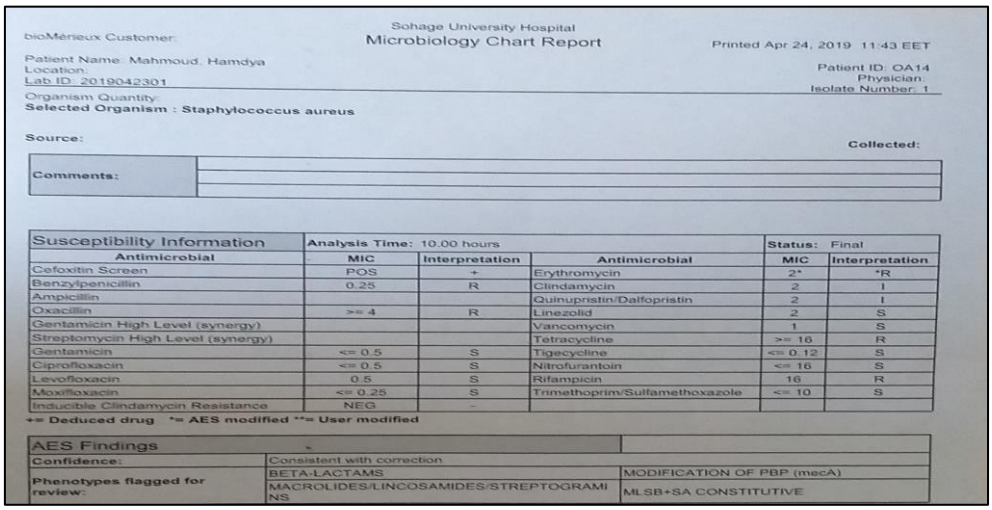
Identification of MRSA isolates by detection of mec A gene:

Methicillin-resistant *staphylococcus aureus* isolates were identified using disc-diffusion method with cefoxitin 30 µg disc according to CLSI *(CLSI, 2016)*, And by detection of the *mec A* gene by PCR; DNA was extracted from the isolates by the use of QIAamp DNA Kits according to the manufacturer's instructions. PCR assay was run using the primer (*mec A*-F 5'-TAA TGC TTT GAT CGG CCT TG-3' and *mec A* -R 5'-TGG ATT GCA CTT CAT CTT GG-3').

PCR were done in a volume of 25 µl; PCR master mix (12.5 µl), PCR grade water (4.5 µl), primer (2 µl of each) and the extracted DNA sample (4 µl). a negative control was included in the experiment, by replacing the DNA template with PCR grade water.

Amplification of the target gene by using a Biometra thermal cycler *(T Gradient PCR system version 4 - Biometra Whatman company, Germany*). The PCR amplification cycle; initial heating at 95°C for 4 min, and then 34 cycles of denaturation at 95°C for 45 s, primer-annealing at 52°C for 45 s, and primer-extention at 72°C for 30 s, followed by a final extension at 72°C for 10 min [12].

The susceptibility of *methicillin-resistant staphylococcus aureus* isolates to different antibiotics was tested by the VITEK 2 system *(BioMerieux, France)* according to manufacturer instructions to the following antibiotics: Benzylpenicillin, Oxacillin, Ciprofloxacin, Levofloxacin, Moxifloxacin, Erythromycin, Clindamycin, Quinupristin/ dalfopristin, Linezolid, Vancomycin, Tetracycline, Tigecycline, Nitrofurantoin, Rifampicin, Trimethoprim/ Sulfamethoxazole. The MIC of different aminoglycosides against MRSA isolates was determined E test ***(Oxoid, UK).***



Sohage University Hospital
Microbiology Chart Report

bioMérieux Customer:
Printed Apr 24, 2019 11:43 EET
Patient Name: Mahmoud, Hamdya
Location:
Lab ID: 2019042301
Patient ID: OA14
Physician:
Isolate Number: 1
Organism Quantity:
Selected Organism : Staphylococcus aureus
Source:
Collected:
Comments:

