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Original Article

High Prevalence of Multi-drug Resistant Methicillin-Resistant *Staphylococcus aureus* in Tertiary Egyptian Hospitals

Abdel-Aty E Alfeky[1], Mahmoud M Tawfick[1,2], Mohamed S Ashour[1], Abdel-Nasser A El-Moghazy[1]

[1] Microbiology and Immunology Department, Faculty of Pharmacy (Boys), Al-Azhar University, Nasr City, Cairo, Egypt
[2] Microbiology and Immunology Department, Faculty of Pharmacy, Heliopolis University, Cairo, Egypt

Abstract

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an emerging cause of morbidity and mortality worldwide. This work aimed to study the occurrence of multidrug-resistant MRSA (MDR-MRSA) in tertiary Egyptian hospitals and determine the antimicrobial susceptibilities and the genetic relatedness of isolates for epidemiological assessment.

Methodology: A total of 170 *S. aureus* isolates were collected from two Egyptian tertiary hospitals in Cairo, between September 2017 and December 2018. MRSA isolates were identified using the conventional microbiological methods and confirmed by the PCR assays targeting *nuc* gene, a surrogate marker of *S. aureus* and the *mecA* gene for genotypic identification of methicillin resistance. Antimicrobial susceptibility was determined using the Kirby-Bauer disk diffusion method and the isolates were grouped into different antibiotypes based on their antibiograms. The genetic relatedness among MDR-MRSA isolates was determined by ERIC-PCR-based molecular typing.

Results: High prevalence of MRSA isolates was identified (138/170, 81.2%) with 79% of isolates (109/138, 79%) being MDR-MRSA. MRSA isolates were resistant to diverse classes of antimicrobials including β-lactams, aminoglycosides and macrolides. Among MRSA isolates, the highest resistance rate was to each cefoxitin and penicillin (100%) and the highest susceptibility was to linezolid (92%). Based on the antibiograms of 109 MDR-MRSA isolates, 52 antibiotypes were determined, and 46 different ERIC fingerprints were identified among MDR-MRSA antibiotypes.

Conclusions: MRSA infections remain a noteworthy problem in Egyptian hospitals. MDR-MRSA isolates showed significant genetic diversity indicating the alarmingly high prevalence. Studies should be performed frequently, even in each healthcare setting, to determine the epidemiology of MRSA isolates and their antimicrobial susceptibility profiles for effective control measures of MRSA infections and better healthcare management.

Key words: Methicillin resistance; *Staphylococcus aureus*; MDR-MRSA; ERIC-PCR; Egypt.

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Introduction

Staphylococcus aureus is a major human Gram-positive pathogen that causes both community and healthcare settings infections [1]. *S. aureus* can cause localized cutaneous infections (such as folliculitis), pneumonia, osteomyelitis, endocarditis, food poisoning, and bacteremia. Further, *S. aureus* infections may progress to life-threatening diseases [2]. For the treatment of *S. aureus* infections, penicillin had been recognized as the drug of choice in the 1940s, however, it becomes non-effective against staphylococcal infections due to the production of the β-lactamase enzyme. These β-lactamase-producing *S. aureus* infections could be treated by penicillinase stable penicillins like cloxacillin [3]. Moreover, *S. aureus* strains that are resistant to oxacillin and/or methicillin are termed methicillin-resistant *S. aureus* (MRSA) [4,5]. MRSA was first identified in 1961, causing life-threatening hospital-acquired infections [3].

In the last two decades, MRSA infections have been emerging as a major cause of morbidity and mortality in healthcare settings worldwide [2]. Methicillin resistance (MR) primarily results from the expression of low-affinity penicillin-binding protein PBP2a, encoded by the *mecA* gene, that is located on the staphylococcal cassette chromosome *mec* (SCC*mec*) mobile genetic element resulting in resistance to β-lactams antimicrobials [4,5]. Thus, β-lactams are not the drugs of choice for the treatment of infections caused by methicillin-resistant members of the *Staphylococcus* species [6]. Thirteen different types of SCC*mec* (I to XIII) have been defined, five of which (I – V) are globally disseminated [7].

The emergence of MRSA has further complicated the healthcare management of patients with staphylococcal infections, increasing the duration of hospital stay and cost and decreasing the therapeutic efficacy of the available antimicrobial drugs [8]. MRSA strains are usually highly resistant to multiple antimicrobial classes including aminoglycoside, lincosamides, macrolides as well as β-lactam drugs. Consequently, the spread of multi-drug resistant methicillin-resistant *S. aureus* (MDR-MRSA) limits the efficacy of therapeutic choices for staphylococcal infections and exacerbates their clinical outcomes; representing a worse condition worldwide [2,7]. Consequently, MDR-MRSA infections have become a serious health challenge for clinicians [9].

In Egypt, many previous studies reported variable prevalences of MRSA infections, for instance, 44% [10], 52% [11], 63% [12], 73.7% [4] and 81.5% [13], the prevalence rates that high among African countries [11,14]. In addition, keeping in view the pathogenic potentials of MRSA and the emergence of multidrug resistance, studies should be performed frequently to determine MRSA epidemiology and antimicrobial susceptibilities for better healthcare management. Accordingly, the present study aimed to investigate the occurrence of MDR-MRSA in Egyptian hospitals, and determine the antimicrobial resistance profiles and the genetic relatedness of MDR-MRSA isolates for epidemiological assessment.

Methodology

Collection and identification of S. aureus isolates

A total of 170 *S. aureus* clinical isolates were collected from two tertiary healthcare hospitals in Cairo, Al-Sayed Galal University hospital (91/170, 53.5%) and Al-Demerdash University hospital (79/170, 46.5%), between September 2017 and December 2018. These *S. aureus* isolates were recovered from different clinical samples, collected from inpatients and outpatients admitted to the study hospitals, including wound swabs, blood, sputum, urine, pus of an abscess, eye swabs, intravenous (IV) catheters, endotracheal aspirates (ETAs), and urinary catheters. Collectively, the frequency of *S. aureus* isolates among males and females in both hospitals was 58.2% (99/170) and 41.8% (71/170), respectively. Regarding age, the rate of isolation of *S. aureus* among patients aged from 0 to 20 years was 17%, from 21 to 40 years was 30.6%, from 41 to 60 years was 30% and patients older than 60 years was 22.4%.

