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Original article

Phenotypic and genotypic detection of macrolide resistance among clinical isolates of staphylococci, Zagazig University Hospitals, Egypt

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ABSTRACT

Background: Macrolide resistance has increased worldwide among Gram-positive cocci including staphylococci, particularly after the irrational use of macrolides during the COVID-19 pandemic. Scarce data exists about the situation in Zagazig University Hospitals. **Aim:** To detect different macrolide resistance phenotypes and genotypes among staphylococcal clinical isolates in Zagazig University Hospitals, one of the tertiary hospitals in Egypt. **Methods:** Antibiotic susceptibility of ninety-two staphylococcal isolates collected from various clinical specimens, was carried out against erythromycin, azithromycin, and clindamycin by disc diffusion method. The D-test was applied to detect inducible macrolide, lincosamides, and streptogramin type B resistance phenotype (iMLS$_B$). Molecular detection of major genes coding for macrolide resistance, including erythromycin ribosomal methylase (*ermA*, *ermB*, and *ermC*), and macrolide-streptogramin resistance gene (*msrA*) was performed using PCR. **Results:** Out of 92 staphylococcal isolates, 37 isolates (40.2%) showed macrolide resistance. The iMLS$_B$ phenotype was identified in 32.4% of the resistant isolates with a rate of 43.7% among methicillin-resistant *Staphylococcus aureus* (MRSA), meanwhile, constitutive resistance was detected in 43.2%. The investigated resistance genes were detected in a total of 89.2% of resistant isolates where the *ermC* was the most frequent (54.1%), followed by the *msrA* gene (45.9%), the *ermA* gene (16.2%), and the *ermB* (5.4%). However, none of the examined genes showed a statistically significant relationship with the resistance phenotypes ($P > 0.05$). **Conclusion:** Our finding revealed increased macrolide resistance, particularly the inducible phenotype among MRSA isolates with wide dissemination of macrolide resistance genes, necessitating continuous monitoring.
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Introduction

Macrolide resistance in staphylococci has been increasingly reported worldwide [1, 2]. However, few studies have addressed this issue in Egypt with one study reporting a resistance rate of 36% among staphylococcal clinical isolates to macrolides [3]. This frequency is expected to have increased dramatically following the COVID-19 pandemic where antibiotics were extensively used for community-acquired pneumonia. Most notably, macrolides have been extensively prescribed for hospitalized, intensive care unit (ICU) patients as well as for non-hospitalized patients in an off-label irrational form, probably due to their possible

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antiviral, anti-inflammatory, in addition to their antibacterial effect [4, 5, 6].

Macrolide antibiotics belong to a group of natural products. Erythromycin A, first discovered in 1952 in the metabolic products of a strain of *Saccharopolyspora erythraea*, was the first clinically used macrolide antibiotic. Other macrolide members include azithromycin, clarithromycin, and spiramycin. Lincosamides such as clindamycin and streptogramins are closely related to macrolides. All have a bacteriostatic effect by inhibiting bacterial protein synthesis through binding to the 23S rRNA moiety of the 50S ribosomal subunit [7].

The macrolide-lincosamide-streptogramin B (MLS_B) antibiotics are widely used to treat Gram-positive infections, particularly in outpatient settings. Furthermore, combinations of macrolides with other antimicrobials have been proven to be useful in eradicating biofilms formed by Gram-negative bacteria [8, 9]. However, their role has been substantially increased with the emergence and widespread resistance to methicillin, and probably vancomycin, among staphylococci. This made the MLS_B antibiotics regarded as a safe alternative for beta-lactam drugs to treat infections caused by methicillin-resistant and multidrug-resistant staphylococci, adding to their role as second-line drugs for patients with beta-lactam allergy or intolerance [10, 11].

