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Research Article

Detection and Characterization of Staphylococcus aureus and Methicillin-resistant S. aureus (MRSA) in Ear Infections in Tanta, Egypt

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ABSTRACT

Ear infection is a common clinical problem worldwide and the main cause of preventable hearing loss in the developing world. The increasing incidence of methicillin-resistant Staphylococcus aureus infections (MRSA) in ear, nose and throat (ENT) diseases is becoming a big clinical concern. This study aimed to investigate *Staphyloccocus aureus* (S. aureus) as a common bacterial causative agent of ear infection, characterize the isolates resistance profiles, investigate the incidence of MRSA among S. aureus isolates, and occurrence of mecA gene among MRSA isolates in Tanta, Egypt. The main isolated bacteria in this study were S. aureus (n=108) accounting for 37.5% of the total bacterial isolates. Antimicrobial susceptibility testing of S. aureus isolates to 15 antimicrobials was performed. All S.aureus isolates (100%) were resistant to penicillin. Moreover, high resistance rates were observed against cefoxitin (63%), rifampin (57.4%), and clindamycin (50.9%). In contrast, ciprofloxacin and moxifloxacin had the lowest resistance rates (3.7% for each). In this study, 68 (63%) S. aureus isolates were recorded as MRSA using the Oxacillin Resistance Screening Agar Base (ORSAB) medium. The presence of mecA gene was detected using polymerase chain reaction (PCR) technique. The incidence of mecA gene among the selected isolates was 92.5%. In conclusion, our data demonstrated that the incidence rate of MRSA is becoming a real threat with potential major public health problems in the management of patients with ear infections in Tanta, Egypt. We recommend the necessity of regular evaluation of the microbiological pattern and antibiogram of S. aureus as one of the major pathogens of ear infection.

Keywords: Egypt, *mecA*, MRSA, Otitis, *Staphylococcus aureus*.

1. INTRODUCTION

Ear infection is a common clinical problem worldwide and the main cause of preventable hearing loss in the developing world.[1,2] Microbial agents can infect the middle and external parts of the ear, including the skin, cartilage, periosteum, ear canal, and tympanic and mastoid cavities.[3] Acute suppurative otitis media (ASOM), chronic suppurative otitis media (CSOM), and otitis externa (OE) are the three types of ear infection.[4] Its chronic form is a serious problem that affects people of all ages and has a low recovery rate. In certain cases this condition can lead to serious life-threatening complications, such as hearing impairment, brain abscesses, or meningitis, mostly in childhood and late in life.[2,4] In 2015, over 5% of the world's population (328 million adults and 32 million children) suffered from serious hearing loss affects, according to the World Health Organization (WHO).[2]

Ear infections can be caused by bacterial, fungal, or viral pathogens. However, the major causative agents of ear infection are bacterial isolates including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Streptococcus pyogenes*, *Klebsiella* spp., *Escherichia coli*, or mixed bacterial infection.[5] In the developed world, the microbiological profiles of ear infection are well documented. However, in most developing countries, few studies have

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been conducted so far.[6,7] Furthermore, the signs and symptoms of earache can sometimes confuse the etiology of infection, making it difficult for the physician to link the condition to the actual etiology. Hence, the physician may defend antibiotic therapy irrespective of the etiology of the disease. If an ear infection is caused by a virus or fungus, this may lead to stress to the patient, unwanted economic loss and foremost antibiotic resistance.[8]

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming a major clinical issue in ENT (ear, nose, and throat) illnesses. MRSA has emerged as a serious problem in a number of diseases such as osteomyelitis, pneumonia, infective endocarditis, skin and soft tissue infections, including sinonasal and ear infections. Widespread use of broadspectrum antibiotics and previous nasal surgeries contribute much to the emergence of MRSA causing ear and sinonasal infections.[9] MRSA usually is multidrug resistant, showing resistance not only to β-lactam antibiotics but also to a wide range of antibiotic classes, including tetracyclines, fluoroquinolones, macrolides, aminoglycosides and lincosamides.[10,11] MRSA is primarily mediated over production of an additional altered penicillin-binding protein (PBP2a) with low affinity for β-lactam antibiotics.[12] The acquisition of *mecA* gene, which codes for the PBP2a involved in bacterial cell wall synthesis, is the major evidence for the detection of resistance to methicillin and to all β-lactam antibiotics in *S. aureus*.[13] Other new *mecA* gene homologs including *mecB* and *mecC* were detected in other species, *mecC* has also been found on the chromosome of *Staphylococcus xylosus*, while *mecB* has not been reported yet in staphylococcal species.[14]

