INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

MOLECULAR CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATES RECOVERED FROM CHILDREN WITH OTITIS MEDIA IN ALEXANDRIA HOSPITALS, EGYPT

Tarek El-Said El-Banna [1], Fatma Ibrahim Sonbol [1], Abeer A. Ghazal [2], Maisra Mohammed El-Bouseary [1], Hebatallah Osama Ellabody *[3]
[1] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Tanta University, Tanta, Egypt
[2] Department of Microbiology, Medical Research Institute, Alexandria University, Alexandria, Egypt
[3] Department of Microbiology, Faculty of Pharmacy and Drug Manufacturing, Pharos University in Alexandria, Alexandria, Egypt
*Corresponding Author Email: hebatallah.ellabody@pua.edu.eg

Article Received on: 05/03/20 Approved for publication: 02/04/20

DOI: 10.7897/2230-8407.110439

ABSTRACT

Clinical infections caused by *Staphylococcus aureus* (*S. aureus*) will likely remain common and serious. Not only there has been an increase in antimicrobial resistance, but also the spectrum of clinical disease continues to increase. Our aim in this research is to determine the prevalence as well as to identify and characterize the pattern of resistance of *S. aureus* isolates recovered from pediatrics with otitis media admitted to tertiary hospitals in Alexandria, Egypt. Out of 180 clinical samples, 193 different bacterial isolates were recovered. Along with *Pseudomonas aeruginosa, S. aureus* was the predominant (18.6%) pathogen isolated. Antibiotic susceptibility testing among *S. aureus* isolates revealed that up to 27 (75%) out of the tested isolates exhibited high (> 0.2) MAR index values. Methicillin-resistant *S. aureus* (MRSA) isolates were determined using the minimal inhibitory concentration (MIC) of cefoxitin. The incidence of MRSA among *S. aureus* isolates was 44.4%. On the other hand, none of the tested isolates was linezolid resistant. The presence of methicillin resistance genes (*mec*A and *fem*A) and aminoglycoside resistance genes (*aac (6')Ie/aph(2'')Ia*, *aph(3')-IIIa* and *ant(4')-Ia*) were detected using Polymerase Chain Reaction (PCR) technique. The incidence of *aac (6')Ie/aph(2'')Ia*, *aph(3')-IIIa* and *ant (4')-Ia* was 38.9%, 36.1% and 30.6%, respectively. Besides, the incidence of *cap*8, *cap*5 and *lukS/F-PV* virulence genes was 33.3%, 25% and 11.1%, respectively. Accordingly, we recommend the necessity of continuous surveillance and control of antimicrobial resistance and virulence level in *S. aureus* as one of the major pathogens of pediatric otitis media in Alexandria, Egypt.

Keywords: Otitis media, *S. aureus*, MRSA, AMEs, capsular polysaccharide, *lukS/F-PV*, Egypt.

INTRODUCTION

Otitis media is an inflammation of the mucous membrane of the middle ear which includes the tympanic cavity, mastoid antrum, mastoid air cells and the eustachian tube. It is characterized by the accumulation of discharge from the ear through a perforation in the tympanic membrane causing suppurative otitis media that is accompanied by bulging of the eardrum and pain in the ear[1]. In childhood, otitis media is a leading cause of health care visits worldwide, as it is the second most common clinical problem after an upper respiratory infection. Different types of otitis media can be manifested including acute otitis media, otitis media with effusion and chronic suppurative otitis media. Otitis media is a multi-factorial disease, with many associated risk factors, including microbial agents, anatomic factors, host characteristics; relating to an immature or defective immunologic status, environmental risk factors and social factors. Bacterial and viral infections are the most important cause of otitis media. Common bacteria isolated from the middle ear fluid includes *S. aureus*, *Streptococcus pyogenes* or Group A *Streptococcus*, *S. epidermidis*, *Pseudomonas aeruginosa*, *Alloiococcus otitidis*, *Escherichia coli* and *Klebsiella pneumonia.*[2]

S. aureus is a Gram-positive bacterium that can cause a wide variety of infections, which range from superficial lesions to a systemic and life-threatening conditions[3,4]. Penicillin was the first-line treatment of *S. aureus* infections. Later on, β-lactamases producing *S. aureus* were identified and as a result, penicillin became inactive against 50% of *S. aureus* infections[5]. Methicillin-resistant *Staphylococcus aureus* (MRSA) causes rapidly progressive, potentially fatal diseases, such as life-threatening pneumonia, necrotizing fasciitis, endocarditis, osteomyelitis, severe sepsis, toxinosis such as toxic shock syndrome and soft tissue infections, including nasal and ear infections[6]. Numerous risk factors contribute to the emergence of MRSA causing ear and sinonasal infections. The major factors are previous nasal surgeries and widespread use of broad-spectrum antibiotics[7].

Aminoglycosides are a class of antibiotics that have a great role in the treatment of *staphylococcal* infections[8]. The resistance of aminoglycosides includes many forms, the main mechanism in *S. aureus* is the inactivation by aminoglycoside-modifying enzymes (AMEs)[8,9]. AMEs are classified into three main groups consistent with their ability to phosphorylate, acetylate or adenylate amino or hydroxyl groups on aminoglycosides structure resulting in a decrease in the binding affinity to its target resulting in loss of its antibacterial efficiency. The most important genes encoded for AMEs are the *aac(6')-Ie + aph(2'')*, encoding aminoglycoside-6'-N-acetyltransferase/2"-O-phosphoryltransferase AAC (6')-Ie + APH (2''), *ant(4')-Ia*, encoding aminoglycoside-4'-O-nucleotidyltransferase I ANT (4')-Ia and *aph(3')-IIIa*, encoding

aminoglycoside-3'-O- phosphoryl transferase III APH (3')-IIIa. In *S. aureus,* AAC (6') and APH (2") is a bi-functional enzyme which is the most common enzyme responsible for the resistance to gentamicin, kanamycin and tobramycin[8,9].