The sample collection and preliminarily identification of *S. aureus* isolates were performed in the included hospitals for the regular medical care of patients by dedicated members. Then, bacterial isolates were collected and transferred to the microbiology laboratory at Faculty of Pharmacy, Al-Azhar University and identified according to Procop *et al.* [15] using conventional microbiological methods including Gram staining, growth characteristics on blood agar and the selective medium mannitol salt agar, and biochemical reactions. Biochemical tests included catalase test, modified oxidase test, bacitracin susceptibility test, coagulase test, deoxyribonuclease (DNase) test, novobiocin susceptibility, carbohydrate fermentation, urease production, Voges Proskauer and nitrate reduction tests [15].

Determination of antimicrobial susceptibility patterns and antibiogram-based typing

Antimicrobial susceptibility patterns of *S. aureus* isolates were determined using the Kirby-Bauer disk diffusion method on Mueller Hinton Agar (MHA) (Oxoid, Hampshire, UK) following Clinical and Laboratory Standards Institute (CLSI) guidelines [16]. Sixteen antimicrobial disks (Oxoid, Hampshire, UK), representing different groups of antimicrobial agents, were used in this study including amikacin (AK, 30 µg), azithromycin (AZM, 15 µg), cefoxitin (FOX, 30 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), clindamycin (DA, 2 µg), doxycycline (DO, 30 µg), erythromycin (E, 15 µg), gentamicin (CN, 10 µg), levofloxacin (LEV, 5 µg), linezolid (LZD, 30 µg), penicillin (P, 10 units), rifampicin (RD, 5 µg), tetracycline (TE, 30 µg), teicoplanin (TEC, 30 µg), and trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg). Results were obtained by measuring the inhibition zones (average of 3 readings at 3 different angles) developed around each antimicrobial disk in millimeter (mm) and interpreted as susceptible (S), intermediate (I) or resistant (R) according to CLSI criteria [16]. The isolate was verified MDR when it shows resistance to at least three different antimicrobial classes [2]. MDR-MRSA isolates were grouped based on their antimicrobial resistance patterns (antibiograms) against the tested 16 antimicrobial agents.

Phenotypic assays for identification of methicillin resistance in S. aureus isolates

Cefoxitin disk diffusion method

The assay was carried out by preparing a suspension of each isolate, equivalent to 0.5 McFarland turbidity standard, and the culture was lawn on Muller Hinton Agar (MHA) plate. A 30 µg cefoxitin disk is placed on the surface of MHA and plates were incubated

aerobically at 37 ºC for 24 hours, then the diameter of the inhibition zone was measured. A zone diameter of ≥ 22 mm was interpreted as sensitive and ≤ 21 mm was considered as resistant [9,17].

Mannitol salt agar-cefoxitin screening test

A suspension of each *S. aureus* isolate, equivalent to 0.5 McFarland, in Muller Hinton broth was inoculated onto mannitol salt agar plates supplemented with cefoxitin (concentration of 6 µg/mL). After incubation for 24 hours at 35 ºC, any growth on the plate was interpreted as a positive result of MR [18].

Oxacillin agar screening test

S. aureus isolates were grown on MHA supplemented with 4% NaCl and oxacillin (concentration of 6 µg/mL). These MHA plates were inoculated by the swabbing of the surface with bacterial suspension (0.5 McFarland). MR was confirmed by bacterial growth after 24 hours of incubation at 37 ºC [17,19].

DNA extraction from S. aureus isolates and PCR oligonucleotide primers

Chromosomal DNA was extracted from isolates using Gene JET Genomic DNA Purification Kit (Thermo Scientific, Waltham, Massachusetts, USA-K0721) according to the manufacturer's instructions. DNA preparations were stored in aliquots at -20 ºC. The PCR oligonucleotide primers used in this study were the product of Willowfort (Birmingham, UK). The lyophilized primers were reconstituted in nuclease-free water and the concentration of each primer was adjusted to 10 pmole/µL.

Molecular identification of MRSA isolates by nuc-directed PCR assay

The *S. aureus* isolates, identified by conventional methods, were confirmed by PCR amplification of the surrogate marker of *S. aureus* species *nuc* gene [2]. The previously published PCR primers were used (forward primer*:* 5′-GCG ATT GAT GGT GAT ACG GTT-3′ and reverse primer: 5′-AGC CAA GCC TTG ACG AAC TAA AGC-3′) [3]. The reaction mixture, set up in a total volume of 20 µL, contained 10 µL of Cosmo PCR Red master mix (Willowfort, Birmingham, UK), 1 µL of template DNA, 1 µL of forward primer, 1 µL of reverse primer and 7 µL nuclease-free water. The PCR conditions: initial denaturation at 94 ºC for 5 minutes, followed by 30 cycles of denaturation at 94 ºC for 30 seconds, annealing at 50 ºC for 30 seconds, extension at 72 ºC for 30 seconds, and final extension at 72 ºC for 7 minutes [3].

Genotypic identification of MRSA isolates by mecA-directed PCR

The isolates were PCR examined for methicillin resistance-encoding gene, *mecA* gene, by PCR. The sequences of the PCR primers (forward primer: 5′-GTG AAG ATA TAC CAA GTG ATT-3′ and reverse primer: 5′-ATG CGC TAT AGA TTG AAA GGA T-3′) were obtained from McClure-Warnier *et al.* [20]. The PCR reaction was set up in a total volume of 20 µL by adding 10 µL of the Cosmo PCR Red master mix (Willowfort, Birmingham, UK), 1 µL of the forward primer and 1 µL of reverse primer, 1 µL of template DNA and the volume was completed to 20 µL by addition of 7 µL nuclease-free water. The PCR conditions: initial denaturation at 94 ºC for 5 minutes, followed by 35 cycles of denaturation at 94 ºC for 30 seconds, annealing at 49 ºC for 30 seconds, extension at 72 ºC for 30 seconds, and final extension at 72 ºC for 7 minutes [20,21].

