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Macrolides and Lincosamides-Resistant Staphylococci Isolated from Healthcare-Associated Illnesses, Mansoura University Hospitals: Phenotypic and Genotypic Characterization

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ABSTRACT

Background: An important public health issue is the rising frequency of infections brought on by macrolides, lincosamides, and streptogramin-B (MLS-B)-resistant staphylococci. Clindamycin can cause resistance and therapeutic failure when used to treat infections caused by inducible (iMLS-B) strains.

Objectives: The goal is to identify staphylococcal isolates resistant to MLS-B based on phenotype and genetics.

Subjects and Methods: This study was scheduled to run from September 2021 through August 2022. Clinical samples were collected from patients admitted to Mansoura University Hospitals with varying signs and symptoms of infection and cultured on the appropriate culture media. Colony morphology, Gram stain, biochemical response, and VITEK2 were used to identify isolated colonies. The VITEK2 system was used to test for antibiotic susceptibility.

Results: Bacterial growth was detected in 753 of the 2450 samples analyzed. 350 of them were Gram-positive bacteria. Of the 350 samples, 200 were staphylococci, and 50 of them were staphylococci with MLS-B resistance. According to a D-test, 53.2% of MLS-B phenotype had constitutive (cMLS-B), 28.2% had iMLS-B, and 18.6% had MS. Staphylococcus aureus exhibited a 9.3% MS phenotype, 15.7% iMLS-B, and 18.7% cMLS-B. ErmA, ermB, ermC, and msrA genes yielded 10%, 32%, 74%, and 4.0%, respectively. In coagulase-negative staphylococci (CoNS), cMLSB was the most common trait and ermC gene dominated, followed by the ermB gene. In S. aureus, the ermC gene dominated the iMLSb phenotype (55%).

Conclusion: The dominant genes among MLS-B resistant isolates are ermC and ermB, while cMLS-B is the prevalent phenotype. To prevent therapeutic failure, it is crucial to identify the iMLS-B phenotype by D-test and find the resistance genes prior to clindamycin administration.

Keywords: Macrolides, Lincosamides, Staphylococci isolated, Healthcare associated illnesses.

INTRODUCTION

Staphylococci are responsible for a variety of diseases, including endocarditis, pneumonia, sepsis, and soft tissue infections [(1)]. Nowadays, macrolides, lincosamides, and streptogramin-B (MLS-B) antibiotics are favored for the treatment of staphylococci due to their superior pharmacokinetic qualities. A class of protein synthesis inhibitors are known as macrolides exhibits broad-spectrum activity [(2)].

Bacterial protein synthesis is stopped when macrolides attach to the 50S ribosomal subunit. Once it binds, the medication blocks the enzyme peptidyl-transferase from adding the succeeding amino acid connected to the tRNA, hence stopping the translation of mRNA and, specifically, the increasing peptide chain [(3)].

Resistance to MLS-B antibiotics is connected to three key mechanisms: active efflux, active rRNA methylation (target modification) based on ribosome structural alterations, and enzymatic inactivation. MLS-B antibiotic resistance phenotypes can be constitutive (cMLS-B), which indicates resistance to all MLS-B, or inducible (iMLS-B), which only emerges in response to antibiotics that induce methylase production [(4)].

Inducers are macrolides with a 14-member ring (M14, erythromycin, for example) or a 15-member ring (M15, azithromycin, for example) [(5)]. Although clindamycin is not an inducer, using it to treat infections caused by iMLSB strains increases the likelihood of resistance and therapeutic failure [(2)].

As a result, the Clinical and Laboratory Standards Institute (CLSI) recommends utilizing the double-disk diffusion method (D-test) to detect Staphylococcus isolates with inducible clindamycin resistance [(4)].

The methylation of adenine in the 23S rRNA ribosomal subunit is carried out by the methylase, which is encoded by the erm (erythromycin ribosome methylation) family of genes. The presence of active efflux-related genes, such as the msr genes in Staphylococcus spp., can also predict the resistance to macrolides and streptogramins B (MSB phenotype) [(6)].

This study set out to identify and characterize the staphylococcal isolates from

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Mansoura University Hospitals in Mansoura, Egypt, that were resistant to MLS-B.