S. aureus produces several virulence factors including capsular polysaccharides (CP) which protect it from complement binding and subsequent phagocytic killing by neutrophils[10,11]. On the contrary, anti-capsular antibodies released by the host can opsonize the pathogen, augmenting the host immune defense mechanisms[10,12]. More than 90% of *S. aureus* harbored CP with 11 different serological types (CP1 to CP11). CP5 and CP8 are the predominant types among *S. aureus* isolates[13–15]. Both polysaccharides consist of similar trisaccharide repeat units of *N*-acetyl mannosaminuronic acid (ManNAc), *N*- acetyl-L-fucosamine (L-FucNAc), and *N*-acetyl-D-fucosamine (D-FucNAc) but differ in the linkages between the sugars and the sites of O-acetylation on the monosaccharide units[10]. In addition, Panton-Valentine leucocidin (PVL) is a bi component leukocidin encoded by two co transcribed genes, namely, *lukS-PV* and *lukF-PV* (*lukS*/*F-PV*), which reside on a prophage and cause leukocyte destruction and tissue necrosis. The presence of PVL in *S. aureus* appears to be associated with increased disease severity, ranging from a cutaneous infection requiring surgical drainage to severe chronic diseases[16]. This research aims to phenotypically and genotypically characterize *S. aureus* isolates from pediatrics suffering from otitis media in Alexandria hospitals, Egypt.

MATERIAL AND METHODS

Sample collection and bacterial identification

A total of 36 *S. aureus* isolates were recovered from 180 otitis media clinical samples recovered from pediatrics with otitis media admitted to tertiary hospitals in Alexandria, Egypt. Bacterial identification was performed using standard morphological and biochemical techniques.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer method according to the guidelines from Clinical and Laboratory Standards Institute (CLSI) 2017[17]. The antibiotics tested included penicillin (P), cefoxitin (FOX), gentamicin (CN), tobramycin (TOB), amikacin (AK), kanamycin (K), tetracycline (TE), azithromycin (AZM), erythromycin (E), linezolid (LZD), chloramphenicol (C), ciprofloxacin (CIP), levofloxacin (LEV) and sulfamethoxazole-trimethoprim (SXT). All antibiotic discs were obtained from Oxoid®, UK. *S. aureus* isolates showing cefoxitin MIC values > 8 µg/ml were identified as MRSA using broth microdilution test and confirmed by the appearance of the intense mauve color of produced colonies on chromogenic MRSA agar (Oxoid®).

Multiple antibiotic resistance (MAR) index studies in *S. aureus* isolates[18]

MAR index values for each isolate were calculated using the following formula:

MAR index for certain isolate =
number of antibiotics to which the isolate was resistant / total number of antibiotics to which the isolate was exposed

Genotypic detection of resistance and virulence genes among *S. aureus* isolates using PCR technique

DNA extraction and gel electrophoresis procedure

Genomic DNA was extracted from *S. aureus* isolates by the boiling method as described by Rahimi (2009)[19]. PCR reaction mixture was prepared as mentioned by Lorenz[20]. Thermal cycler conditions were carried out as described in other reports with specific primers. The amplified products were loaded on 2% agarose gel along with 100 bp DNA ladder (Gene Direx, USA) and electrophoresed at 100 volts for 45 min using Mupid-exU System gel electrophoresis equipment (ADVANCE). Ethidium bromide-stained bands were visualized under UV transilluminator[20].

Detection of antibiotic resistance genes

All *S. aureus* isolates were tested for *mec*A and *fem*A by multiplex PCR. The primers used to amplify each of the genes were (*mec*A-F): CTGGAACTTGTTGAGCAGAG, (*mec*A-R): TGGCTATCGTGTCACAATCG, (*fem*A-F): CTTACTTACTGCTGTACCTG and (*fem*A-R): ATCTCGCTTGTTATGTGC[21]. Also, aminoglycoside resistance among tested isolates was confirmed by the detection of AMEs. Multiplex PCR was used for amplification of *aac(6')Ie/aph(2")Ia* and *ant(4')-Ia* genes. The primers used to amplify *aac(6')Ie/aph(2")Ia* were *(aac(6')Ie/aph(2")Ia*-F): CAGAGCCTTGGGAAGATGAAG and *(aac(6')Ie/aph(2")Ia*-R): CCTCGTGTAATTCATGTTCTGGC[22]. While, the primers used for *ant(4')-Ia* gene were designed by primer BLAST as following, (*ant(4')-Ia*-F)*:* CGGTGAGTGGAAGGTGGAAG and (*ant(4')-Ia*-R): GCACAAATCGCATCGTGGAA. Conventional PCR was used for amplification of *aph(3')-IIIa* gene, the primers used for amplification of this gene were (*aph (3')-IIIa*-F): CTGATCGAAAAATACCGCTGC and (*aph (3')-IIIa*-R): TCATACTCTTCCGAGCAAAGG[23].