Susceptibility Information	Analysis Time: 10.00 hours			Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	POS	+	Erythromycin	2*	*R
Benzylpenicillin	0.25	R	Clindamycin	2	I
Ampicillin			Quinupristin/Dalfopristin	2	I
Oxacillin	>= 4	R	Linezolid	2	S
Gentamicin High Level (synergy)			Vancomycin	1	S
Streptomycin High Level (synergy)			Tetracycline	>= 16	R
Gentamicin	<= 0.5	S	Tigecycline	<= 0.12	S
Ciprofloxacin	<= 0.5	S	Nitrofurantoin	<= 16	S
Levofloxacin	0.5	S	Rifampicin	16	R
Moxifloxacin	<= 0.25	S	Trimethoprim/Sulfamethoxazole	<= 10	S
Inducible Clindamycin Resistance	NEG	-			

+= Deduced drug *= AES modified **= User modified

AES Findings		
Confidence:	Consistent with correction	
Phenotypes flagged for review:	BETA-LACTAMS	MODIFICATION OF PBP (mecA)
	MACROLIDES/LINCOSAMIDES/STREPTOGRAMINS	MLSB+SA CONSTITUTIVE

Fig. 1: Report of antibiotic sensitivity testing by VITEK 2.

Table 1: MIC interpretive criteria to different aminoglycosides according to CLSI 2016

Antimicrobial agent	MIC Interpretive Criteria (µg/mL)		
	S	I	R
Gentamicin	≤4	8	≥16
Amikacin	≤16	32	≥64
Kanamycin	≤16	32	≥64

Molecular detection of genes encoding AMEs aac 6'-Ie/aph 2" and aph 3'-IIIa by PCR:

AMEs genes were detected by Polymerase Chain Reaction (PCR) for detection of *aac 6'-Ie/aph 2"* and *aph 3'-IIIa genes* coding for aminoglycoside resistance in our *Staphylococcus aureus isolates*.

Sample treatment & DNA extraction was done according to the manufacturer's instructions by the use of DNA extraction by DNA purification kit (Thermo Fisher scientific, California). Prior destruction of the bacterial cell wall of the isolates was done by a lysis solution composed of 20 mM Tris-HCl, 2 mM EDTA, 1.2% Triton X-100, and lysozyme 20 mg/mL at pH 8.0. 2×10^9 bacterial cells were harvested in a 1.5 or 2 mL microcentrifuge tube and centrifuged for 10 mins at 5000 ×g, then the sediment pellet was suspended in 180 µL of Gram-positive bacteria lysis buffer, incubated at 37 °C for 30 min. 200 µL of Lysis Solution and 20 µL of Proteinase K were added and mixed thoroughly by vortexing to obtain a homogenous suspension, and then the sample was incubated at 56 °C during vortexing occasionally until the cells were completely lysed (30 min).

Primers

Oligonucleotide primer sequences used (synthesized by metabion international AG, Germany) were as follows: The 2 oligonucleotide primers 1 and 2 resulting in the amplification of a 491-bp PCR fragments for detection of *aac (6')/aph (2")* gene; Primer 1 (*aac (6')/aph (2") F*): 5′-GAAGTACGCAGAAGAGA-3′. Primer 2 *(aac (6')/aph (2") R)*: 5′-ACATGGCAAGCTCTAGGA-3′. The 2 oligonucleotide primers B1 and B2 resulting in the amplification of a 242 -bp PCR fragments for detection of *aph (3')-IIIa* gene; Primer B1 *(aph (3')-IIIa-F)*: 5′-AAATACCGCTGCGTA-3′. Primer B2 *(aph (3')-IIIa-R)*: 5′--CATACTCTTCCGAGCAA-3′.[13].

PCR

PCR reaction was carried out in the same previously mentioned quantities. Amplification of ***aac(6')-Ie/aph(2")*** gene using the following cycling conditions: 5 min of denaturation at 95 °C. (1 cycle), followed by 35 cycles of amplification; each of heat denaturation at 95 °C for 60 s, primer annealing at 48 °C for 60 s, and DNA extension at 72 °C for 45 s then one cycle for final extension at 72°C for 10 minutes.