ERIC-PCR-based molecular typing of the selected MDR-MRSA isolates

Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) DNA fingerprinting was carried out to determine the genetic diversity among MDR-MRSA isolates. ERIC primers sequences ERIC-1 primer (5′-ATG TAA GCT CCT GGG GAT TCA C-3′) and ERIC-2 primer (5′-AAG TAA GTG ACT GGG GTG AGC G -3′), were obtained from Candan *et al.* [22]. The PCR reactions were prepared in total volumes of 25 µL, contained 12.5 µL of Cosmo PCR Red master mix (Willowfort, Birmingham, UK), 1 µL of template DNA, 1 µL of ERIC-1 primer, 1 µL of ERIC-2 primer and the volume was completed to 25 µL by adding nuclease-free water. The PCR amplifications were carried out in the thermal cycler (Biometra UNO-Thermoblock, Germany) programmed for an initial denaturation at 94 ºC for 5 minutes and 40 cycles of denaturation at 95 ºC for 1 minute, primer annealing at 45 ºC for 1 minute and extension at 72 ºC for 8 minutes followed by a final extension at 72 ºC for 10 minutes [22,23].

Detection of amplified PCR products by TBE (Tris-borate-EDTA) agarose gel electrophoresis

PCR products were resolved through TBE agarose gel (0.8%) electrophoresis prepared using molecular biology grade agarose (GIBCO Bethesda Research Lab.; Life Technologies, Grand Island, NY, USA) in 1 × TBE buffer (Thermo Scientific, Waltham,

Massachusetts, USA). DNA fragments were electrophoresed (at 100 V and 90 mA for 30 minutes) in the horizontal gel electrophoresis apparatus (Cole Parmer, Germany), stained with ethidium bromide (Alliance Bio, Bothell, Washington, USA), and visualized by placing on a UV transilluminator (Biometra, Göttingen, Germany) and photographed directly. For the sizing of the separated DNA fragments, Gene Ruler 1 Kb DNA ladder (Thermo Scientific, Waltham, Massachusetts, USA) was used.

ERIC-based patterns analyses

The obtained ERIC patterns were clustered by dendrogram generated with the Dice similarity coefficient and the unweighted pair group method with arithmetic averages (UPGMA) clustering method using the DendroUPGMA tool available at http://insilico.ehu.es/dice_upgma/.

Statistical analysis

Results were presented as descriptive statistics in terms of relative frequency and percentages. ERIC-based fingerprints were analyzed using the Dice similarity coefficients of similarity and a dendrogram was constructed using the UPGMA method.

Results

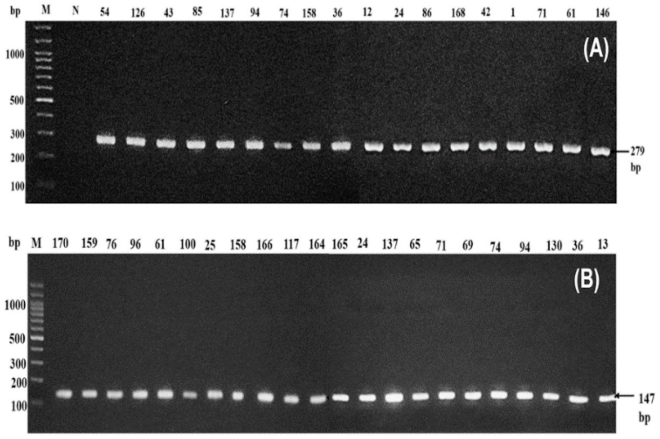
Identification and frequencies of S. aureus isolates recovered from different clinical samples

The 170 *S. aureus* isolates recovered in this study showed the cultural characteristics and positive biochemical reactions of *S. aureus* species. Furthermore, *nuc* gene, the surrogate marker of *S. aureus* was detected in all 170 phenotypically identified isolates that produced the expected amplicon of 279 bp, consequently, they were identified as *S. aureus* species (Figure 1A). Concerning the included hospitals, the frequencies of collected *S. aureus* isolates were 53.5% (91/170) from Al-Sayed Galal University hospital and 46.5% (79/170) from Al-Demerdash University hospital. The prevalence of *S. aureus* isolates regarding the source of clinical specimens showed that the highest percentage of isolates was from wound swabs (34.1%), followed by blood 15.3%, each IV catheters and sputum (10%), each urine and pus of abscess (8.8%), each eye swabs and ETAs (5.9%) and urinary catheters (1.2%) (Table 1).

Antimicrobial resistance profiles of S. aureus isolates

The antimicrobial susceptibility testing of all *S. aureus* isolates revealed that the highest resistance rate was against each cefoxitin and penicillin of 81.2%, followed by gentamicin (49.4%), doxycycline (48.8%) and tetracycline (47.6%). The antimicrobials linezolid, teicoplanin and rifampicin showed the highest susceptibility rates of 93.5%, 82.9% and 75.2%, respectively. According to the antimicrobial

Figure 1. PCR-based detection of *nuc* and *mecA* genes in *S. aureus* isolates.



Representative agarose gel (0.8%) electrophoresis of PCR products of amplified *nuc* gene (A) and *mecA* gene (B) from *S. aureus* and MRSA isolates, respectively. Lane M; 100-bp ladder size marker, lane N; Negative control, and other lanes in each panel are *nuc*- and *mecA*-directed PCR positive results giving the expected PCR products of 279 bp and 147 bp, respectively.

Table 1. The frequencies of *S. aureus* isolates from different clinical samples.