Detection of *S. aureus* virulence factors: Capsular polysaccharides *cap*5 and *cap*8 were detected in all *S. aureus* isolates by multiplex PCR while, *lukS*/*F-PV* gene was detected by conventional PCR. The primers used to amplify *cap*5 and *cap*8 genes were (*cap*5-F): GAAAGTGAACGATTAGTAGAA, (*cap*5-R): GTACGAAGCGTTTTGATAGTT, (*cap*8-F): GTGGGATTTTTGTAGCTTTT and (*cap*8-R): CGCCTCGCTATATGAACTAT[24]. While, the primers used for amplification of *lukS*/*F-PV* genes were (*lukS*/*F-PV*-F): ATCATTAGGTAAAATGTCTGGACATGATCCA and (*lukS*/*F-PV*-R): ATCATTAGGTAAAATGTCTGGACATGATCCA[16].

RESULTS

A total of 193 bacterial isolates were collected from 180 pediatrics suffering from otitis media. Fifty-two isolates were identified as *Staphylococci* by catalase test and microscopical examination. Among these, 36 isolates were identified as *S. aureus* by tube coagulase test and 35 out of these were mannitol fermenters.

The susceptibility of *S. aureus* isolates to various agents is shown in Table 1. Penicillin showed the highest resistance (91.7%) followed by kanamycin (80.6%) and tobramycin (55.6%). On the other hand, none of the tested isolates was linezolid resistant. Up to 31 different antibiotic resistance patterns were observed among 36 *S. aureus* isolates as shown in Table 2. Determination of MAR index values of bacterial isolates revealed that 27 (75%) out of the tested isolates exhibited a high (>0.2) MAR index value. A

total of 16 (44.4%) *S. aureus* isolates showing cefoxitin MIC ≥ 8 µg/ml and intense mauve color of produced colonies on chromogenic MRSA agar were recorded as MRSA.

PCR results revealed the presence of *mec*A and *fem*A genes in 47.2% and 25% of the isolates respectively (Figure 1). All 16 MRSA isolates with MIC ≥ 8 µg/ml were also *mec*A positive, on the other hand only one isolate with MIC < 8 µg/ml harbored *mec*A gene. Genes encoding AMEs including *aac(6')Ie/aph(2'')Ia* (38.9%), *ant(4')-Ia* (30.6%) and *aph(3')-IIIa* (36.1%) regardless of gene combinations were detected in 28/36 isolates (77.8%) (Figures 2 and 3). Twenty-eight isolates were positive for at least one AME gene. Interestingly two phenotypically resistant isolates were negative for all aminoglycosides resistant genes (Table 3). None of the tested aminoglycosides resistant genes were detected in 8 isolates, 6 isolates of them were aminoglycoside sensitive phenotypically. Virulence genes: *cap*5, *cap*8 and *lukS*/*F-PV* were present in 25%, 33.3% and 11.1% of the *S. aureus* isolates, respectively (Figures 4 and 5). Each of the tested isolates harbored one of these virulence genes however, one isolate was positive for both *cap*5 and *cap*8 genes. Two isolates produced amplicons of the expected sizes for both *cap*5 and *lukS*/*F-PV*.

Table 1: The percentage of resistance of the recovered *S. aureus* isolates against tested antibiotics

Class of Antibiotic	Antibiotic (µg/disc.)	Percentage of Resistance
β lactams	Penicillin (10 µg)	91.7%
	Cefoxitin (30 µg)	55.6%
Aminoglycosides	Gentamicin (10 µg)	50%
	Tobramycin (10 µg)	52.8%
	Amikacin (30 µg)	41.7%
	Kanamycin (30 µg)	80.6%
Tetracyclines	Tetracycline (30 µg)	44.4%
Macrolides	Azithromycin (15 µg)	22.2%
	Erythromycin (15 µg)	33.3%
Oxazolidinones	Linezolid (30 µg)	0%
Chloramphenicol	Chloramphenicol (30 µg)	5.6%
Quinolones	Ciprofloxacin (5 µg)	22.2%
	Levofloxacin (5 µg)	19.4%
Folates	Sulphamethoxazole/ Trimethoprim (25 µg)	13.9%

Table 2: Antimicrobial resistance patterns of *S. aureus* isolates

Pattern code	Antimicrobial resistance pattern*	No. (%) of isolates	MAR index for isolates
PI a	P-FOX-E-AZM-AK-K-TOB-CN-CIP-LEV-SXT	1 (2.8)	0.78
PI b	P-FOX-TE-E-AK-K-TOB-CN-CIP-LEV-SXT	1 (2.8)	0.78
PII	P-FOX-E-AK-K-TOB-CN-CIP-LEV-SXT	1 (2.8)	0.71
PIII a	P-FOX-TE-AK-K-TOB-CN-CIP-LEV	1 (2.8)	0.64
PIII b	P-TE-E-AZM-C-K-TOB-CIP-LEV	1 (2.8)	0.64
PIII c	P-FOX-TE-E-AZM-K-CN-CIP-LEV	1 (2.8)	0.64
PIV a	P-FOX-TE-E-AK-K-TOB-CN	1 (2.8)	0.57
PIV b	P-AZM-AK-K-TOB-CN-CIP-LEV	1 (2.8)	0.57
PV a	P-FOX-TE-AK-K-TOB-CN	4 (11.1)	0.5
PV b	P-FOX-E-AZM-K-TOB-CN	1 (2.8)	0.5
PV c	P-FOX-TE-K-TOB-CN-CIP	1 (2.8)	0.5
PV d	P-TE-E-AZM-K-TOB-CN	1 (2.8)	0.5
PVI a	P-FOX-TE-K-TOB-CN	1 (2.8)	0.42
PVI b	P-TE-AK-K-TOB-CN	1 (2.8)	0.42
PVII a	P-TE-AK-K-TOB	1 (2.8)	0.35
PVII b	P-E-AZM-AK-K	1 (2.8)	0.35
PVII c	P-K-TOB-CN-SXT	1 (2.8)	0.35
PVIII	AK-K-TOB-CN	1 (2.8)	0.64
PIX a	P-TE-K	1 (2.8)	0.21
PIX b	P-FOX-TE	1 (2.8)	0.21
PIX c	P-FOX-K	1 (2.8)	0.21
PIX d	P-E-AZM	1 (2.8)	0.21
PIX e	P-AZM-K	1 (2.8)	0.21
PIX f	AK-K-CN	1 (2.8)	0.21
PX a	P-FOX	1 (2.8)	0.14
PX b	P-SXT	1 (2.8)	0.14
PX c	P-K	3 (8.3)	0.14
PX d	P-C	1 (2.8)	0.14
PX e	P-E	1 (2.8)	0.14
PXI a	P	1 (2.8)	0.07
PXI b	K	1 (2.8)	0.07