The MLS_B resistance in staphylococci is mediated by three mechanisms including target site modification by methyltransferases encoded by erythromycin ribosomal methyltransferase (*erm*) genes. This confers cross-resistance to MLS_B antibiotics and could be constitutive ($cMLS_B$) or inducible ($iMLS_B$). A second mechanism is the active efflux of antibiotics mediated by macrolide-streptogramin resistance (*msr*) genes conferring resistance to macrolides and streptogramin B sparing lincosamides (MS_B phenotype), and enzymatic inactivation conferring resistance to lincosamides which is less prevalent in staphylococci [12].

This study aims to detect the frequency of different macrolide resistance phenotypes and genotypes among clinical isolates of staphylococci to help establish adequate therapy for staphylococcal infections in Zagazig University Hospitals.

Material and Methods:

This cross-sectional study was conducted over 6 months (September 2023- March 2024) in the Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University and Clinical Pathology Department, Zagazig University Hospitals.

This study was approved by the Institutional Review Board (IRB), Faculty of Medicine, Zagazig University (ZU-IRB#10999-5/9-2023) and carried out according to updated 2013 Helsinki declarations. Informed consent was obtained from each patient or the guardians of unconscious patients.

Different specimens were obtained from infected inpatients mainly in ICUs and referred to the Bacteriology labs of Zagazig University Hospitals. Specimens included pus aspirate or wound swabs, sputum or bronchoalveolar lavage, blood, urine, cerebrospinal fluid (CSF), and conjunctival swabs. Specimens that yielded staphylococcal growth were included meanwhile, specimens that yielded growth other than staphylococci or mixed growth were excluded from the current study.

Sample size calculation: about 500 patients are attending the ICU during the study period (six months), and the expected frequency of staphylococcal infection is 8% [13], the sample size will be 92 cases at 80% power and 95% CI. (Epi info, version 6).

Bacterial isolates:

Specimens were immediately transported to the laboratory. Isolation and identification of bacterial strains were performed using routine microbiological tests. *S. aureus* isolates were distinguished from CoNS by giving yellow colonies after culture on mannitol salt agar and being coagulase-positive [14]. The tube coagulase test was performed using rabbit plasma (Biomed, Poland). The obtained staphylococcal isolates were maintained in glycerol broth (20%) at -20º C until use.

Antimicrobial susceptibility testing:

The disc diffusion method (Modified Kirby-Bauer technique) using Mueller Hinton agar was performed according to the **CLSI guidelines (2022)** [15]. Susceptibility of the collected staphylococcal isolates was tested against three antibiotics; macrolide antibiotics including erythromycin (15 ug), and azithromycin (15ug), and

one lincosamide antibiotic which is clindamycin (2 ug). Antibiotic discs were obtained from Oxoid Co. (Oxoid Limited, Basingstoke, Hampshire, England). *S. aureus* ATCC®25923 strain was used as a quality control strain for susceptibility tests (American Type Culture Collection Global Bioresource Center, Manassas, VA, USA).

Phenotypic detection of macrolide resistance:

The (D-test) was applied to detect the inducible resistance phenotype ($iMLS_B$) as described previously [16]. During the performance of the antibiotic susceptibility test, the disk of clindamycin (2 μ g), was placed near the disk of erythromycin (15 μ g), at 15-26 mm (edge to edge) and incubated at 35°C for 16-18 h.

Isolates were considered macrolide-resistant if showing resistance to one or more of the macrolides used. Then, according to the results of the D-test, three phenotypes were identified as follows; isolates showing resistance to both erythromycin and clindamycin were recognized as having $cMLS_B$ phenotype. The $iMLS_B$ or inducible clindamycin resistance (ICR) phenotype was identified by resistance to the erythromycin disc and flattening of the inhibition zone around the clindamycin disk in the area between the two discs (positive D test). The MS_B phenotype was determined by resistance to erythromycin disk with no flattening of the zone around clindamycin (negative D test) [17].

Detection of methicillin resistance:

Methicillin resistance was detected among the macrolide-resistant strains using the cefoxitin disc (30 ug) in the disc diffusion method [18].