Sample type	*S. aureus* isolates	MRSA isolates	MDR-MRSA isolates
Wound swabs	58[1] (34.1%)[2]	47[1] (34.1%)[3]	36[1] (33%)[4]
Blood	26 (15.3%)	23 (16.7%)	19 (17.4%)
Sputum	17 (10%)	12 (8.7%)	8 (7.3%)
Urine	15 (8.8%)	12 (8.7%)	10 (9.2%)
Pus of abscess	15 (8.8%)	13 (9.4%)	10 (9.2%)
Eye swabs	10 (5.9%)	6 (4.3%)	5 (4.6%)
IV catheters	17 (10%)	13 (9.4%)	11 (10.1%)
Urinary catheters	2 (1.2%)	2 (1.4%)	2 (1.9%)
ETAs[5]	10 (5.9%)	10 (7.3%)	8 (7.3%)
Total	170 (100%)	138 (100%)	109 (100%)

[1] Number of isolates; [2] Percentage calculated to the total number of *S. aureus* isolates (n = 170); [3] Percentage calculated to the total number of MRSA isolates (n = 138); [4] Percentage calculated to the total number of MDR-MRSA isolates (n = 109); [5] ETAs, endotracheal aspirates.

Table 2. Antimicrobial resistance profiles of all *S. aureus* isolates included in this study.

Antimicrobial agent	Susceptible (S)		Intermediate (I)		Resistant (R)	
	No.	%[1]	No.	%	No.	%
Amikacin	122	71.7	11	6.5	37	21.8
Azithromycin	120	70.6	4	2.4	46	27
Cefoxitin	32	18.8	–	–	138	81.2
Chloramphenicol	127	74.7	2	1.2	41	24.1
Ciprofloxacin	116	68.2	9	5.3	45	26.5
Clindamycin	109	64.1	11	6.5	50	29.4
Doxycycline	70	41.2	17	10	83	48.8
Erythromycin	86	50.6	20	11.8	64	37.6
Gentamicin	72	42.4	14	8.2	84	49.4
Levofloxacin	107	62.9	20	11.8	43	25.3
Linezolid	159	93.5	–	–	11	6.5
Penicillin	32	18.8	–	–	138	81.2
Rifampicin	128	75.2	21	12.4	21	12.4
Tetracycline	78	45.9	11	4.5	81	47.6
Teicoplanin	141	82.9	19	11.2	10	5.9
Trimethoprim/Sulfamethoxazole	124	72.9	27	15.9	19	11.2
MDR isolates	109/170 (64.1%)					

[1]Percentage calculated to the total number of isolates (n = 170).

susceptibility profiles, 64.1% (109/170) of *S. aureus* isolates in this study were MDR (Table 2).

Prevalence of methicillin resistance among S. aureus isolates

Based on the results of phenotypic assays for identification of MR among 170 *S. aureus* isolates, 81.2% (138/170) of isolates were MRSA with the same rate from both hospitals of study; while 18.8% were methicillin-sensitive *S. aureus* (MSSA). The phenotypically identified 138 MRSA isolates harbored the methicillin resistance-encoding gene *mecA*, as indicated by the detection of the expected amplicon of 147 bp in the *mecA*-directed PCR assay (Figure 1B).

The distribution of MRSA isolates from different clinical samples were 34.1% from wounds, 16.7% from blood, 9.4% from each abscess and IV catheters, 8.7% from each sputum and urine, 7.4% from ETAs, 4.3% from eye swabs, and 1.4% from urinary catheters (Table 1). The specimen-wise distribution showed that MSSA vs MRSA in blood was (11.5% vs 88.5%), in wounds (19% vs 81%), in sputum (29.5% vs 70.6%), in urine (20% vs 80%), in abscess (13.3% vs 86.7%), in eye swabs (40% vs 60%), in IV catheters (23.5% vs 76.5%), and in urinary catheters and ETAs (0% vs 100%) (Figure 2).

Similar to the entire collection of isolates, the antimicrobial resistance patterns of MRSA isolates showed that the highest resistance rate was for each

Table 3. Antimicrobial resistance profiles of MRSA isolates.

Antimicrobial agent	Susceptible (S)		Intermediate (I)		Resistant (R)	
	No.	%[1]	No.	%	No.	%
Amikacin	93	67.4	9	6.5	36	26.1
Azithromycin	89	64.5	3	2.2	46	33.3
Cefoxitin	–	–	–	–	138	100
Chloramphenicol	100	72.5	–	–	38	27.5
Ciprofloxacin	88	63.8	7	5.1	43	31.1
Clindamycin	80	58	8	5.7	50	36.3
Doxycycline	47	34.1	12	8.7	79	57.2
Erythromycin	62	44.9	12	8.7	64	46.4
Gentamicin	50	36.3	14	10.1	74	53.6
Levofloxacin	78	56.5	19	13.8	41	29.7
Linezolid	127	92	–	–	11	8
Penicillin	–	–	–	–	138	100
Rifampicin	96	69.6	21	15.2	21	15.2
Tetracycline	52	37.7	8	5.8	78	56.5
Teicoplanin	109	79	19	13.8	10	7.2
Trimethoprim/Sulfamethoxazole	95	68.8	24	17.4	19	13.8
MDR-MRSA	109/138 (79%)					

[1] Percentage calculated to the total number of MRSA isolates (n = 138).

cefoxitin and penicillin (100%), followed by doxycycline (57.2%), tetracycline (56.5%) and gentamicin (53.6%), while the highest susceptibility was to linezolid (92%), followed by teicoplanin (79%) and chloramphenicol (72.5%). The rate of MDR-MRSA isolates was 109/138 (79%) (Table 3). The highest frequency of MDR-MRSA isolates was found among those isolates recovered from wounds 33% (36/109), followed by blood 17.4% (19/109), and IV catheters 10.1% (11/109) (Table 1).

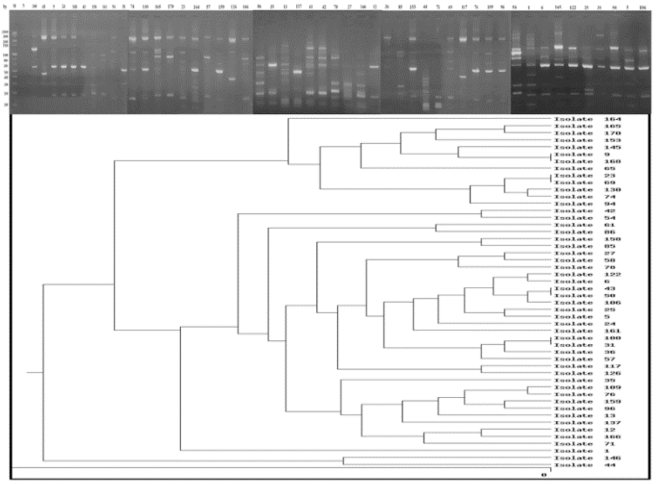
Antibiogram-based typing of MDR-MRSA isolates

Based on the antibiogram patterns of the 109 MDR-MRSA isolates identified in this study, 52 antibiotypes were determined. These antibiotypes were arbitrarily given a number from 1 to 52. The more frequent antibiotype was antibiotype 16 included 10 isolates (10/109, 9.2%) followed by antibiotypes 5 and 17 which each included 9 isolates (9/109, 8.2%) (Table 4).