Vancomycin has been recommended as a miracle cure for MRSA for the past two decades. Nevertheless, decreased susceptibility limits vancomycin usage to eradicate serious infections caused by MRSA especially for MRSA pneumonia, due to suboptimal penetration of vancomycin in the alveolar lining fluid.[15,16] Resistance has already emerged to the newest antibiotics approved to treat MRSA infections, daptomycin and linezolid.[11,17,18] The delayed diagnosis and treatment of MRSA infections lead to poorer clinical outcomes. Rapid diagnostic tests may help to reduce mortality, hospitalisation, and expenditures by providing better management strategies.[19]

This study aimed to investigate *S. aureus* as a common bacterial causative agent of ear infection and to characterize the resistance profiles of the recovered isolates. Also, we investigate the incidence of MRSA among *S. aureus* isolates and occurrence of *mecA* gene among MRSA isolates in Tanta, Egypt.

2. MATERIALS AND METHODS

2.1. Study design

This paper is part of a larger study entitled “Microbiological and Epidemiological Studies on Microbes Isolated from Patients Suffering from Ear Infections”. A total of 202 patients with ear infection were included in this study from the out-patient clinic of ENT department of Tanta University Teaching Hospital. The study took place at Faculty of Pharmacy, Tanta University, Egypt.

2.2. Ethical considerations

Informed written consent was obtained from all patients included in the study. In case of children, written informed consent was obtained from the parents. Patients were not charged for participating in the study, and those who did not consent were also treated, and their refusal had no bearing on the treatment they received. Confidentiality was upheld as well. The methodology used in this research adheres to the ethical guidelines of "The Research Ethics Committee, Faculty of Pharmacy, Tanta University, Egypt".

2.3. Sampling

Ear swab specimens were aseptically collected unilaterally (one ear) or bilaterally (both ears). Specimens were collected by physicians, placed in nutrient broth (Oxoid, UK) as a transport media and transported within 1 hour in an ice box to the microbiology laboratory.

2.4. Isolation and identification

Each clinical specimen was inoculated onto Mannitol Salt Agar (Oxoid, UK) plates followed by incubation at 35-37°C for 20 hours. *S. aureus* was defined as yellow colonies. Isolates were identified according to standard laboratory methods followed by using matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI–TOF MS) for further confirmation (Bruker, Germany).

2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of all *S. aureus* isolates to 15 antimicrobials representing 11 different classes were tested using the following antimicrobial discs: β-lactams [penicillin, P (10 U.) and cefoxitin, FOX (30 µg)], glycopeptides [vancomycin, VA (30 µg)], aminoglycosides [gentamicin, CN (10 µg) and amikacin, AK (30 µg)], macrolides [erythromycin, E (15 µg)], tetracyclines [tetracycline TE (30 µg)], fluoroquinolones [ciprofloxacin, CIP (5 µg); ofloxacin, OFX (5 µg) and moxifloxacin, MXF (5 µg)], lincosamides [clindamycin, DA (2 µg)], folate pathway inhibitors [trimethoprim-sulfamethaxole, SXT (25 µg)], phenicols [chloramphenicol, C (30 µg)], ansamycins [rifampin, RD (5 µg)] and oxazolidinones [linezolid, LZD (30 µg)] (Oxoid, UK). This test was conducted following the Kirby-Bauer disk diffusion method,[20] the inhibition zones were measured and the results were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2015 guidelines.[21] *S. aureus* (ATCC 25923) was considered as the control strain. This strain was obtained from Naval Medical Research Unit-3 (NAMRU 3), Cairo, Egypt. Isolates that test resistant by cefoxitin disk test was further streaked onto Oxacillin

Resistance Screening Agar Base (ORSAB) plates (Oxoid, UK) for confirmation of MRSA. Isolates showed intense blue colonies on ORSAB were recorded as MRSA.