The PCR amplification cycling profile ***aph (3')-IIIa*** gene was 5 min of denaturation at 95°C (1 cycle), followed by 35 cycles of amplification; each of heat denaturation at 95 °C for 60 s, primer annealing at 48 °C for 60 s, and DNA extension at 72 °C for 60 s then one cycle for final extension at 72°C for 10 minutes [13]. The amplified target gene was detected by agarose gel electrophoresis *(Electrophoresis power supply-Biometra Whatman company, Germany)*, using 5% agarose stained with ethidium bromide, and visualized under UV transillumination.

RESULTS

Demographics of the studied population presented in (Table 2). The age distribution of patients with HAI caused by *Staphylococcus aureus* was significantly different than patients with CAIs. The mean age ± SD was 39.05 ± 17.85 for HAI cases and 16.25 ± 14.53 for CAI cases (*P value <0.001*).

There was a highly significant difference between the rate of Isolation of Staphylococcal strains from patients with invasive devices and patients without ***(P-value 0.001*)** such as: urinary catheter, infected tracheostomy wound, surgical sutures, infected intravascular devices and surgical drain. The presence or absence of co-morbid conditions didn't significantly affect the rate of isolation of Staphylococcal strains neither from patients with HAI nor patients with CAI ***(P value 0.1).***

Table 2: Comparison between the study groups regarding presence of comorbidities and invasive devices

Variable	Community acquired infection (N=24)	Hospital acquired infection (N=38)	P-value
Invasive device use			
Urinary catheter	0 (0.0%)	10 (26.3%)	**0.001**
Infected tracheostomy wound	0 (0.0%)	7 (18.4%)	
surgical sutures	0 (0.0%)	12 (31.6%)	
infected intravascular devices	0 (0.0%)	4 (10.5%)	
Surgical drain	0 (0.0%)	5 (13.2%)	
No	24 (100%)		
Comorbidities			
Anemia	5 (20.8%)	2 (5.3%)	
Asthma	0 (0.0%)	2 (5.3%)	
Diabetes	2 (8.3%)	1 (2.6%)	0.1
Diabetes, hypertension	1 (4.2%)	4 (10.5%)	
Diabetes, hypertension, asthma	0 (0.0%)	1 (2.6%)	
Hepatic disease	0 (0.0%)	2 (5.3%)	
Hypertension	1 (4.2%)	9 (23.7%)	
Renal disease	0 (0.0%)	1 (2.6%)	
No	15 (62.5%)	16 (42.1%)	

P value was calculated by Chi square test, P-value <0.05 is statistically significant.

On testing the susceptibility pattern of the CAIs strains to the previously mentioned antibiotics it was found that: the highest resistance rate was to Benzylpenicillin and Oxacillin (100%) followed by Tetracycline (58.3%), while the highest sensitivity was to Linezolid (100%) followed by, Vancomycin (87.5%). The antibiotic susceptibility profile of the isolated CA Staphylococcal strains to different aminoglycosides was as follows: (33.3%) were resistant to Amikacin, (20.8%) to Kanamicin and (37.5%) to Gentamicin.

Table 3: The susceptibility pattern of CA isolates to different aminoglycosides (N= 24).

Antibiotics	Sensitive NO. (%)	Intermediate NO. (%)	Resistant NO. (%)
Amikacin	16(66.7%)	0 (0.0%)	8 (33.3%)
Kanamicin	17(70.8%)	2 (8.3%)	5 (20.8%)
Gentamicin	12 (50%)	3 (12.5%)	9 (37.5%)

On other hand, the susceptibility testing of the HA-strains to the same antibiotics revealed that; the highest resistance rate was to Benzylpenicillin and Oxacillin (100%) followed by Tetracycline (84.2%), while the highest sensitivity was to Linezolid (97.4%) followed by, Tigecycline (76.3%). While The antibiotic susceptibility profile of the isolated Staphylococcus aureus strains to aminoglycosides among HAI was as follows: (65.8%) were resistant to Amikacin, (73.7%) to Kanamycin and (71.1%) to Gentamicin.