*P: penicillin, FOX: cefoxitin, E: Erythromycin, AZM: azithromycin, AK: amikacin, K: kanamycin, TOB: tobramycin, CN: gentamicin, CIP: ciprofloxacin, LEV: levofloxacin, SXT: sulfamethoxazole/ trimethoprim, TE: tetracycline, C: chloramphenicol.

Table 3: Frequency of Aminoglycoside resistance genes among the tested *S. aureus* isolates

Pattern code	Aminoglycoside resistance genes			Phenotype*	No. (%) of samples
	aac(6')Ie/aph(2'')Ia	*an(4')-Ia*	*aph(3')-IIIa*		
1	+	+	+	AK, K, TOB, CN	2 (5. 6%)
2	+	+	−	K, CN	1 (2.8%)
3a	+	−	−	AK, K, TOB, CN	3 (8.3%)
3b	+	−	−	K, TOB, CN	3 (8.3%)
3c	+	−	−	K	1 (2.8%)
4	−	+	+	AK, K, TOB, CN	1 (2.8%)
5a	−	+	−	AK, K, TOB	1 (2.8%)
5b	−	+	−	K, TOB, CN	1 (2.8%)
5c	−	+	−	AK, K	1 (2.8%)
5d	−	+	−	K, TOB	1 (2.8%)
5e	−	+	−	K	1 (2.8%)
5f	−	+	−	−	2 (5.6%)
6a	−	−	+	K, TOB, CN	1 (2.8%)
6b	−	−	+	AK, K	1 (2.8%)
6c	−	−	+	K	3 (8.3%)
6d	−	−	+	−	1 (2.8%)
7	+	−	+	AK, K, TOB, CN	4 (11.1%)
8a	−	−	−	AK, K, TOB, CN	2 (5.6%)
8b	−	−	−	−	6 (16.7%)

*AK: amikacin, K: kanamycin, TOB: tobramycin, CN: gentamicin

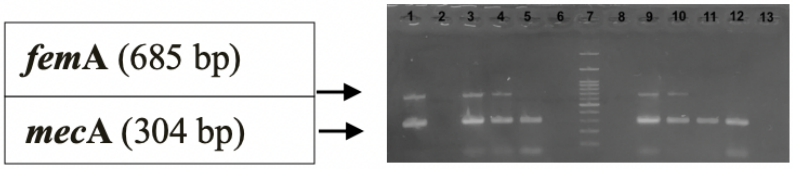

Figure 1: **Multiplex PCR-generated DNA of *mec*A and *fem*A genes in *S. aureus* isolates Lane 1, 3, 4, 9, 10: *mec*A and *fem*A; lane 5, 11, 12: *mec*A only; lane 13: negative control and lane 7: 100 bp DNA ladder**

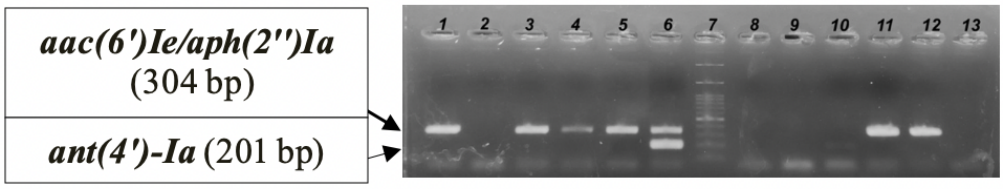

Figure 2: Multiplex PCR-generated DNA of *aac(6')Ie/aph(2'')Ia* and *ant(4')-Ia* genes in *S. aureus* isolates. Lane 1, 3, 4, 5, 11 and 12: *aac(6')Ie/aph(2'')Ia*; lane 6: both *aac(6')Ie/aph(2'')Ia* and *ant(4')-Ia*; lane 13: negative control and lane 7: 100 bp DNA ladder

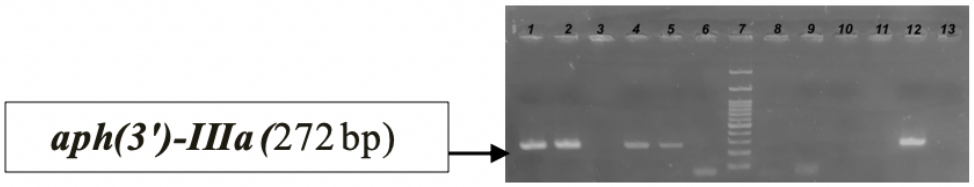

Figure 3: Conventional PCR-generated DNA of *aph(3')-IIIa* gene in *S. aureus* isolates. Lane 1, 2, 4, 5 and 12: *aph(3')-IIIa*; lane 13: negative control and lane 7: 100 bp DNA ladder