Genotypic detection of macrolide resistance:

The macrolide resistance genes *emrA*, *ermB*, *ermC*, and *msrA* were screened for by polymerase chain reaction (PCR) among macrolide-resistant isolates. Bacterial DNA was extracted using QIAamp® DNA Mini Kit (Qiagen GmbH, Hilden, Germany). PCR reactions were done using *Taq* PCR Master Mix (Qiagen GmbH, Hilden, Germany). Primer sequences, amplicons size and PCR amplification conditions are listed in Table 1. Each PCR reaction was performed with a final volume of 25 µl and contained 12.5 µl of *Taq* PCR Master Mix, 1 µl of each forward and reverse primer (concentration between 0.1-1.0 mM), 1 µl of DNA (50-200 ng), and 9.5 µl of RNase-free water. Then the amplicons were subjected to electrophoresis on 2% agarose gel (1xTRIS-acetate-EDTA, 120 mV, 40 min) containing ethidium bromide to visualize the amplified bands under UV and compare them with a molecular size marker (Gene RulerTM 100 bp DNA Ladder, Fermentas, ThermoScientific, USA).

Statistical Analysis:

Collected data were analyzed using SPSS version 22 software (SpssInc, Chicago, ILL Company). Categorical data were presented as numbers and percentages. The Fisher exact was used to analyze categorical variables. $P < 0.05$ was considered significant.

Results:

Ninety-two non-duplicated staphylococcal isolates including 65 *S. aureus* (70%), and 27 CoNS (29%) were obtained from different clinical samples.

Of 92 staphylococcal isolates, 37 (40.2%) were macrolide-resistant and included in this study. Of them, 26 (70.3%) were *S. aureus*, while 11 (29.7%) were CoNS. The highest ratio of resistant isolates (35.1%, n=13) was obtained from patients in the surgical ICU **(Supplementary data)**. The clinical source of those isolates is shown in **Fig 1.** where the highest rate of macrolide resistance was found in isolates recovered from pus (32.4%) and blood (27%).

Methicillin resistance was detected in a total of 59.5% of macrolide-resistant isolates (n=22) where 61.5% (16/26) of *S. aureus* were found to be methicillin-resistant (MRSA). While out of 11 macrolide-resistant CoNS isolates, 6 (54.5%) were methicillin-resistant.

Concerning the frequency of different macrolide resistance phenotypes, it was found that 16 (43.2%) staphylococcal isolates exhibited the $cMLS_B$ phenotype. The inducible phenotype ($iMLS_B$), detected by a positive D-test, was found in 12 isolates (32.4%). However, only 9 (24.3%) isolates showed resistance to macrolide only with a negative D-test (MS_B phenotype) **(Table 2)**.

The most frequent resistance phenotype in *S. aureus* isolates was the $cMLS_B$ phenotype (38.5%, n=10), followed by the $iMLS_B$ phenotype (34.6%, n=9), and then the MS_B phenotype (26.9%, n=7). Similarly, the $cMLS_B$ phenotype was the most frequent among CoNS isolates being detected in 54.5% (n=6) of isolates, followed by the $iMLS_B$ phenotype in 27.3% (n=3) of isolates, and lastly the MS_B phenotype in 18.2% (n=2) of isolates. There was no statistically significant difference between *S.*

aureus and CoNS as regards the resistant phenotype (P>0.05) **(Table 2)**.

The distribution of resistance phenotypes and their relationship with methicillin resistance are demonstrated in **Table 3**. The inducible phenotype was the most frequent among MRSA isolates (43.7%) compared to the constitutive $cMLS_B$ and the MS_B phenotypes (37.5% and 18.8%, respectively). However, most MR CoNS isolates (83.3%) exhibited the $cMLS_B$ phenotype. Despite this, no statistically significant difference has been detected (P>0.05).