Table 4: The susceptibility pattern of HA isolates to different aminogycosides (N= 38):

Antibiotics	Sensitive NO. (%)	Intermediate NO. (%)	Resistant NO. (%)
Amikacin	13(34.2%)	0 (0.0%)	25 (65.8%)
Kanamycin	10(26.3%)	0 (0.0%)	28 (73.7%)
Gentamicin	9(23.7%)	2 (5.3%)	27 (71.1%)

Generally, the isolated staphylococcal strains of HAIs were more resistant than strains of CAIs to different antibiotics. The antimicrobial resistance rates were significantly higher in HAI compared to CAI (68.4% vs. 37.5%) for Erythromycin ***(P value 0.04)*** and (39.5% vs. 8.3%) for Quinupristin/dalfopristin ***(P value 0.022)*** and (71.1% vs. 37.5%) for Ciprofloxacin ***(P value 0.033)*** However, resistance rates to Benzylpenicillin and Oxacillin were similar among HAI and CAI (100% vs. 100%, respectively).

Table 5: Comparison between study groups regarding antibiotics sensitivity profile

Antibiotic	Community acquired infection (N=24)	Hospital acquired infection (N=38)	P-value
Benzylpenicillin			
Sensitive	0 (0.0%)	0 (0.0%)	NA
Intermediate	0 (0.0%)	0 (0.0%)	
Resistant	24 (100%)	38 (100%)	
Oxacillin			
Sensitive	0 (0.0%)	0 (0.0%)	NA
Intermediate	0 (0.0%)	0 (0.0%)	
Resistant	24 (100%)	38 (100%)	
Ciprofloxacin			
Sensitive	12 (50%)	9 (23.7%)	0.033
Intermediate	3 (12.5%)	2 (5.3%)	
Resistant	9 (37.5%)	27 (71.1%)	
Levofloxacin			
Sensitive	15 (62.5%)	18 (47.4%)	0.287
Intermediate	3 (12.5%)	3 (7.9%)	
Resistant	6 (25%)	17 (44.7%)	
Moxifloxacin			
Sensitive	14 (58.3%)	22 (57.9%)	0.566
Intermediate	2 (8.3%)	1 (2.6%)	
Resistant	8 (33.4%)	15 (39.5%)	
Erythromycin			
Sensitive	14 (58.3%)	10 (26.3%)	**0.04**
Intermediate	1 (4.2%)	2 (5.3%)	
Resistant	9 (37.5%)	26 (68.4%)	
Clindamycin			
Sensitive	13 (54.2%)	11 (28.9%)	0.137
Intermediate	1 (4.2%)	2 (5.3%)	
Resistant	10 (41.6%)	25(65.8%)	
Quinupristin/ dalfopristin			
Sensitive	20 (83.4%)	22 (57.9%)	**0.022**
Intermediate	2 (8.3%)	1 (2.6%)	
Resistant	2 (8.3%)	15 (39.5%)	
Linezolid			
Sensitive	24 (100%)	37 (97.4%)	1*
Intermediate	0 (0.0%)	0 (0.0%)	
Resistant	0 (0.0%)	1 (2.6%)	
Vancomycin			
Sensitive	21 (87.5%)	27 (71.1%)	0.286
Intermediate	1 (4.2%)	2 (5.3%)	
Resistant	2 (8.3%)	9 (23.7%)	
Tetracycline			
Sensitive	8 (33.4%)	4 (10.5%)	0.064
Intermediate	2 (8.3%)	2 (5.3%)	
Resistant	14 (58.3%)	32 (84.2%)	
Tigecycline			
Sensitive	20 (83.4%)	29 (76.3%)	0.279
Intermediate	2 (8.3%)	1 (2.6%)	
Resistant	2 (8.3%)	8 (21.1%)	
Nitrofurantoin			
Sensitive	18 (75%)	26 (68.4%)	0.304
Intermediate	3 (12.5%)	2 (5.3%)	
Resistant	3 (12.5%)	10 (26.3%)	
Rifampicin			
Sensitive	17 (70.8%)	19 (50%)	0.065
Intermediate	3 (12.5%)	2 (5.3%)	
Resistant	4 (16.7%)	17 (44.7%)	
Trimethoprim/Sulfamethoxazole			
Sensitive	17 (70.8%)	25 (65.8%)	0.611
Intermediate	3 (12.5%)	3 (7.9%)	
Resistant	4 (16.7%)	10 (26.3%)	