SUBJECTS AND METHOD

Study design and subjects:

Between September 2021 and August 2022, a prospective study was conducted in which clinical samples were collected from patients admitted to Mansoura University Hospitals with various signs and symptoms of infection. All erythromycin-resistant staphylococcal isolates were subjected to D-test to identify MLS-B phenotypes and PCR to detect the resistance genes (ermA, ermB, ermC, and msrA).

I-Collection of Clinical Samples

Prior to the administration of antibiotics, samples were taken under strictly aseptic circumstances.

II-Processing of Clinical Samples

A-Culture

The samples were cultured on the proper culture media (blood samples on blood culture media, swabs, body fluids, and sputum on blood and MacConkey media, urine on CLED), and incubated at 37°C for 48 hours. Isolated colonies were recognized using the Vitek2 System, gram stain, biochemical responses, and colony morphology. The Vitek2 System **(Biomérieux, Marcy-l'Étoile, France)** tested the susceptibility of bacteria to antibiotics.

B-Detection of clindamycin resistance:

The disc diffusion method was used to test all erythromycin-resistant staphylococcal isolates for clindamycin resistance. A standard 0.5 McFarland suspension was prepared from isolated colonies. With the discs of erythromycin (15 µg) and clindamycin (2 µg) spaced around 15 mm from edge to edge, the inoculum was aseptically plated over a Muller Hinton agar plate and incubated aerobically at 37°C for 24 hours.

The inducible resistance phenotype was discovered in isolates with erythromycin resistance (zone of inhibition 13 mm) and flattening of the zone (D-zone) around the clindamycin disc (zone of inhibition 21 mm). Constitutive resistance phenotypes (cMLS-B) were defined as isolates resistant to both erythromycin and clindamycin, and MS phenotypes were defined as isolates resistant to erythromycin but susceptible to clindamycin with no D-zone (Figure 1).

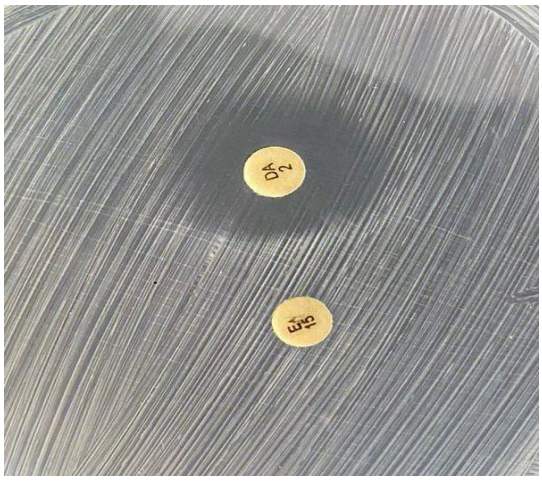

Figure (1): Inducible MLS-B resistance phenotype (D-shaped sensitivity)

C-Conventional PCR for MLS-B resistance genes:

Qiagen extraction kit was used to extract DNA from pure colonies of all Staphylococcus isolates, which were then frozen at -20°C until use. PCR was done to identify the ermA, ermB, ermC, and msrA genes using primers with the following sequences:

ErmA
F:AAGCGGTAAACCCCTCTGA,
R:TTCGCAAATCCCTTCTCAAC,

ErmB
F:CGTTTACGAAATTGGAACAGGTAAAGG GC,
R:GAATCGAGACTTGAGTGTGC,

ErmC
F:GCTAATATTGTTTAAATCGTCAATTCC,
R:GGATCAGGAAAAGGACATTTTAC

MsrA
F:GGCACAATAAGAGTGTTTAAAGG,
R:AAGTTATATCATGAATAGATTGTCCTG TT.

Amplification was carried out in a 25 µL PCR mixture (Qiagen) containing 5 µL of extracted DNA, 12.5 µl of PCR master mix**,** 0.1 µl of each primer (forward and reverse)**,** and 7.3 µl of nuclease free water. After 30 cycles of PCR (30 s at 94°C; 30 s at 52°C; 1 min at 72°C), Ten µL of PCR product was resolved at 90V for 1 hour on a 2% agarose gel containing 0.5 mg/mL ethidium bromide and viewed in a gel documentation system. ermA gene was seen at 190bp, ermB gene was seen at 345bp, ermC at 550bp and msrA at 910bp. DNA ladder at 50bp was used (Figure 2).