2.6. Antimicrobial resistance profiles analysis

Multi Drug Resistance (MDR) and Extensively Drug Resistance (XDR) character were identified as the isolate that showed acquired resistance to at least one agent in ≥ three antimicrobial categories was considered MDR, whereas the isolate that was acquired resistance to at least one antimicrobial agent in all, but ≤ two antimicrobial categories was considered XDR.[22] When defining antimicrobial resistance for a *S. aureus* isolate that is an MRSA, a special rule was used in the MDR definition. Finding an isolate resistant to oxacillin or cefoxitin predicts non-susceptibility to all categories of β-lactam antimicrobials, with the exception of the anti-MRSA cephalosporins (i.e. all categories of penicillins, cephalosporins, β-lactamase inhibitors and carbapenems, approved up until 25 January 2011). An MRSA isolate thus will always be characterized as MDR.[22]

2.7. Multiple antimicrobial resistance (MAR) indexing

To quantify the multi-resistance of *S. aureus* isolates, the multiple antimicrobial resistances (MAR) indexing was used as Equation (1) follows:

$$MAR\ index = \frac{a}{b}$$

Where, "a" represents the number of antimicrobials to which the microorganism was resistant and "b" represents the total number of antimicrobials tested on the microorganism.[23]

2.8. Multiple antimicrobial resistance (MAR) indexing

Conventional Polymerase Chain Reaction (PCR) technique was performed to detect the presence of *mecA* gene. PCRs were run on Thermal Cycler (Thermo fisher, USA). Total DNA of the tested MRSA isolates was extracted by denaturation of a few fresh colonies suspended in sterile water at 98°C for 15 min and then centrifuged at 13,000 rounds per minute (rpm) for 30 seconds. The supernatant was employed as template for amplification in PCR.[24] The thermal profile consisted of an initial denaturation step at 95ºC for 5 min, followed by 35 cycles of denaturation step at 95ºC for 1 min, annealing at 52ºC for 1 min, extension at 72ºC for 1 min, finally an extension step at 72ºC for 5 min and store at 4°C.[25] The primers used were (Thermo Fisher Scintefic, USA):

Forward primer sequence (*mecA*-F):

"ACGAGTAGATGCTCAATATAA"

Reverse primer sequence (*mecA*-R):

"CTTAGTTCTTTAGCGATTGC"

The PCR was performed using Taq DNA Polymerase Kit (Thermo Fisher Scintefic, USA) according to the manufacturer's instructions. The PCR products were run on 1.5% agarose gel (Bioline, UK) to visualize the amplified bands using horizontal gel electrophoresis apparatus (Mupid-exU System gel electrophoresis, Mupid CO., Japan). The gels were stained by ethidium bromide (Sigma, USA) and photographs recorded under UV using Syngene G-BOX documentation system (Syngene, UK). DNA fragment size was determined by comparison with a 200 bp DNA ladder band sizes (Bioline, USA). Bands with approximate size of 293 bp were detected as *mecA* gene positive. PCR-grade nuclease free water (Thermo Fisher Scintefic, USA) without template was served as a negative control.

3. RESULTS

3.1. Prevalence of *S. aureus* in the examined specimens

Out of 212 clinical specimens (since there were 10 patients whom collected bilaterally) a total of 288 bacterial pathogens were isolated. The main isolated bacteria in this study was S. aureus (n=108) accounting for 37.5% of the total bacterial isolates. The prevalences of S. aureus in patients with CSOM, ASOM and OE were 62.04%, 35.2% and 2.8% respectively

3.2. Method validation

Analysis of the antimicrobial resistance of 108 *S. aureus* isolates against the 15 tested antimicrobial agents demonstrated that all isolates (100%) were resistant to penicillin (Table 1). Moreover, high resistance rates were observed against cefoxitin (63%), followed by rifampin (57.4%) and clindamycin (50.9%). On the other hand, our results showed that ciprofloxacin and moxifloxacin had the lowest resistance rates against the tested isolates (3.7% resistance for each). A total of 68 (63%) *S. aureus* isolates showing cefoxitin resistance and intense blue colonies on ORSAB were recorded as MRSA. The prevalences of MRSA in patients with CSOM, ASOM and OE were 60.3%, 39.7% and 0% respectively.

Table 1: Incidence of antimicrobial resistance among *S. aureus* isolates.