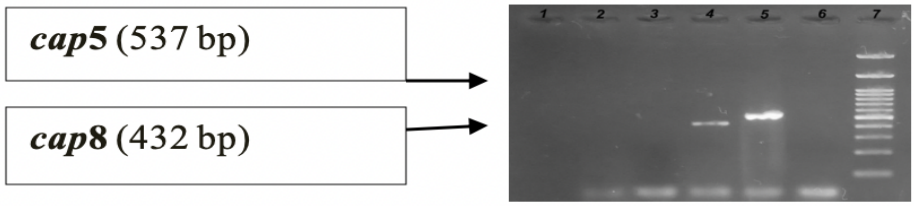

Figure 4: Multiplex PCR-generated DNA of Capsular Polysaccharides *cap*5 and *cap*8 genes in *S. aureus* isolates. Lane 4: *cap*8; Lane 5: *cap*5; Lane 1: negative control and lane 7: 100 bp DNA ladder

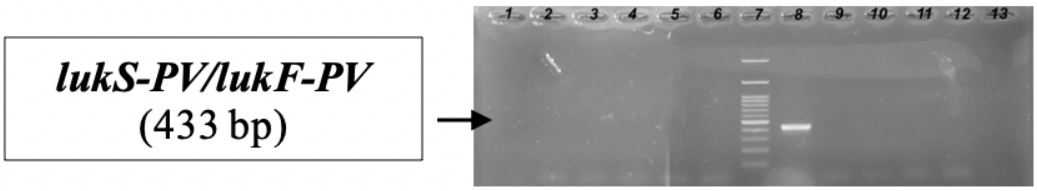

Figure 5: Conventional PCR-generated DNA of Panton-Valentine leucocidin encoding *lukS-PV/lukF-PV* gene in *S. aureus* isolates. Lane 8: encoding *lukS-PV/lukF-PV*; lane 13: negative control and lane 7: 100 bp DNA ladder

DISCUSSION

Otitis media infection is one of the most common infections in pediatrics and a major cause of childhood use of antibiotics[25]. In most of the developing countries, no available guidelines are concerning the use of antibiotic treatment in otitis media[26]. *S. aureus* is considered as the predominant bacterial cause of otitis media and shows a global concern in resistance to the majority of available treatment options[5]. In the present study, the prevalence of *S. aureus* isolates recovered from children suffering from otitis media was investigated in Alexandria inpatients' and outpatients' ENT clinics. In this study 35/36 *S. aureus* isolates (97.2%) were mannitol positive. Similarly, the presence of mannitol negative *S. aureus* was also reported by Danielle Caldeira in Brazil among 15% of his *S. aureus* isolates[27]. Because of genetic variation, some coagulase-positive *S. aureus* lack the ability to ferment mannitol[28]. Regarding antibiotic resistance results, it was found that, penicillin showed the highest antibacterial resistance (91.7%) followed by kanamycin (80.6%) and tobramycin (55.6%). Our results were comparable with the findings reported in other studies performed in several countries including Egypt[29,30]. A study in the Ismailia governorate showed that 90% of *S. aureus* isolates were resistant to penicillin[29]. These findings indicate that these antibiotics are no longer effective against *S. aureus* infections in Egypt. Our tested *S. aureus* isolates showed high susceptibility rates against linezolid and chloramphenicol (100% and 94.4% respectively), these results were consistent with the findings reported in other studies[30,31]. Chloramphenicol susceptibility indicated that routine exposure of bacteria to newly developed antibiotics cause a reversal of susceptibility to outdated antibiotics. Moreover, Linezolid became one of the most important antibiotics worldwide in the treatment of *S. aureus* infections[5]. However, Linezolid must be used with caution to prevent future resistance as *S. aureus* isolate resistant to linezolid was firstly discovered in the United states in July 2001[32]. Another study conducted in Pakistan in 2017 showed that the incidence of MRSA and MSSA resistance to linezolid was 48.1% and 29.2% respectively[33].

Methicillin-resistant *S. aureus* (MRSA) is responsible for a great number of antibiotic-resistant infections worldwide[34,35]. In the present study, 16 (44.4%) MRSA isolates were detected using chromogenic MRSA agar media and confirmed using the cefoxitin broth microdilution method with MIC ranging from 8 to 256 µg/ml. Moreover, MRSA isolates were confirmed by the detection of *mec*A and *fem*A genes using multiplex PCR. The *mec*A gene was detected in 17 isolates (47.2%). Similarly, another study performed in Cairo, Egypt revealed that 43.97% of *S. aureus* were found to be MRSA[36]. A controversy was obvious where a higher incidence of MRSA in Menoufia (73.5%)[37] and a lower rate (24%) was found in Minia were reported among recovered *S. aureus* isolates[38]. Another study in Tanta, Egypt performed on otitis media patients reported a lower percentage of MRSA (25.6%) among their isolates[39]. The variation in the incidence of MRSA concerning Egypt could be due to different study designs, different populations, geographical locations and the quality of hospital sampling carried out[9].

Aminoglycosides have good activity against *S. aureus*, including methicillin-resistant, vancomycin-intermediate and resistant isolates. Gentamicin is known to be one of the most common antibiotics used worldwide for the treatment of *S. aureus* infections in combination with other antibiotics[40]. Concerning aminoglycosides resistance, it was found that 50%, 41.7%, 80.6% and 52.9% of our tested *S. aureus* isolates were resistant to gentamicin, amikacin, kanamycin and tobramycin respectively. A study in Iran showed a higher resistance percentage of MRSA isolates against amikacin, kanamycin, gentamycin and tobramycin where, 77.6%, 86.3%, 84.5% and 82% respectively were resistant[41]. Another study in Egypt revealed that 30% and 90% of MRSA isolates were resistant to gentamicin and kanamycin respectively[42]. Ameen demonstrated that 27% of *S. aureus* isolates showed resistance to gentamicin[29].