P value was calculated by Chi square test, * P value was calculated by Fisher's Exact Test, P value < 0.05 is statistically significant, and NA (not applicable)

As regarding to the presence or absence of the tested genes of aminoglycoside- modifying enzymes; the *aac (6')-Ie/aph (2")* gene was found in 58.3 % of the strains of CAIs and in 68.4% of the strains of HAIs. There is no significant difference between HAIs and CAIs harboring *aac (6')-Ie/aph (2")* gene *(p value 0.419).* While *aph (3)-IIIa* gene was found in 45.8% of the strains of CAIs and in 44.7% of the strains of HAIs. There is no significant difference between HAIs and CAIs harboring *aph (3)-IIIa* gene *(p value 0.933).*

Table 6: Comparison between the study groups regarding AME genes.

AME genes	Community acquired infection (N=24)	Hospital acquired infection (N=38)	P-value
AME genes (aac (6')-Ie/aph (2") No Yes	 10 (41.7%) 14 (58.3%)	 12 (31.6%) 26 (68.4%)	0.419
AME genes (aph (3)-IIIa) No Yes	 13 (54.2%) 11 (45.8%)	 21 (55.3%) 17 (44.7%)	0.933

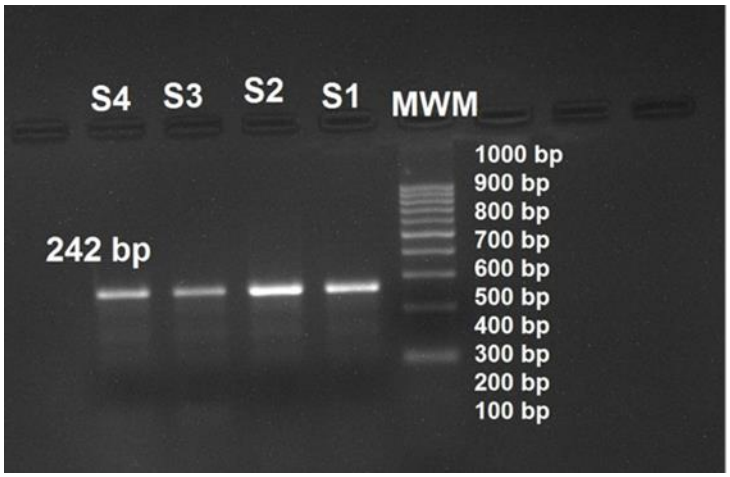


Fig. 2: Agarose gel electrophoresis of PCR products after amplification of *aph (3')-IIIa* gene. Molecular weight marker (100 bp DNA ladder, Biomatik), S1, S2, S3 and S4 of S. aureus positive for *aph (3')-IIIa* gene (*aph (3')-IIIa* gene products at 242 bp).