Antimicrobial group	Antimicrobial agent	Resistant isolates No. (%)*
β-lactams	Penicillin	108 (100%)
	Cefoxitin	68 (63%)
Glycopeptides	Vancomycin	19 (17.6%)
Aminoglycosides	Gentamicin	20 (18.5%)
	Amikacin	5 (4.6%)
Macrolides	Erythromycin	48 (44.4%)
Tetracyclines	Tetracycline	9 (8.3%)
Fluoroquinolones	Ciprofloxacin	4 (3.7%)
	Ofloxacin	9 (8.3%)
	Moxifloxacin	4 (3.7%)
Lincosamides	Clindamycin	55 (50.9%)
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	7 (6.5%)
Phenicols	Chloramphenicol	33 (30.6%)
Ansamycins	Rifampin	62 (57.4%)
Oxazolidinones	Linezolid	14 (13%)

*Percentages were calculated relative to the total number of *S. aureus* isolates (n=108).

3.3. Antimicrobial resistance profiles and MAR indices of *S.aureus* isolates

The antimicrobial resistance patterns of the *S. aureus* (n=108) resistant isolates were grouped according to the number and type of exhibited resistance markers. In general, multiple antimicrobial resistances were common among the tested isolates where *S. aureus* exhibited 53 antimicrobial resistance patterns. Moreover, *S. aureus* tested isolates were very heterogeneous where not more than nine isolates shared the same resistance pattern. Based on the antimicrobial resistance patterns of these isolates, MAR index values were calculated and revealed that 78.7% of *S. aureus* isolates had MAR index of 0.2 and above. MDR and XDR characters were identified (Table 2). An MRSA is always considered MDR. Interestingly, it was noticed that 74 (68.5%) isolates of *S. aureus* were MDR and 4 (3.7%) were XDR isolates

Table 2: Antimicrobial resistance patterns among S. aureus isolates.