PCR is a reliable tool for the identification of AME genes in *S. aureus*[23]. Consequently the incidence of *aac(6')Ie/aph(2'')*, *ant(4')-Ia* and *aph(3')-IIIa* resistant genes was 38.9%, 30.6% and 36.1%, respectively among *S. aureus* isolates. A study in Iran[8] showed that *aac(6')Ie/aph(2'')* was the most predominant AME gene among MRSA isolates (45.2%) followed by *aph(3')-IIIa* (19%) and *ant(4')-Ia* (14.3%). It was found that *aac(6')Ie/aph(2'')Ia* gene was dominant, which is consistent with other studies[8,41,43]. On the contrary to our results, a study in Japan reported a higher prevalence of *ant(4')-Ia* gene (84.5%) than that of the other two AME genes[44]. The difference between these reported results from different geographical could be due to differences among the isolates, dissimilar study design and different antibiotic treatment strategies.

In the present study, six isolates were susceptible to all the tested aminoglycosides antibiotics and were also negative for all of the AMEs genes. Moreover, all isolates positive for *aph(3')-IIIa* gene were resistant to kanamycin except for one isolate which was susceptible to all tested aminoglycosides. On the other hand, two aminoglycoside susceptible isolates harbored the *ant(4')-Ia* gene. Contrary, the three AMEs genes were absent in two isolates (5.6%) although they were phenotypically resistant to all aminoglycosides. Another finding was that 12 MRSA isolates (75%) harbored one or more of aminoglycosides resistance genes except one isolate which was susceptible to all tested aminoglycosides, but harbored the *ant(4')-Ia* gene. According to a justification mentioned by researchers in Poland[45], the failure to detect the three AMEs genes in two isolates which demonstrate phenotypic resistance against aminoglycosides may be due to either the presence of variant AME genes that cannot be detected with the primers used or the possible presence of a new aminoglycoside resistance gene among the *S. aureus* population. On the other hand, the detection of resistance genes in antibiotic susceptible isolates may be due to the amplification of repressed antibiotic resistance genes or AME of these strains display a relatively lower enzymatic activity.

Several types of virulence factors were produced by *S. aureus* strains, capsular polysaccharides (CP) types 5 and 8 are the prevalent types of CP among *S. aureus* isolates[13–15]. Therefore, in our study all *S. aureus* isolates were characterized by the detection of *cap*5 and *cap*8 specific genes. The results showed that *cap*8 was more predominant than *cap*5*;* this finding was consistent with another study in Western Australia which reported a higher percentage of *cap*8 than *cap*5 in *S. aureus* isolates (45.2% and 38.7% respectively)[11]. Another study in Nigeria showed that *cap*8 and *cap*5 were present in 94% and 6% of *S. aureus* isolates respectively[46]. On the other hand, the incidence of *lukS-PV/lukF-PV* was found to be 11.1% in *S. aureus* isolates. Comparing to another study in Nigeria it was found that the level of *lukS-PV/lukF-PV* was higher than our findings (80.2%)[46]. Another study in the USA reported that 33% of *S. aureus* isolates harbored *lukS-PV/lukF-PVL* gene[47].

CONCLUSION

This study highlights the prevalence of *S. aureus* among pediatrics with otitis media admitted to ENT clinics, Alexandria, Egypt. The tested isolates exhibited multidrug resistance with significant heterogenous resistance profiles. MRSA was confirmed in 44.4% of the tested *S. aureus* isolates. The presence of *mec*A gene was confirmed in all phenotypically methicillin-resistant isolates. The *aac(6')Ie/aph(2'')Ia* might be considered the most common gene responsible for aminoglycosides resistance among *S. aureus* isolates. Moreover, *cap*8 is found to be the most common virulence factor responsible for otitis media. Further investigations are essential to develop novel antibacterial and/or anti virulence drugs against *S. aureus* isolate harboring resistance as well as virulence genes aiming to minimize the incidence or indeed prevent otitis media pathogenesis and its long-term consequences for pediatrics all over the world. Moreover, the cautious use of antibiotics in many infections for improving patients' quality of life without compromising their health.