ERIC-PCR-based genotyping of the selected MDR-MRSA isolates

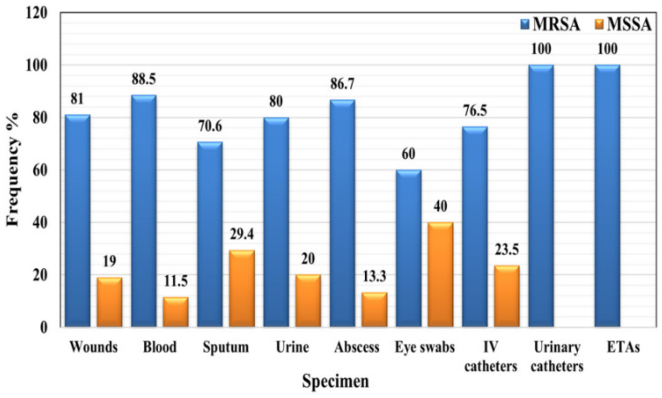
ERIC-PCR-based genotyping of 50 MDR-MRSA isolates, representing highly resistant antibiotypes, revealed a significant molecular heterogeneity of these isolates, which is indicated by 46 different ERIC-based patterns or fingerprints. These fingerprints were arbitrarily given a number from 1 to 46. Moreover, four patterns only included 2 isolates (2/50, 4%) in each pattern and the other patterns included only one isolate (1/50, 2%) (Figure 3).

Figure 3. ERIC-based PCR patterns of the selected 50 MDR-MRSA isolates representing different antibiotypes.



Dendrogram of ERIC patterns-based typing of MDR-MRSA in this study. n; the number of isolates in each ERIC fingerprint. Cluster analysis was generated with the Dice similarity coefficient and the UPGMA clustering method.

Figure 2. The distribution of MRSA and MSSA isolates from various clinical specimens.



Discussion

Globally, *S. aureus* infections exhibit an increasing prevalence of MRSA strains. In addition, MRSA has become typically resistant to various classes of antimicrobials, particularly almost all available β-lactams because MRSA strains are acquiring resistance genes [3]. Thus, the effectiveness of the available antimicrobial therapeutic agents is limited for staphylococcal infections and worsens their clinical outcomes. Consequently, in the last two decades, MRSA has become a major cause of morbidity and mortality in both hospitalized patients and healthy individuals [9]. Accordingly, this study was carried out to assess the prevalence of MDR-MRSA among *S. aureus* isolates collected from tertiary Egyptian hospitals, investigate their antimicrobial susceptibility patterns, as well as study the genetic diversity of the isolates.

In this study, 170 *S. aureus* isolates, recovered from different clinical samples, were collected from two tertiary hospitals in Cairo. The identification of *S. aureus* species isolates in the current study was confirmed by the detection of *nuc* gene (100%) which is considered a surrogate marker for the molecular-based quick and reliable method for identification of *S. aureus* species [3,24,25]. The highest frequency of isolates, based on the type of samples, was from wound infections (34.1%), followed by bloodstream infections (15.3%), then respiratory tract infections (10%). These results revealed that wound infections or wound swabs were the major sources of *S. aureus* isolates in the current study. This finding is consistent with the results of the Hadyeh *et al.* [26] study from Palestine which reported the highest number of *S. aureus* isolates was recovered from wound infections or swabs (35.7%), followed by bloodstream infections (12.5%). In

addition, the results of the present study were consistent with the Pakistani study of Akram *et al.* [27] which reported the higher percentage of *S. aureus* isolates was 49% from wound infections. However, another study from Nepal by Sapkota *et al.* [28] reported that the frequency of *S. aureus* isolates from wound infections (23.3%) was second to that from the skin infections at 55.6%, then bloodstream infections (11.3%).

The results of the antimicrobial susceptibility testing in the current study demonstrated high resistance profiles of *S. aureus* isolates. The 170 *S. aureus* isolates exhibited the highest resistance frequencies to the β-lactam antimicrobial agents of 81.2% to each cefoxitin and penicillin. That is could be partly attributed to the extensive use of these safest antimicrobials, particularly as over counter drugs in Egypt. Many previous studies from other developing

Table 4. Antibiogram pattern-based typing of MDR-MRSA isolates.