Pattern code	Antimicrobial resistance pattern[a]	Isolates No. (%)[b]	MAR index	Character of resistant strains[c]
S I	P	5 (4.6%)	0.07	-
S II a	P-FOX	4 (3.7%)	0.13	MDR
S II b	P-E	9 (8.3%)	0.13	-
S II c	P-DA	2 (1.9%)	0.13	-
S II d	P-RD	3 (2.8%)	0.13	-
S III a	P-FOX-E	2 (1.9%)	0.2	MDR
S III b	P-FOX-DA	5 (4.6%)	0.2	MDR
S III c	P-FOX-C	1 (0.9%)	0.2	MDR
S III d	P-FOX-RD	7 (6.5%)	0.2	MDR
S III e	P-E-DA	6 (5.6%)	0.2	-
S III f	P-MXF-DA	1 (0.9%)	0.2	-
S III g	P-DA-RD	2 (1.9%)	0.2	-
S III h	P-C-RD	1 (0.9%)	0.2	-
S IV a	P-FOX-CN-SXT	1 (0.9%)	0.27	MDR
S IV b	P-FOX-E-DA	2 (1.9%)	0.27	MDR
S IV c	P-FOX-E-RD	2 (1.9%)	0.27	MDR
S IV d	P-FOX-TE-DA	1 (0.9%)	0.27	MDR
S IV e	P-FOX-DA-RD	5 (4.6%)	0.27	MDR
S IV f	P-FOX-C-RD	6 (5.6%)	0.27	MDR
S IV g	P-CN-AK-E	1 (0.9%)	0.27	-
S IV h	P-E-DA-RD	3 (2.8%)	0.27	MDR
S IV i	P-TE-DA-RD	1 (1.9%)	0.27	MDR
S V a	P-FOX-CN-E-RD	2 (1.9%)	0.33	MDR
S V b	P-FOX-CN-DA-C	1 (0.9%)	0.33	MDR
S V c	P-FOX-CN-DA-RD	1 (0.9%)	0.33	MDR
S V d	P-FOX-CN-C-RD	1 (0.9%)	0.33	MDR
S V e	P-FOX-E-DA-RD	2 (1.9%)	0.33	MDR
S V f	P-FOX-DA-C-RD	1 (0.9%)	0.33	MDR
S V g	P-CN-TE-DA-RD	1 (0.9%)	0.33	MDR
S V h	P-E-DA-C-RD	5 (4.6%)	0.33	MDR
S VI a	P-FOX-VA-CN-AK-E	1 (0.9%)	0.4	MDR
S VI b	P-FOX-VA-C-RD-LZD	2 (1.9%)	0.4	MDR
S VI c	P-FOX-CN-E-SXT-RD	1 (0.9%)	0.4	MDR
S VI d	P-FOX-E-TE-DA-C	1 (0.9%)	0.4	MDR
S VI e	P-FOX-E-DA-C-RD	1(0.9%)	0.7	MDR
S VII a	P-FOX-VA-CN-DA-C-LZD	1 (0.9%)	0.47	MDR
S VII b	P-FOX-VA-OFX-DA-C-LZD	1 (0.9%)	0.47	MDR
S VII c	P-FOX-VA-DA-C-RD-LZD	1 (0.9%)	0.47	MDR
S VII d	P-FOX-CN-E-DA-C-RD	1 (0.9%)	0.47	MDR
S VII e	P-FOX-TE-DA-SXT-C-RD	1 (0.9%)	0.47	MDR
SVIII a	P-FOX-VA-CN-AK-E-OFX-DA	1 (0.9%)	0.53	MDR
SVIII b	P-FOX-VA-E-OFX-C-RD-LZD	1 (0.9%)	0.53	MDR
SVIII c	P-FOX-VA-TE-DA-C-RD-LZD	1 (0.9%)	0.53	MDR
SVIII d	P-FOX-VA-DA-SXT-C-RD-LZD	1 (0.9%)	0.53	MDR
S IX a	P-FOX-VA-CN-AK-E-OFX-DA-RD	1 (0.9%)	0.6	MDR
S IX b	P-FOX-VA-CN-AK-CIP-OFX-DA-RD	1 (0.9%)	0.6	MDR
S IX c	P-FOX-VA-CN-E-OFX-C-RD-LZD	1 (0.9%)	0.6	MDR
S IX d	P-FOX-VA-CN-E-DA-C-RD- LZD	1 (0.9%)	0.6	MDR
S X a	P-FOX-VA-CN-E-TE-MXF-DA-RD-LZD	1 (0.9%)	0.67	XDR
S X b	P-FOX-VA-CN-E-DA-SXT-C-RD-LZD	1(0.9%)	0.67	XDR
S X c	P-FOX-VA-E-TE-CIP-OFX-MXF-C-RD	1 (0.9%)	0.67	MDR
S XI	P-FOX-VA-CN-E-CIP-OFX-SXT-C-RD-LZD	1 (0.9%)	0.73	XDR

S XII	P-FOX-VA-E-TE-CIP-OFX-MXF-DA-SXT-RD-LZD	1 (0.9%)	0.8	XDR

[a]: P; Penicillin, FOX; Cefoxitin, VA; Vancomycin, CN; Gentamicin, AK; Amikacin, E; Erythromycin, TE; Tetracycline, CIP; Ciprofloxacin, OFX; Ofloxacin, MXF; Moxifloxacin, DA; Clindamycin, SXT; Trimethoprim-sulfamethoxazole, C; Chloramphenicol, RD; Rifampin, LZD; Linezolid.
[b]: Percentages were calculated relative to the total number of *S.aureus* isolates (n=108).
[c]: MDR; Multi Drug Resistance, XDR; Extensively Drug Resistance

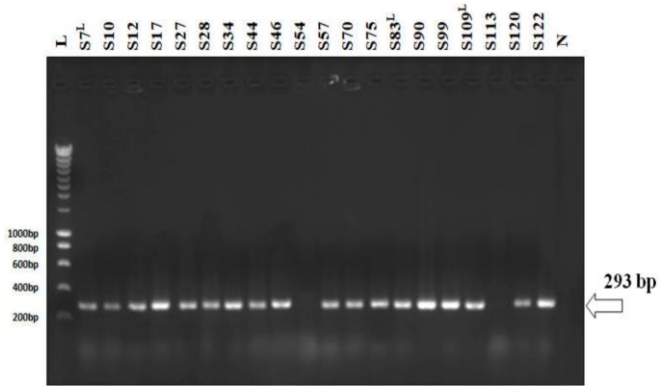

Figure 1: Electrophoregram showing detection of *mecA* gene among MRSA isolates using PCR technique. Lane L; (200 bp) DNA ladder. Isolates 7^L, 10, 12, 17, 27, 28, 34, 44, 46, 57, 70, 75, 83^L, 90, 99, 109^L, 120 and 122 showed a band at approximately 293 bp that corresponds to *mecA* gene, lane N: negative control.