REFERENCES

1. Ilechukwu GC, Ilechukwu CGA, Ubesie AC, Ojinnaka CN, Emechebe GO, Iloh KK. Otitis Media in Children: Review Article. Open Journal of Pediatrics 2014; 4(1): 47–53.
2. Ngo CC, Massa HM, Thornton RB, Cripps AW. Predominant bacteria detected from the middle ear fluid of children experiencing otitis media: A systematic review. PLoS one 2016; 11(3): e0150949.
3. El-banna TE, Sonbol FI, El-aziz AAA. Characterization of Vancomycin Resistant *Staphylococcus aureus* in Tanta University Hospital. International Journal of Current Microbiology and Applied Sciences 2015; 4(10): 1–11.
4. Bien J, Sokolova O, Bozko P. Characterization of Virulence Factors of *Staphylococcus aureus*: Novel Function of Known Virulence Factors That Are Implicated in Activation of Airway Epithelial Proinflammatory Response. Journal of Pathogens 2011; 2011: 601905.
5. J. Fishovitz, J.A. Hermoso, M. Chang SM. Penicillin-Binding Protein 2a of Methicillin-Resistant *Staphylococcus aureus*. National institute of health 2014; 66(8): 572–577.
6. Garoy EY, Gebreab YB, Achila OO, Tekeste DG, Kesete R, Ghirmay R, *et al*. Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among Patients - A Multicenter Study in Asmara, Eritrea. Canadian Journal of Infectious Diseases and Medical Microbiology 2019; 2019: 8321834.
7. Sachithanandam ST. Rising Methicillin-Resistant *Staphylococcus aureus* Infections in Ear, Nose, and Throat Diseases. Case Reports in Otolaryngology 2014; 2014: 253945.
8. Seyedi-Marghaki F, Kalantar-Neyestanaki D, Saffari F, Hosseini-Nave H, Moradi M. Distribution of Aminoglycoside-Modifying Enzymes and Molecular Analysis of the Coagulase Gene in Clinical Isolates of Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus*. Microbial Drug Resistance 2019; 25(1): 47–53.
9. Rahimi F. Characterization of resistance to aminoglycosides in methicillin-resistant *Staphylococcus aureus* strains isolated from a tertiary care hospital in Tehran, Iran. Jundishapur Journal of Microbiology 2016; 9(1): e29237.
10. Mohamed N, Timofeyeva Y, Jamrozy D, Rojas E, Hao L, Monerri NCS De, *et al*. Molecular epidemiology and expression of capsular polysaccharides in *Staphylococcus aureus* clinical isolates in the United States. PLoS ONE 2019; 14(1): e0208356.
11. Waryah CB, Gogoi-tiwari J, Wells K, Costantino P, Al-salami H, Sunagar R, *et al*. Serological versus molecular typing of surface- associated immune evading polysaccharide antigens-based phenotypes of *Staphylococcus aureus*. Journal of Medical Microbiology 2014; 63: 1427–31.
12. Nanra JS, Buitrago SM, Crawford S, Ng J, Fink PS, Hawkins J, *et al*. Capsular polysaccharides are an important immune evasion mechanism for *Staphylococcus aureus*. Human Vaccines and Immunotherapeutics 2013; 9(3): 480–7.
13. Thakker M, Park J, Carey V, Lee JC. *Staphylococcus aureus* Serotype 5 Capsular Polysaccharide Is Antiphagocytic and Enhances Bacterial Virulence in a Murine Bacteremia Model. Infection and Immunity 1998; 66(11): 5183–9.
14. Sau S, Lee CY. Cloning of Type 8 Capsule Genes and Analysis of Gene Clusters for the Production of Different Capsular Polysaccharides in *Staphylococcus aureus*. Journal of Bacteriology 1996; 178(7): 2118–26.
15. Sau S, Bhasin N, Wann ER, Lee JC, Foster TJ, Lee CY. The *Staphylococcus aureus* allelic genetic loci for serotype 5 and 8 capsule expression contain the type-specif ic genes flanked by common genes. Microbiology 1997; 143(7): 2395–405.
16. Louie T, Hutchins W, McClure J-A, Elsayed S, Lau V, Zhang K, *et al*. Novel Multiplex PCR Assay for Detection of the *Staphylococcal* Virulence Marker Panton-Valentine Leukocidin Genes and Simultaneous Discrimination of Methicillin-Susceptible from -Resistant *Staphylococci*. Journal of Clinical Microbiology 2006; 44(3): 1141–4.
17. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100, editor. PA: Clinical and Laboratory Standards Institute Wayne; 2017.
18. Sandhu R, Dahiya S, Sayal P. Evaluation of multiple antibiotic resistance (MAR) index and Doxycycline susceptibility of *Acinetobacter* species among inpatients. Indian Journal of Microbiology Research 2016; 3(3): 299–304.
19. Rahimi F, Bouzari M, Maleki Z, Rahimi F. Antibiotic susceptibility pattern among *Staphylococcus spp*. with emphasis on detection of mecA gene in methicillin resistant *Staphylococcus aureus* isolates. Iranian Journal of Clinical Infectious Diseases 2009; 4(3): 143–50.
20. Lorenz TC. Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies. Journal of Visualized Experiments 2012; (63): e3998.
21. Ardic N, Sareyyupoglu B, Ozyurt M, Haznedaroglu T, Ilga U. Investigation of aminoglycoside modifying enzyme genes in methicillin-resistant *staphylococci*. Microbiological Research 2006; 161(1): 49–54.
22. Shokravi Z, Mehrad L, Ramazani A. Detecting the frequency of aminoglycoside modifying enzyme encoding genes among clinical isolates of methicillin-resistant *Staphylococcus*