Antibiotype	Antibiogram pattern	Isolates, N (%)[1]
1	Resistant to P, FOX, E and DA	2 (1.8%)
2	Resistant to P, FOX, AZM and CIP	4 (3.7%)
3	Resistant to P, FOX, AK, E and DA	1 (0.9 %)
4	Resistant to P, FOX, CN, CIP and LEV	3 (2.7%)
5	Resistant to P, FOX, CN, TE and DO	9 (8.3%)
6	Resistant to P, FOX, TE, DO and C	7 (6.4%)
7	Resistant to P, FOX, CN, E and TE	3 (2.7%)
8	Resistant to P, FOX, TE, DO and CIP	2 (1.8%)
9	Resistant to P, FOX, AZM, E and SXT	1 (0.9%)
10	Resistant to P, FOX, E, CIP and C	1 (0.9%)
11	Resistant to P, FOX, CN, AZM and TE	1 (0.9%)
12	Resistant to P, FOX, AK, AZM, E and DA	1 (0.9%)
13	Resistant to P, FOX, CN, TE, DO and CIP	3 (2.7%)
14	Resistant to P, FOX, CN, TE, DO and SXT	1 (0.9%)
15	Resistant to P, FOX, AZM, TE, CIP and RD	1 (0.9%)
16	Resistant to P, FOX, CN, E, TE, DO and DA	10 (9.2%)
17	Resistant to P, FOX, CN, E, TE, DO and C	9 (8.3%)
18	Resistant to P, FOX, CN, AK, AZM, CIP and LEV	4 (3.7%)
19	Resistant to P, FOX, AK, AZM, TE, CIP and LEV	1 (0.9%)
20	Resistant to P, FOX, CN, E, TE, DO, DA and RD	1 (0.9%)
21	Resistant to P, FOX, AZM, E, TE, DO, LEV and C	1 (0.9%)
22	Resistant to all antimicrobials except C	1 (0.9%)
23	Resistant to all antimicrobials except RD	1 (0.9%)
24	Resistant to all antimicrobials except LZD	2 (1.8%)
25	Resistant to all antimicrobials except TEC	1 (0.9%)
26	Resistant to all antimicrobials except TE and DA	1 (0.9%)
27	Resistant to all antimicrobials except RD, TEC and LZD	1 (0.9%)
28	Resistant to all antimicrobials except SXT, C and LZD	2 (1.8%)
29	Resistant to all antimicrobials except SXT, C and TEC	2 (1.8%)
30	Resistant to all antimicrobials except C, TEC and LZD	1 (0.9%)
31	Resistant to all antimicrobials except C, RD and LZD	3 (2.7%)
32	Resistant to all antimicrobials except DA, C and LZD	1 (0.9%)
33	Resistant to all antimicrobials except CIP, LEV and C	1 (0.9%)
34	Resistant to all antimicrobials except E, DA, C and TEC	1 (0.9%)
35	Resistant to all antimicrobials except SXT, C, TEC and LZD	2 (1.8%)
36	Resistant to all antimicrobials except AK, AZM, C and LZD	1 (0.9%)
37	Resistant to all antimicrobials except CN, E, DA and LZD	1 (0.9%)
38	Resistant to all antimicrobials except CIP, LEV, C and LZD	1 (0.9%)
39	Resistant to all antimicrobials except AK, AZM, SXT and LZD	1 (0.9%)
40	Resistant to all antimicrobials except CN, E, DA, SXT and LZD	1 (0.9%)
41	Resistant to all antimicrobials except AK, TE, CIP, C and LZD	1 (0.9%)
42	Resistant to all antimicrobials except AK, CIP, DA, TEC and LZD	1 (0.9%)
43	Resistant to all antimicrobials except AK, SXT, C, TEC and LZD	2 (1.8%)
44	Resistant to all antimicrobials except E, TE, DO, DA, C and LZD	2 (1.8%)
45	Resistant to all antimicrobials except AK, AZM, CIP, C, TEC and LZD	4 (3.7%)
46	Resistant to all antimicrobials except E, TE, DO, C, RD and LZD	1 (0.9%)
47	Resistant to all antimicrobials except CN, TE, DO, LEV, C, RD and LZD	1 (0.9%)
48	Resistant to all antimicrobials except AK, AZM, CIP, LEV, SXT, RD and LZD	1 (0.9%)
49	Resistant to all antimicrobials except AK, AZM, CIP, LEV, SXT, TEC and LZD	2 (1.8%)
50	Resistant to all antimicrobials except AK, AZM, CIP, LEV, RD, TEC and LZD	1 (0.9%)
51	Resistant to all antimicrobials except CN, E, TE, DA, RD, TEC and LZD	1 (0.9%)
52	Resistant to all antimicrobials except AK, TE, DO, C, RD, TEC and LZD	1 (0.9%)

[1] Percentage calculated to the total number of MDR-MRSA isolates (n = 109). AK: amikacin; AZM: azithromycin; C: chloramphenicol; CIP: ciprofloxacin; CN: gentamicin; DA: clindamycin; DO: doxycycline; E: erythromycin; FOX: cefoxitin; LEV: levofloxacin; LZD: linezolid; P: penicillin; RD: rifampicin; SXT: trimethoprim/sulfamethoxazole; TE: tetracycline and TEC: teicoplanin.

countries showed almost comparable higher rates of resistance to penicillins [2,28-30]. *S. aureus* isolates showed a resistance frequency of 49.4% to gentamicin in the current study. This rate is relatively close to the frequencies recorded in previous studies where the rates of resistance to gentamicin were 46.8%, 52% or 54.5% [28,31,4]. However, the frequency in this study was inconsistent with studies of Yu *et al.* [29], Raut *et al.* [5] and Naimi *et al.* [30] as they revealed lower resistance rates to gentamicin of 21.9%, 25.5% and 32.9%, respectively. This may be due to the frequent use of aminoglycosides including gentamicin in the hospitals included in this study. On the other hand, there was a low rate of resistance to amikacin of 21.8% among *S. aureus* isolates which is consistent with the studies of Sapkota *et al.* [28] and Raut *et al.* [5] which showed rates of resistance to amikacin of 15.96% and 7.5%, respectively. The high activity of amikacin can be explained by the presence of the amino hydroxy butyryl group, which usually prevents the enzymatic modification of amikacin at multiple positions without interfering with binding to the A site of rRNA [32].