The PCR results revealed that 33 (89.2%) isolates had one or more macrolide resistance genes. However, 4 (10.8%) isolates did not harbor any tested genes **(Table 4)**. The distribution and combination of different genes among the resistant isolates are presented in **Table 4** and **Fig 2**. It has been shown that 59.4% (n=22) of the resistant isolates carried only one resistance gene, 27.1% (n=10) carried two genes, and one (2.7%) isolate carried three different resistance genes (*ermA*, *ermC*, and *msrA*).

The frequency of different macrolide-resistance genes among macrolide-resistant isolates is demonstrated in **Table 5**. The *ermC* gene was the most frequently detected being present in 54.1% (n=20) of isolates, followed by the *msrA* gene (45.9%, n=17), then the *ermA* gene (16.2%, n=6), and lastly the *ermB* gene (5.4%, n=2). No statistically significant difference has been detected between *S. aureus* and CoNS regarding the frequency of macrolide-resistance genes (P>0.05).

The distribution of macrolide-resistance phenotypes and genotypes among the tested isolates and their relationship are demonstrated in **Table 6**. It indicates that isolates having *ermC* gene mostly exhibited a constitutive resistance phenotype, whether *S. aureus* (57.2%) or CoNS (100%). Meanwhile, isolates having *msrA* exhibited mainly an inducible phenotype whether *S. aureus* (45.4%) or CoNS (50%). However, the MS_B phenotype was the most frequent among *S. aureus* harboring the *ermB* gene (50%) and CoNS having *ermA* and *ermB* (50% for each). Despite this, no statistically significant difference has been detected (P>0.05).

Table 1. Primer sequences and PCR reaction conditions used to detect macrolide resistance genes.

Gene	Primer sequence (5'-3')	PCR conditions	Product size (bp)	References
ermA	F: TCTAAAAAGCATGTAAAAGAA R: CTTCGATAGTTTATTAATATTAGT	35 cycles (30 s at 94ºC, 1 min at 48ºC, 2 min at 72ºC)	645	[19]
ermB	F: GAAAAGGTACTCAACCAAATA R: AGTAACGGTACTTAAATTGTTTAC	35 cycles (30 s at 94ºC, 30 s at 50ºC, 2 min at 72ºC)	639	[19]
ermC	F: AGTACAGAGGTGTAATTTCG R: AATTCCTGCATGTTTTAAGG	35 cycles (55 s at 94ºC, 1 min at 53ºC, 1 min at 72ºC)	642	[19]
msrA	F: GGCACAATAAGAGTGTTTAAAGG R:AAGTTATATCATGAATAGATTGTCC TGTT	25 cycles (1 min at 94ºC, 1 min at 50ºC, 90 s at 72ºC)	399	[19]

Table 2. Macrolide resistance phenotypes among the obtained isolates (*S. aureus* and CoNS).

Phenotype	**All isolates (n=37)**	***S. aureus* (n=26)**	**CoNS (n=11)**	**P Value***
$cMLS_B$	16 (43.2%)	10 (38.5%)	6 (54.5%)	0.37
$iMLS_B$	12 (32.4%)	9 (34.6%)	3 (27.3%)	0.66
MS_B	9 (24.3%)	7 (26.9%)	2 (18.2%)	0.69

***** Fisher exact test, $P \leq 0.05$ is statistically significant. **Abbreviations:** CoNS; coagulase-negative staphylococci, $cMLS_B$; constitutive macrolide, lincosamide, and streptogramin B resistance, $iMLS_B$; inducible resistance, MS_B; macrolide and streptogramin B resistance

Table 3. Macrolide-resistance phenotypes and methicillin resistance in the examined isolates.