aureus. BioImpacts 2015; 5(2): 87–91.
23. Schmitz FJ, Fluit AC, Gondolf M, Beyrau R, Lindenlauf E, Verhoef J, *et al*. The prevalence of aminoglycoside resistance and corresponding resistance genes in clinical isolates of *staphylococci* from 19 European hospitals. Journal of Antimicrobial Chemotherapy 1999; 43(2): 253–9.
24. Goerke C, Esser S, Kümmel M, Wolz C. *Staphylococcus aureus* strain designation by agr and cap polymorphism typing and delineation of agr diversification by sequence analysis. International Journal of Medical Microbiology 2005; 295(2): 67–75.
25. DeAntonio R, Yarzabal JP, Cruz JP, Schmidt JE, Kleijnen J. Epidemiology of otitis media in children from developing countries: A systematic review. International Journal of Pediatric Otorhinolaryngology 2016; 85: 65–74.
26. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrobial Resistance and Infection Control 2017; 6(47).
27. dos Santos DCM, da Costa TM, Rabello RF, Alves FA, de Mondino SSB. Mannitol-negative methicillin-resistant *Staphylococcus aureus* from nasal swab specimens in Brazil. Brazilian Journal of Microbiology 2015; 46(2): 531–3.
28. Shittu A, Lin J, Morrison D. Molecular identification and characterization of mannitol-negative methicillin-resistant *Staphylococcus aureus*. Diagnostic microbiology and infectious disease 2007; 57(1): 93–5.
29. Ameen F, Reda SA, El-shatoury SA, Riad EM, Enany ME, Alarfaj AA. Prevalence of antibiotic resistant mastitis pathogens in dairy cows in Egypt and potential biological control agents produced from plant endophytic *actinobacteria*. Saudi Journal of Biological Sciences 2019; 26(7): 1492–8.
30. Wurster JI, Bispo PJM, Tyne D Van, Cadorette JJ, Boody R, Gilmore MS. *Staphylococcus aureus* from ocular and otolaryngology infections are frequently resistant to clinically important antibiotics and are associated with lineages of community and hospital origins. plos one 2018; 13(12): e0208518.
31. Shariq A, Tanvir SB, Zaman A, Khan S, Anis A, Khan MA, *et al*. Susceptibility profile of methicillin-resistant *Staphylococcus aureus* to linezolid in clinical isolates. International Journal of Health Sciences 2017; 11(1): 9–12.
32. Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, *et al*. Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. The Lancet 2001; 358(9277): 207–8.
33. Azhar A, Rasool S, Haque A, Shan S, Saeed M, Ehsan B, *et al*. Detection of high levels of resistance to linezolid and vancomycin in *Staphylococcus aureus*. Journal of Medical Microbiology 2017; 66(9): 1328–31.
34. Kaur DC, Chate SS. Study of Antibiotic Resistance Pattern in Methicillin Resistant *Staphylococcus aureus* with Special Reference to Newer Antibiotic Dardi. Journal of Global Infectious Diseases 2015; 7(2): 78–84.
35. CDC. Antibiotic Resistance Threats in the United States, 2019. U.S. Department of Health and Human Services. Atlanta, GA; 2019.
36. Elshimy R, Khattab RA, Zedan H, Hosny AES, Elmorsy TH. Study on prevalence and genetic discrimination of methicillin-resistant *Staphylococcus aureus* (MRSA) in Egyptian hospitals. African Journal of Microbiology Research 2018; 12(27): 629–46.
37. Melake NA, Eissa NA, Keshk TF, Sleem AS. Prevalence of multidrug-resistant bacteria isolated from patients with burn infection. Menoufia Medical Journal 2015; 28(3): 677–84.
38. Ahmed EF, Gad GFM, Abdalla AM, Hasaneen AM, Abdelwahab SF. Prevalence of methicillin resistant *Staphylococcus aureus* among egyptian patients after surgical interventions. Surgical Infections 2014; 15(4): 404–11.
39. Elbouseary M, El-Banna TE, Sonbol FI. Prevalence of MRSA among *Staphylococcus aureus* Isolates Recovered From Patients with Otitis Media. Nature and Science 2018; 16(6): 48–55.
40. Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: An overview. Cold Spring Harbor Perspectives in Medicine 2016; 6(6): a027029.
41. Khosravi AD, Jenabi A, Montazeri EA. Distribution of genes encoding resistance to aminoglycoside modifying enzymes in methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Kaohsiung Journal of Medical Sciences 2017; 33(12): 587–93.
42. Elmonir W, Aglan H, Elmahallawy EK, El-Tras WF. Antimicrobial phenotypes of geographically matched *Staphylococcus aureus* isolated from buffalo's milk and clinical human cases in Egypt: Potential zoonotic risks. Slov Vet Res 2019; 56(suppl 22): 755–63.
43. Mohammadi S, Sekawi Z, Monjezi A, Maleki MH, Soroush S, Sadeghifard N, *et al*. Emergence of SCCmec type III with variable antimicrobial resistance profiles and spa types among methicillin-resistant *Staphylococcus aureus* isolated from healthcare and community-acquired infections in the west of Iran. International Journal of Infectious Diseases 2014; 25: 152–8.
44. Ida T, Okamoto R, Shimauchi C, Okubo T, Kuga A, Inoue M. Identification of Aminoglycoside-Modifying Enzymes by Susceptibility Testing: Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in Japan. Journal of Clinical Microbiology 2001; 39(9): 3115–21.
45. Hauschild T, Sacha P, Wieczorek P, Zalewska M, Kaczyñska K, Tryniszewska E. Aminoglycosides resistance in clinical isolates of *Staphylococcus aureus* from a University Hospital in Bialystok, Poland. Folia Histochem Cytobiol 2008; 46(2): 225–8.
46. Ayepola OO, Olasupo NA, Egwari LO, Becker K. Molecular Characterization and Antimicrobial Susceptibility of *Staphylococcus aureus* Isolates from Clinical Infection and Asymptomatic Carriers in Southwest Nigeria. PLOS one 2015; 10(9): e0137531.
47. Sutter DE, Summers AM, Keys CE, Taylor KL, Frasch CE, Braun LE, *et al*. Capsular serotype of *Staphylococcus aureus* in the era of community-acquired MRSA. FEMS Immunology and Medical Microbiology 2011; 63(1): 16–24.

Cite this article as:

Tarek El-Said El-Banna *et al*. Molecular Characterization of *Staphylococcus aureus* isolates recovered from Children with Otitis Media in Alexandria Hospitals, Egypt. Int. Res. J. Pharm. 2020;11(4):36-42 http://dx.doi.org/10.7897/2230-8407.110439

Source of support: Nil, Conflict of interest: None Declared