The resistance rate to chloramphenicol among *S. aureus* isolates was 24.1%, which is rather lower than other studies from Afghanistan, Brazil and Pakistan that recorded frequencies of 30%, 35.2% and 58.6%, respectively [30,31,33], although, it was higher than rates recorded by the studies of Yu *et al.* [29] and Raut *et al.* [5] of 10.2% and 15.2%, respectively. The low level of resistance to chloramphenicol in the current study may be owing to chloramphenicol is not being widely used in the treatment of bacterial infections as in previous ages. *S. aureus* isolates showed almost similar resistance rates to fluoroquinolones drugs ciprofloxacin and levofloxacin of 26.5% and 25.3%, respectively. This finding is similar to the rate recorded in the Chinese study of Yu *et al.* [29] which revealed a resistance rate of 25% to ciprofloxacin. This low rate of resistance can be explained by the infrequent use of fluoroquinolones for the treatment of staphylococcal infections. However, other previous studies showed higher rates of resistance to ciprofloxacin among *S. aureus* isolates ranging from 33% to 100% [5,28,30,31,33,34]. While Adeiza *et al.* [9] study from Nigeria reported a low rate of resistance to levofloxacin of 12%. The rate of resistance to the macrolide drugs erythromycin and azithromycin were 37.6% and 27%, respectively. The erythromycin resistance rate was relatively similar to the rates recorded by the studies of Adeiza *et al.* [9] and Raut *et al.* [5] of 33.3%, and 48%, respectively. In contrast, other studies showed higher resistance rates to erythromycin ranging from 60% to 96% [28-31]. Regarding the tetracyclines, slightly high rates of resistance among *S. aureus* isolates to doxycycline and tetracycline of 48.8% and 47.6%, respectively, were determined. Other studies by Caboclo *et al.* [31] from Brazil and Malik *et al.* [6] from Pakistan showed higher resistance rates to tetracycline and doxycycline of 61.6% and 70.5%, respectively, while other studies from Nepal, Nigeria and Afghanistan showed lower rates of resistance to tetracyclines [5,9,30]. The present study showed that *S. aureus* isolates exhibited a resistance rate to clindamycin of 29.4%. This rate is higher than the rate of resistance of 22.5% recorded by the study of Raut *et al.* [5]. However, the results of previous studies revealed higher rates of resistance to clindamycin ranging from 39.8% to 89.9% [34,9,29,31]. In addition, the current study revealed a low resistance rate to trimethoprim/sulfamethoxazole (11.2%). This finding is consistent with the study of Eksi *et al.* [34] from Turkey which showed a resistance rate to trimethoprim/sulfamethoxazole of 11%. This may be attributed to less usage of this drug in hospitals of study as it is formed only in oral dosage forms which are infrequently used in hospitalized patients, especially in ICUs patients. However, other studies showed higher resistance rates to trimethoprim/sulfamethoxazole ranging from 23.4% to 61.6% [5,28-31]. These differences in resistance rates to different antimicrobials are also a result of the different outlines of using antimicrobial drugs across different countries and/or ages as well as the emergence of more resistant strains.

Importantly, this study revealed the most effective antimicrobial agents against *S. aureus* isolates are linezolid 93.5%, followed by teicoplanin 82.9% and rifampicin 75.2%. This finding is consistent with many previous studies that showed almost all *S. aureus* isolates were susceptible to linezolid and teicoplanin [2,5,28,29,31]. Another study from Afghanistan by Naimi *et al.* [30] showed that one of the most effective antimicrobial agents against *S. aureus* isolates was rifampicin (95.2%), which is relatively consistent with the results of the current study. Consequently, although antimicrobial classes like glycopeptides, oxazolidinones, and rifamycins can be used as empirical therapy, they should be carefully used only in MRSA critical cases, as these antibiotics should be saved for use in the future due to the insufficient resources in developing countries like Egypt.

MR in staphylococci is mediated by the *mecA* gene, which encodes for PBP2a, with decreased affinity for the β-lactam antibiotics [2]. PBP2a enables

transpeptidase activity in the presence of β-lactams, preventing them from inhibiting cell wall synthesis. The *mecA* gene is extensively distributed among *Staphylococcus* species, which may be due to horizontal transmissions among *Staphylococci* strains [26,33]. According to CLSI guidelines, isolates that test resistant to oxacillin and cefoxitin should be reported as methicillin-resistant [16], however molecular detection of the *mecA* gene is the most reliable method for the identification of MRSA [1]. Although oxacillin has been the agent recommended by CLSI for the prediction of MR, routine oxacillin tests usually fail to detect very heterogeneous MRSA populations especially low-level MR strains which consequently are considered MSSA. Therefore, cefoxitin has been also reported as a surrogate marker for the detection of MR by disk diffusion method due to the greatest accuracy for MR characterization [8,19,35]. In this study, MR was detected phenotypically by disk diffusion method using cefoxitin (30 μg) disk and confirmed by PCR-amplification of the *mecA* gene; this is consistent with the Iranian study carried out by Sahebnasagh *et al.* [3]. Mannitol salt agar-cefoxitin screening test and oxacillin agar screening test has been extensively used for the prediction of MR with wide variation in the reported sensitivity [19]. The current study revealed that the cefoxitin disk was the best predictor of MR where 100% of cefoxitin resistant isolates harbored *mecA* gene as detected by PCR. Regarding oxacillin, it was less sensitive as it showed false-negative results in 9% of MRSA isolates as shown in the study of Boubaker *et al.* [36] from Tunisia where they concluded that the cefoxitin disk test (specificity 100%, sensitivity 96.5%) was better than the oxacillin disk methods (specificity 99.1%, sensitivity 90.4%). Moreover, considering that detection of the *mecA* gene by PCR method is a gold standard method for identifying MRSA isolates. In this study, all tested MRSA isolates harbored *mecA* gene which is consistent with many previous studies [3,19,21,25,29,37].

In the present study, 81.2% of *S. aureus* isolates were MRSA. This finding is almost comparable to the percentages recorded in many previous studies from different countries, for instance, Polish study of Rozanska *et al.* [38], Nepali study of Sapkota *et al.* [28], Egyptian study of Hassan *et al.* [4] and Iranian study of Sahebnasagh *et al.* [3], revealed the percentages of MRSA isolates were 67%, 70.64%, 73.3%, and 78.2%, respectively. While other studies from Iran and India showed higher rates of MRSA of about 90% [2,39]. This can be attributed to the continuous development of MRSA due to the persistent misuse of antibiotics. On the other hand, other studies showed lower resistance rates ranging from 43.6% to 55.3% [1,5,30,34]. These findings suggested that β-lactams are not suitable for the initiation of empirical therapy of staphylococcal infections in the hospitals of study. This may be explained by the overuse of these safe antimicrobial agents in developing countries like Egypt. Moreover, resistance to cefoxitin, penicillin and other β-lactams among staphylococcal isolates in this study may be also related to increasing the use of other β-lactam antibiotics in hospitals or acquisition of resistance during hospitalization [40]. Regarding the source samples, the prevalence of MRSA isolates was significantly different among various clinical samples with the highest rate of MRSA was recovered from wound swabs (34%). This finding is consistent with previous studies by Hassan *et al.* [4], Goudarzi *et al.* [2], Garoy *et al.* [41] and Fatholahzadeh *et al.* [37] which showed that the highest frequencies of MRSA isolates were from wound swabs of 32.9%, 30%, 35.6% and 59.6%, respectively.