Phenotype	**MRSA (n=16)**	**MSSA (n=10)**	**MR CoNS (n=6)**	**MS CoNS (n=5)**	**P Value***
$cMLS_B$	6 (37.5%)	4 (40%)	5 (83.3%)	1 (20%)	0.15
$iMLS_B$	7 (43.7%)	2 (20%)	1 (16.7%)	2 (40%)	0.48
MS_B	3 (18.8%)	4 (40%)	0 (0%)	2 (40%)	0.24

*****Fisher exact test, $P \leq 0.05$ is statistically significant. **Abbreviations:** MRSA; methicillin-resistant *S. aureus*, MSSA; methicillin-sensitive *S. aureus*, MR CoNS; methicillin-resistance coagulase-negative staphylococci, MS CoNS; methicillin-sensitive coagulase-negative staphylococci, $cMLS_B$; constitutive macrolide, lincosamide, and streptogramin B resistance, $iMLS_B$; inducible resistance, MS_B; macrolide and streptogramin B resistance

Table 4. Distribution and Combinations of macrolide-resistance genes among the tested isolates.

Gene distribution	**Genes**	**Isolates n (%)**
No genes detected		4 (10.8%)
One gene, 22 (59.4%)	*ermA*	1 (2.7%)
	ermB	1 (2.7%)
	ermC	11 (29.7%)
	msrA	9 (24.3%)
Two genes, 10 (27.1%)	*ermC, msrA*	5 (13.6%)
	ermC, ermA	3 (8.1%)
	ermB, msrA	1 (2.7%)
	ermA, msrA	1 (2.7%)
Three genes, 1 (2.7%)	*ermA, ermC, msrA*	1 (2.7%)
One gene or more		33 (89.2%)

Table 5. Frequency of the investigated macrolide resistance genes among the tested isolates.

Genotype	**All isolates (n=37)**	***S. aureus* (n=26)**	**CoNS (n=11)**	**P Value***
ermA	6 (16.2%)	4 (15.4%)	2 (18.2%)	1.00
ermB	2 (5.4%)	2 (7.7%)	0 (0%)	0.34
ermC	20 (54.1%)	14 (53.8%)	6 (54.5%)	0.96
msrA	17 (45.9%)	11 (42.3%)	6 (54.5%)	0.49

*Fisher exact test, $P \leq 0.05$ is statistically significant.
CoNS; coagulase-negative staphylococci.

Table 6. Number and percentage of macrolide-resistance phenotypes and genotypes among the examined isolates.

Staphylococcal Resistant Isolates					
Phenotype	***ermA*** **(n=6)**	***ErmB*** **(n=2)**	***ermC*** **(n=20)**	***msrA*** **(n=17)**	**P Value***
$cMLS_B$	3 (50%)	0 (0%)	14 (70%)	6 (35.3%)	0.08
$iMLS_B$	1 (16.7%)	1 (50%)	3 (15%)	8 (47.1%)	0.14
MS_B	2 (33.3%)	1 (50%)	3 (15%)	3 (17.6%)	0.54
S. aureus **Resistant Isolates**					
	ermA **(n=4)**	***ErmB*** ***(n=2)***	***ermC*** **(n=14)**	***msrA*** **(n=11)**	**P Value***
$cMLS_B$	2 (50%)	0 (0%)	8 (57.2%)	4 (36.4%)	0.42
$iMLS_B$	1 (25%)	1 (50%)	3 (21.4%)	5 (45.4%)	0.57
MS_B	1 (25%)	1 (50%)	3 (21.4%)	2 (18.2%)	0.79
CoNS Resistant Isolates					
	ermA **(n=2)**	***ErmB*** **(n=0)**	***ermC*** **(n=6)**	***msrA*** **(n=6)**	**P Value***
$cMLS_B$	1 (50%)	0 (0%)	6 (100%)	2 (33.3%)	0.11
$iMLS_B$	0 (0%)	0 (0%)	0 (0%)	3 (50%)	0.17
MS_B	1 (50%)	0 (0%)	0 (0%)	1 (16.7%)	0.37

***Fisher exact test, $P \leq 0.05$ is statistically significant. **Abbreviations:** CoNS; coagulase-negative staphylococci, $cMLS_B$; constitutive macrolide, lincosamide, and streptogramin B resistance, $iMLS_B$; inducible resistance, MS_B; macrolide and streptogramin B resistance

Figure 1. Clinical source of macrolide-resistant staphylococcal isolates (N=37).