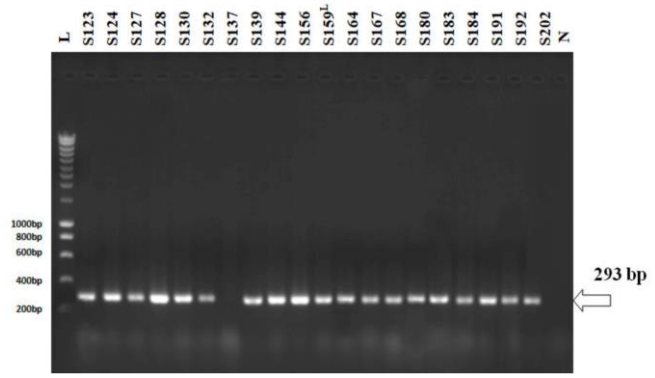

Figure 2: Electrophoregram showing detection of *mecA* gene among MRSA isolates using PCR technique. Lane L; (200 bp) DNA ladder. Isolates 123, 124, 127, 128, 130, 132, 139, 144, 156, 159^L, 164, 167, 168, 180, 183, 184, 191, 192 and 202 showed a band at approximately 293 bp that corresponds to *mecA* gene, lane N: negative control.

3.4. Detection of *mecA* gene among MRSA isolates

Conventional PCR technique was performed on total DNA extract of each selected isolate (n=40) representatives to all MRSA different resistance patterns. Bands with approximate size of 293 bp for *mecA* gene were detected **(Figure 1** and **2)**. It was found that 37 (92.5%) of the selected isolates were *mecA* positive.

4. DISCUSSION

Ear infection is a more common treatable health care problem around the world, but if left untreated, it can lead to a serious complication like speech development disorder, hearing loss, distress in patients and their family quality of life, and economic burden on the health care system.[2] Due to poor living conditions and sanitary conditions, as well as a lack of proper nutrition, the burden and prevalence of ear infection are higher in developing countries.[5,26,27] As a result, highlighting the etiologies of ear infection and their antibiotic susceptibility patterns will aid in reducing the severity of infection complications and guiding the empirical antibiotic prescribed by the physicians, especially for developing countries.[26,28] *S. aureus* is considered one of the predominant bacterial cause of ear infections and shows a global concern in resistance to the majority of available treatment options.[29]

In the present study, the prevalence of *S. aureus* isolates recovered from patients with ear infection was investigated in out-patient clinic of ENT department of Tanta University Teaching Hospital. Our results demonstrated that the prevalence of *S. aureus* was 37.5% of the total bacterial isolates. *S. aureus* was the most common isolate. These results are consistent with previous reports which have reported *S. aureus* as the most common isolate in Singapore (33.3%)[30] and Nepal (32.2%).[31] Moreover, *S. aureus* was found to be the second commonest in Nigeria[32] and Palestine.[33] On the other hand, some studies have reported *P. aeruginosa* as the most common isolate in Pakistan (40%),[34] Nigeria (31.3%)[5] and India (35%).[35] This variation in result could be due to effect of climate and variation of organisms in different community and locality.

Regarding the antimicrobial resistance results, it was found that, penicillin showed the highest antibacterial resistance (100%). Our results were comparable with the findings reported in other studies performed in several governorates in Egypt. A study in the Ismailia governorate showed that (90%) of *S. aureus* isolates were resistant to penicillin.[36] Another study in the Alexandria governorate showed (91.7%) resistance to penicillin.[37] These findings indicate that this antibiotic are no longer effective against *S. aureus* infections in Egypt. Our research demonstrated a high level of resistance against cefoxitin (63%) among *S. aureus* isolates. Similarly, a high resistance rate against cefoxitin (55.6%) was also reported in Egypt.[37] Rifampin had a higher resistance level (57.4%) against our tested isolates in comparing with another report in Egypt, where 32.5% of the tested isolates were resistant.[38] Clindamycin also had a higher resistance level (50.9%) in comparing with other reports in Ethiopia and Pakistan where (26% and 10%, respectively) of the tested isolates were resistant.[39,40]