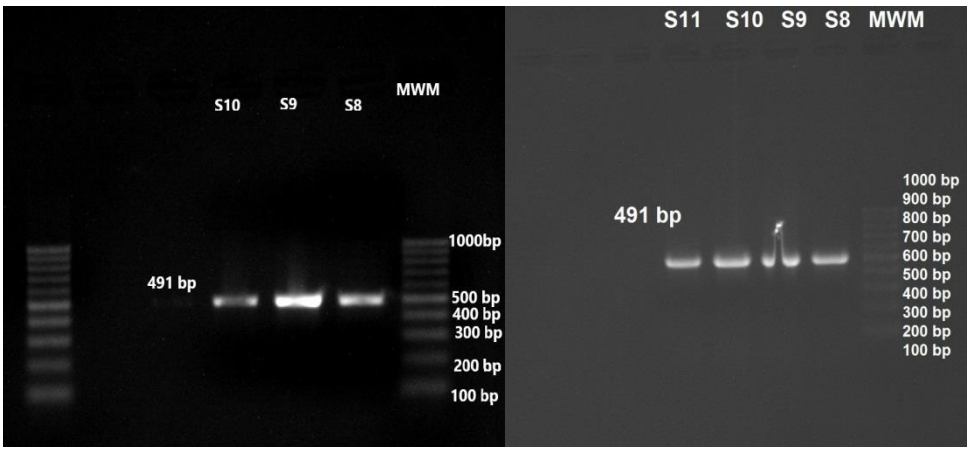


Fig. 3: Agarose gel electrophoresis of PCR products after amplification of *aac (6')/aph (2")* gene. Molecular weight marker (100 bp DNA ladder, Biomatik); S8, S9, S10 and S11 of *S. aureus* positive for *aac (6')/aph (2")* gene at 491 bp.

DISCUSSION

Due to the high prevalence of MRSA, those isolates have aquired resistance to many other antibiotic groups, such as tetracyclines, aminoglycosides, and lincosamides, and become difficult to treat [14]. Aminoglycosides are valuable antibiotics used against a variety of staphylococcal infections; these antibiotics are often used in combination with beta-lactams and glycopeptides to treat infections caused by staphylococci and enterococci [15].

The most important mechanism of aminoglycoside resistance in *S. aureus* is the production of aminoglycoside modifying enzymes [AMEs]. These enzymes, depending on the functional group, can be classified into three groups: AG N-acetyltransferases (AAC), AG O-nucleotidyltransferases (ANT), and oraz AG O-phosphotransferases (APH) [16]. Respectively to acetylate, adenylate, or phosphorylate the orginal aminoglycosides have much lower affinity with the target site of action on bacterial ribosome.

In this study 150 samples were collected from patients with different types of infections admitted at different departments and outpatients at Sohag University Hospitals; (36) samples from Chest department representing (24%) of all collected samples, (27) samples from General surgery department (18%) of all collected samples, (22) from ICU representing (14.7%), (31) from General Surgery clinic representing (20.7%), (29) from Dermatology clinic representing (19.3%) of all collected samples and (5) samples from Vascular surgery department representing (3.3%).

In our study, the highest resistance rate was to Benzylpenicillin and Oxacillin (100%) followed by Tetracycline (58.3%), while the highest sensitivity was to Linezolid (100%) followed by, Vancomycin (87.5%). While among HAI strains it was found that: the highest resistance rate was to Benzylpenicillin and Oxacillin (100%) followed by Tetracycline (84.2%), while the highest sensitivity was to Linezolid (97.4%) followed by, Tigecycline (76.3%).The antimicrobial resistance rates were significantly higher in HAI compared to CAI (68.4% vs. 37.5%) for Erythromycin and (39.5% vs. 8.3%) for Quinupristin/ dalfopristin and (71.1% vs. 37.5%) for Ciprofloxacin.

Resistance to penicillin and oxacillin were 100% and resistance to Linezolid was 2.6 % among HAI and 0 % among CAI and these results are similar to that of the study of Sekawi et al. [18]. Resistance to Clindamycin, Rifampicin and Tetracycline were higher in HAI than CAI which is similar to that of the study of Sekawi et al.[19]. Resistance to Erythromycin (51.6%), Clindamycin (29%) and rifampin (17.7%) were lower in the study of Sekawi et al.[18] than our study which were (68.4%, 65.8% and 44.7%) respectively. Resistance to Erythromycin (68.4%) and to Clindamycin (65.8%) among HAI which is similar to the results of the study of Mahdiyoun et al.[19]. Resistance to Ciprofloxacin was (36.8%) among HAI which is lower than the results of the study of Mahdiyoun et al. [19]. Possible reasons for the variety of antibiotic resistance rates in the different studies was not understood, but it may reflect the amount of antibiotics used in various settings.