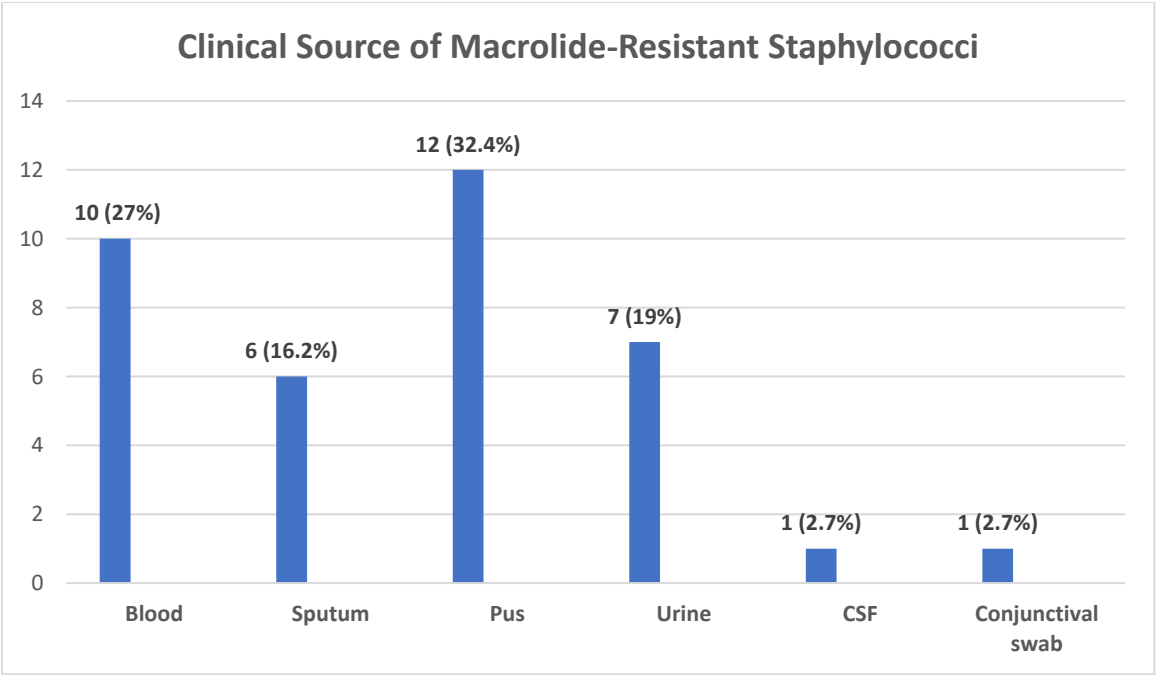
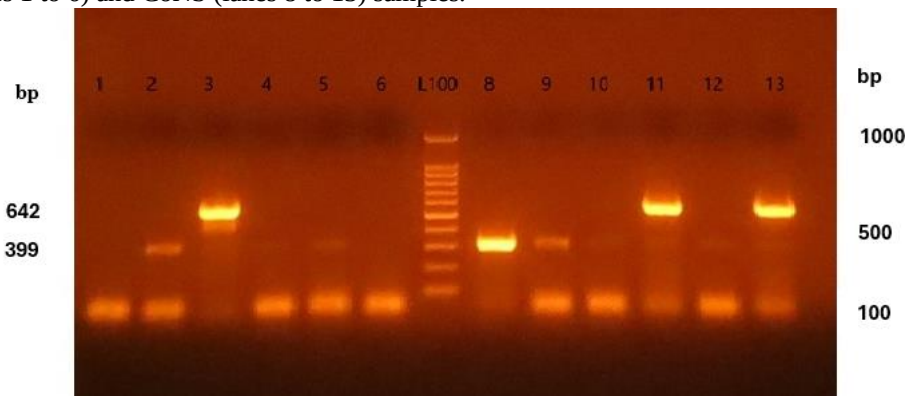


Figure 2. Agarose gel electrophoresis of the PCR product showing *erm*C (642 bp), and *msr*A (399 bp) genes in *S. aureus* (lanes 1 to 6) and CoNS (lanes 8 to 13) samples.