On the other hand, our results showed high susceptibility rates against ciprofloxacin and moxifloxacin (96.3% for each). These results were consistent with the findings reported in other studies.[39-41] Quinolones are considered to be the most effective, with high sensitivities for most isolated *S. aureus* in this study. They would hence,

provide a viable option for the treatment; as they are available in oral, injectables and topical ear drops as well.[42]

The high resistance of *S. aureus* isolates to the antimicrobials in the present study might be due to the widespread and the uncontrolled use of these agents in human and animal treatments. This gives a reflection about the extent of using these antimicrobials in Egypt and therefore proposes a challenge to the management of infections. Nowadays, the emergence of MDR *S. aureus* isolates especially MRSA isolates is becoming a growing challenge as it can impair the effective therapy of *S. aureus* infections.[43] In the current study, there were MDR (68.5%) and XDR (3.7%) *S. aureus* isolates. It is interesting to note that 78.7% of *S. aureus* isolates in this study had MAR index of 0.2 and above, which indicates a high frequency of antibiotics usage in Egypt. This is in accordance with another report in Egypt.[37]

MRSA is responsible for a great number of antibiotic resistant infections worldwide.[44,45] In the present study, 68 (63%) *S. aureus* isolates were recorded as MRSA using ORSAB medium. Comparable rates were reported in different studies conducted in Egypt including Cairo (50%)[46] and (60.5%).[47] Abdel-maksoud et al.,[48] reported an overall higher incidence rate of 76.6% in 12 hospitals in Egypt from 2005 to 2013. Comparing our findings with that obtained by other workers in Egypt, a controversary was obvious where a lower incidence of MRSA in Cairo (25%)[49] and in Tanta (26%)[50] was reported. However, a markedly lower incidence of MRSA was reported in Assiut (18.9%)[51] and Sohag (4.6%).[52]

Reports from Middle Eastern countries also revealed higher rates in the incidence of MRSA according to reports from Saudi Arabia (77.5%)[53] and from Libya (54–68%).[54] However, lower rates of below 10% in North Africa and Malta were reported.[55,56] In Tunisia, the prevalence of MRSA increased from 16% to 41% between 2002–2007,[57] while in Libya it was 31% in 2007.[58] In South Africa, the incidence rate fell from 36% in 2006 to 24% between 2007 and 2011.[59] Between 2000 and 2007, the prevalence in Botswana ranged from 23 to 44%.[60,61] The former findings indicate that the incidence of MRSA keeps changing every year. 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.

The main evidence for the detection of MRSA isolate is the recognition of the *mecA* gene. This was approved by many investigators all over the world including Egypt,[62] Europe[63,64] and Nepal.[65] In the present study, conventional PCR technique was performed for detection of *mecA* gene. The *mecA* gene was detected in (92.5%) of the tested MRSA isolates. This is in accordance with another reports in Egypt, where (85.7%)[66] and (90.5%) of MRSA isolates were *mecA*-positive.[50] These results are lower compared to those obtained by Elshimy et al.,[38] who reported a percentage of 85.7%. However, PCR based detection of MRSA is srongly recommended. The absence of *mecA* gene in a significant proportion of MRSA isolates necessitates further research into alternative genetic options connected to the resistance phenomena.[67]

5. CONCLUSIONS

In conclusion, our data demonstrated that the relatively high isolation rate of MRSA isolates associated with ear infections in Tanta, Egypt is an alarming situation with potential serious consequences to the health. Absence of *mecA* gene in some MRSA isolates recommended the investigation of alternative genetic options related to methicillin resistance phenomena. The need for continuous surveillance of MRSA in endemic regions to obtain a more comprehensive and detailed knowledge of epidemiology is highly recommended. We suggest a preventive approach through health education on early presentation and diagnosis to prevent chronicity and to limit disability from hearing loss.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

F.I.S and T.E.E designed the study. F.I.S and O.F.F carried out the laboratory work. All the authors contributed to the analysis and interpretation of the results. F.I.S and O.F.F wrote the manuscript in consultation with A.A.A. All authors revised, approved the final manuscript and agreed to be responsible for all aspects of the work.

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