Resistance to Linezolid was (2.6%), Tigecycline (21.1%), Vancomycin (23.7%). These results showed that these antibiotics, especially Linezolid, are highly effective against staphylococcal infections.

The antibiotic susceptibility profile of the isolated *Staphylococcus aureus* strains to aminoglycosides among HAI was as follows: (65.8%) were resistant to Amikacin, (73.7%) to Kanamycin and (71.1%) to Gentamicin. These results were close to that of the study of Choi et al. [13] except for resistance to Amikacin which was higher in our study.

The antibiotic susceptibility profile of the isolated *Staphylococcus aureus* strains to aminoglycosides among CAI was as follows: (33.3%) were resistant to Amikacin, (20.8%) to Kanamycin and (37.5%) to Gentamicin which is lower than the results of the study of Sekawi al. [18].

The MIC values obtained from the isolated *Staphylococcus aureus* were in the following ranges: for Gentamicin, 0.5-64 mg/L, for Amikacin , 4-64 mg/L, and for Kanamycin 4-64 mg/L in our study while the ranges were: for Gentamicin, 0.25-256 mg/L, for Amikacin 2-256 mg/L in the study of Szymanek-Majchrzak et al.[11].

In this study we used simple qualitative PCR for detection of AME genes (*aac (6')-Ie/aph (2")* and *aph (3)-IIIa*) in the staphylococcal isolates that are resistant to aminoglycosides. The aac (6')-Ie/aph (2") gene was found in 68.4% of the strains of HAIs while aph (3)-IIIa gene was found in 44.7% of the strains of HAIs which were similar to the results of the study of Khosravi et al. [20] which were (64% and 42.2% respectively). These results were also similar to the results of Sorour et al. [21] in which aph (3)-IIIa gene was found in 45.8% of the isolates.

Our results were lower than the results reported by Fatholahzadeha et al., 2009 in which aac (6')-Ie/aph (2") gene was found in 83% of the strains of HAIs while aph (3)-IIIa gene was found in 71% of the strains of HAIs and they were also lower than the results of Choi et al. [13] which reported that aac (6')-Ie/aph (2") gene was found in 65% of the strains of HAIs while aph (3)-IIIa gene was found in 9% of the strains of HAIs. The change in the prevalence of genes encoding AME may be due to changes in antibiotic policy and the type of used aminoglycoside, the introduction and consequent inter-hospital spread of resistant strains, especially MRSA or the possibility that these resistance genes could be originated from an environmental source.

CONCLUSION

In this study a high level of resistance of *Staphylococcus aureus* to multiple classes of antibiotics and upward trend in emergence of CAI with *Staphylococcus aureus* strains as well as in hospitals was observed. This has become a threat to public health. In order to prevent transmission of these community acquired strains, accurate and rapid monitoring techniques should be administered in both clinical and community settings with the wise use of antibiotics and avoid unnecessary treatment with broad spectrum antibiotics for *Staphylococcus aureus* without antibiotic sensitivity testing.

It's important to reduce the spread of HAI by strict infection control measures as hand hygiene practice, proper sterilization of the equipment, cleaning hospital environment and regular surveillance for resistant strains of *Staphylococcus aureus* with isolation of infected patients to prevent transmission of infection.It is important to control development of antibiotic resistance by monitoring potential developing of new aminoglycoside resistant genes that may be produced within *S. aureus* population. This will help to establish effective antibiotic therapies and prevent nosocomial infection as well as environmental spread of resistant strains.

PCR assays allow for the faster establishment of effective antibiotic therapies, and will lead to improved therapeutic success and reduced empirical treatments with broad-spectrum antibiotics, which are costly and have high toxicities, and eventually slow potential development of antibiotic resistant organisms. In terms of infection control programs, such rapid detection of resistance could be used to prevent nosocomial spread of MRSA in advance.

Conflicts of interest:

The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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