Discussion

Though being older generation antibiotics, the idea of using macrolides with staphylococcal infections has become compelling as it may constitute a safe alternative to treat infections caused by methicillin-resistant strains and even vancomycin-resistant strains, particularly after their rising prevalence in community-acquired infections [20, 21].

Therefore, it was necessary to determine the exact frequency of macrolide resistance and to investigate the prevalence of genetic determinants coding for this resistance among staphylococcal clinical isolates.

In this study, a macrolide resistance frequency of 40.2% was recorded among 92 staphylococcal clinical isolates obtained from different clinical specimens from Zagazig University Hospitals. This is slightly higher than previous Egyptian studies, which recorded 36% and 38.5% frequencies among staphylococcal clinical isolates [3, 22]. However, higher rates have been recorded worldwide, particularly in *S. aureus* where in a previous Iranian study 56.4% of the isolates expressed resistance to erythromycin [23] and an even higher rate (82.28%) was recorded in Vietnam [1]. This may be due to the frequent use of these drugs as a first-line choice in some countries.

Pus and blood constituted the main specimens that yielded macrolide-resistant staphylococcal isolates (32.4% and 27%, respectively). This aligns with previous reports showing increased macrolide resistance in staphylococcal isolates obtained from bloodstream infections [24, 25].

The MLS_B resistance among staphylococci can be mediated by different mechanisms with several genes coding for these mechanisms. Identifying the phenotype of MLS_B resistance is of utmost importance and could be very helpful to the treating physician. This is particularly needed with the inducible phenotype which upon the excessive use of clindamycin, can be converted to a constitutive phenotype resulting in treatment failure [26].

In the current study, the $cMLS_B$ phenotype was the most frequent among resistant staphylococcal isolates (43.2%) as well as among *S. aureus* isolates (38.5%), meanwhile, the $iMLS_B$ phenotype was detected in 32.4% of all staphylococcal isolates and was the most frequent in CoNS isolates (54.5%). The MS_B phenotype was less frequent with 24.3%, 26.9%, and 18.2% frequencies among all staphylococcal isolates, *S. aureus*, and CoNS, respectively.

Similar findings were reported previously in Egypt and different parts of the world, where the $cMLS_B$ phenotype was the most frequently recorded among macrolide-resistant staphylococci [3, 22, 23, 26-29].

Concerning the inducible resistance, the records ranged from 2.9% to 44% among African countries with Egypt recording one of the highest (44%) among *S. aureus* isolates [30]. The $iMLS_B$ phenotype had an even higher frequency (33.4%) compared to the $cMLS_B$ phenotype (8.9%) among resistant staphylococcal isolates in Serbia [31]. Several factors may contribute to the reported differences such as the different geographical regions of the studies, the source of specimens, the frequency of macrolide administration, the local

resistance mechanisms, and the co-occurrence of methicillin resistance [32, 33].

In the current study, inducible resistance was higher among MRSA isolates compared to MSSA (43.7% versus 20%), though this was not evident with MR-CoNS (16.7%) and MS-CoNS (40%). This comes higher than the frequencies reported among MRSA isolates in other parts of the world such as Japan (38.7%%) and Iran (20.5%) [34, 35]. However, higher frequencies have been found in Tanzania (61%) [33] and Jordan (76.7%) [36]. Despite a previous Egyptian report that recorded a rate of 77.8% among MRSA recovered from oncology patients suffering from afebrile neutropenia [37], which comes much higher than the current result, the current finding constitutes a warning that warrants continuous monitoring and judicious use of MLS_B antibiotics in healthcare settings.