Comparing the frequencies of both MRSA and MSSA based on sample type, the prevalence rate of MRSA was significantly higher than MSSA among isolates from each sample type. This finding was consistent with previous studies by Naimi *et al.* [30] and Sahebnasagh *et al.* [3] which showed that the prevalence of MRSA strains in all clinical samples was higher than MSSA, however, in the Nepali study of Raut *et al.* [5], the prevalence of MSSA was higher than MRSA. This difference in MRSA prevalence among *S. aureus* recovered from different samples may be due to the discrepancy in antibiotics utilization, infection control procedures and extended antimicrobial treatment of hospitalized patients. In addition, comparing the antimicrobial sensitivity patterns of the MRSA and the MSSA in this study revealed that the MRSA had a higher level of resistance to various classes of antimicrobials than the MSSA. Many studies have reported similar findings showing higher antimicrobial resistance profiles among MRSA isolates [28-30,42]. In this study, the highest rate of resistance among MRSA isolates was to each cefoxitin and penicillin of 100%, while the highest susceptibility rate was to linezolid (92%), followed by teicoplanin (79%). This finding is consistent with the results of Hassan *et al.* [4] and Fatholahzadeh *et al.* [37] studies from Egypt and Iran.

The development of MDR bacteria to diverse antimicrobials has increased at a terrifying rate worldwide. The regular overuse and misuse of antimicrobials, imprecise diagnosis, improper

prescribing, and non-compliance with antimicrobial therapy by patients have all encouraged the rapid spread of resistance even to modern antimicrobial agents [8,43]. The percentage of MDR isolates in the present study was 79% among MRSA isolates. This rate of MDR-MRSA isolates is higher than the rates recorded by other studies by He *et al.* [44] from China and Goudarzi *et al*. [2] from Iran as they showed that the prevalence of MDR isolates was 60.08% and 57.1%, respectively. Although, this finding is consistent with the rate of MDR-MRSA isolates of 86.36% recorded by the study carried out in Egypt by Hassan *et al.* [4], and Yu *et al.* [29] study revealed a MDR-MRSA percentage of 72.7%. However, Naimi *et al.* [30] study showed a slightly higher rate of MDR-MRSA isolates of 91.4%. This can be attributed to the continuous development of MDR-MRSA strains due to the continued misuse of antibiotics worldwide, in addition to different outlines of antibiotic usage among countries. The high level of MDR bacteria, especially in developing countries, is due to the massive and/or misuse of antimicrobial agents in these countries which results in very limited therapeutic options [45]. It should not be ignored that MDR-MRSA strains can serve as a reservoir of resistance genes and can spread to other microorganisms. In addition, this decrease in the susceptibility of all the previous antimicrobial agents may be due to increasing or cumulative use, or the lack of observance of infection control practices by hospitals. Therefore, to prevent the further spread of MDR-MRSA, the use of antibiotics should be monitored and the implementation of restricted infection control measures. On the other hand, continued use of antibiotics for the treatment of staphylococcal infections should be supported by monitoring antimicrobial susceptibility to prevent the spread of resistant isolates and reduce the use of antibiotics for long times.

Concerning the genetic relatedness of MDR-MRSA isolates, the ERIC-based genotyping data obtained from 50 MDR-MRSA isolates indicated a significant genetic diversity of MDR-MRSA isolates in this study providing that these bacteria were polyclonal and not transmitted between patients in hospitals, since the banding patterns of the isolates from different patients showed extensive diversity. That may help in the epidemiological investigation and understanding more about the disease acquisition and transmission, in addition to studying if there was patient-to-patient transmission or a common source of infection as an exogenous source or if it mostly was of the endogenous source. A total of 46 different ERIC types (fingerprints) were distinguished for the 50 MDR-MRSA isolates. The results of this study also showed that most MRSA isolates produced different genomic fingerprint patterns, therefore, the dissemination source of infection is different. This finding is consistent with the Iranian study of Abdollahi *et al.* [23] who reported that the ERIC–PCR profiles allowed typing of 90 isolates into 75 ERIC types, most of the isolates showed unique patterns which indicate that the rate of transmission of resistant strains was very low in their study hospitals.

Conclusions

This study demonstrates a high prevalence of MDR-MRSA among *S. aureus* isolates causing infections in tertiary care Egyptian hospitals. The PCR-based identification of MRSA isolates, by detection of *nuc* and *mecA* genes, provides rapid detection and identification methods of MRSA and offers a very specific, sensitive and rapid alternative to conventional assays. Because of the high rates of MR, β-lactams should not be the first drugs of choice for the treatment of hospital and community-associated infections caused by MRSA. Antimicrobial classes like glycopeptides, oxazolidinones and rifamycins should be carefully used in MRSA infections. These antibiotics should be saved as a reservoir for use in the future. In this study, amikacin and azithromycin could be considered empirical therapy for staphylococcal infections and other useful antimicrobials include ciprofloxacin and cotrimoxazole. ERIC-PCR-based typing indicated that there was no occurrence of bacterial spread among patients as there was no genetic relatedness among isolates. The implementation of periodic active surveillance for MRSA will be useful to monitor the changes in epidemiology and antimicrobial susceptibility profiles of these bacteria for effective antibiotic selection and infection control practices.

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Corresponding author

Mahmoud M. Tawfick, PhD
Associate Professor of Microbiology and Immunology,
Faculty of Pharmacy, Al-Azhar University.
1 El-Mokhayam El-Daem Street, Nasr City, Cairo, Egypt. 11751.
Phone: 00201157336676
Fax: (+202) 3837-1543
E-mail: mahmoud_tawfick@azhar.edu.eg

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