The MS_B phenotype was less frequently detected in the current work which agrees with previous studies where frequencies ranging from 2.2% to 16% were reported [23, 27, 38]. However, the MS_B phenotype was reported as the most frequent among staphylococcal isolates in India [39].

In the current study, PCR results revealed a wide dissemination of macrolide resistance genes among resistant isolates where 89.2% harbored one or more resistance genes where 59.4%, 27.1%, and 2.7% had one, two, and three genes, respectively. Meanwhile, 10.8% of the examined isolates did not harbor any of the investigated resistance genes.

Similar findings were reported in a previous Egyptian study where 51.8%, 37.1%, and 11.1% of resistant staphylococcal isolates had one, two, and three resistance genes, respectively [3].

However, the current study did not investigate all possible variants of methylase genes such as *ermY* and *ermF*, or the newly documented efflux pump genes [40] which could explain the absence of resistance genes in 10.8% of resistant isolates.

The *ermC* gene was the most prevalent among all resistant staphylococci in the current study (54.1%) with a frequency of 53.8% among *S. aureus*. Similarly, it was the most frequent, along with *msrA* gene, among resistant CoNS, (54.5% for each).

High frequency of *ermC* gene was reported previously by different studies whether in staphylococci (79.2% - 82.6%) [3, 22, 27], or in *S. aureus* (35.2%) [23]. The high prevalence of the *ermC* gene over the other genes coding for macrolide resistance could be attributed to its easy transmission from resistant to susceptible strains being carried on a small plasmid [41].

The low frequency of the *ermB* gene reported in the current study (5.4%) is consistent with the observation that this gene is present mainly in streptococci and enterococci [42].

However, the distribution of *erm* genes depends largely on the geographic region. Where *ermC* gene is mostly reported as the most prevalent, the *ermB* gene demonstrated a higher prevalence in some studies from China and Egypt particularly in *S. aureus* [43]. On the other hand, the *ermA* gene was more prevalent in South America [44].

Concerning the distribution of phenotypes and genotypes among resistant isolates, the current results demonstrated that isolates with *ermC* gene exhibited mostly the constitutive phenotype whether *S. aureus* (57.2%) or CoNS (100%). Meanwhile, those with *msrA* exhibited mainly the inducible type, either *S. aureus* (45.4%) or CoNS (50%). However, the MS phenotype was the most frequent among *S. aureus* having *ermB* (50%) and CoNS having *ermA*.

Similar findings have been previously reported where *ermC* gene was more prevalent in isolates exhibiting constitutive phenotype [31, 38]. However, in other studies, the *ermA* gene predominated among isolates with constitutive phenotype [28, 34].

The predominance of *msrA* among isolates with inducible phenotype demonstrated in the current study comes different from previous reports that found both *ermA* and *ermC* to be the predominant genes among this phenotype [28, 31] and from another study that documented the *msrA* gene to be the most prevalent among the MS phenotype [38].

These discrepancies could be attributed to the differences in the population studied, sample size, or the study location [45]. Furthermore, they demonstrate the genetic variability associated with macrolide-resistant strains as previously shown [46].

Among the limitations of this study are that the work did not include isolates from outpatients and the inability to assess all possible genes and all mechanisms responsible for macrolide resistance. Another limitation is the inability to assess

macrolide resistance genes in susceptible isolates which could give a better idea about the extent of gene dissemination among those isolates.

In conclusion, the current finding revealed increased macrolide resistance, particularly the inducible phenotype among MRSA isolates compared to previous Egyptian studies with wide dissemination of macrolide resistance genes. This finding intensifies the importance of performing the D test and emphasizes the need for detecting MLS_B resistance phenotype and genotype particularly among MRSA isolates.

Conflict of interest

The authors report no conflicts of interest.

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