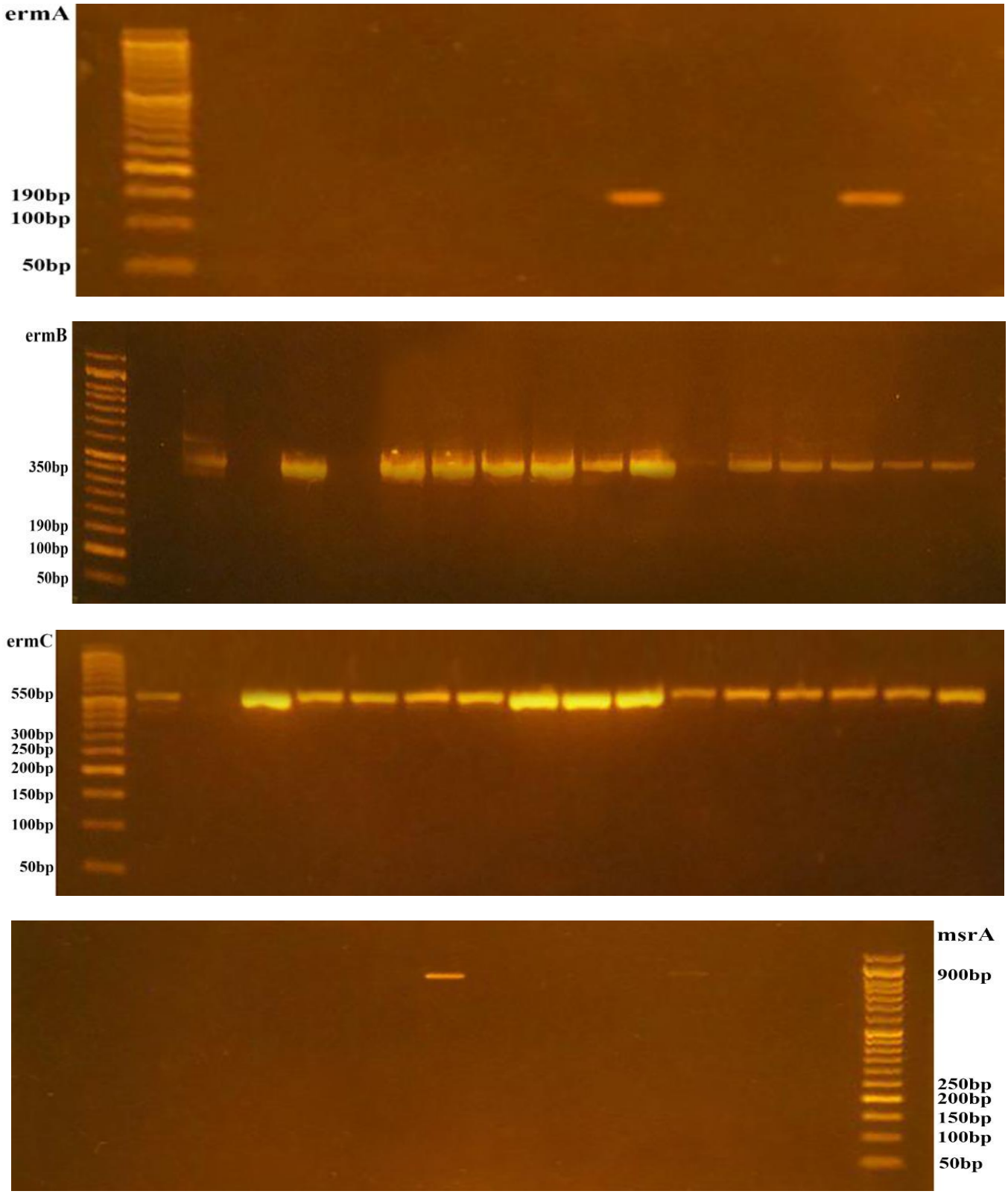

Figure (2): Phenotype image of ermA, ermB, ermC, and msrA genes among MLSBi isolates.

Ethical approval:

Mansoura Medical Ethics Committee of the Mansoura Faculty of Medicine gave its approval to this study. All participants gave written consent after receiving all information. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis:

Windows® SPSS v. 22 was used. The frequencies and relative percentages were used to depict the qualitative data, which were compared by Chi square test (χ^2) or Fisher exact test. To indicate statistical significance, the P value was set at 0.05, and a result <0.001 was considered highly significant.

RESULTS

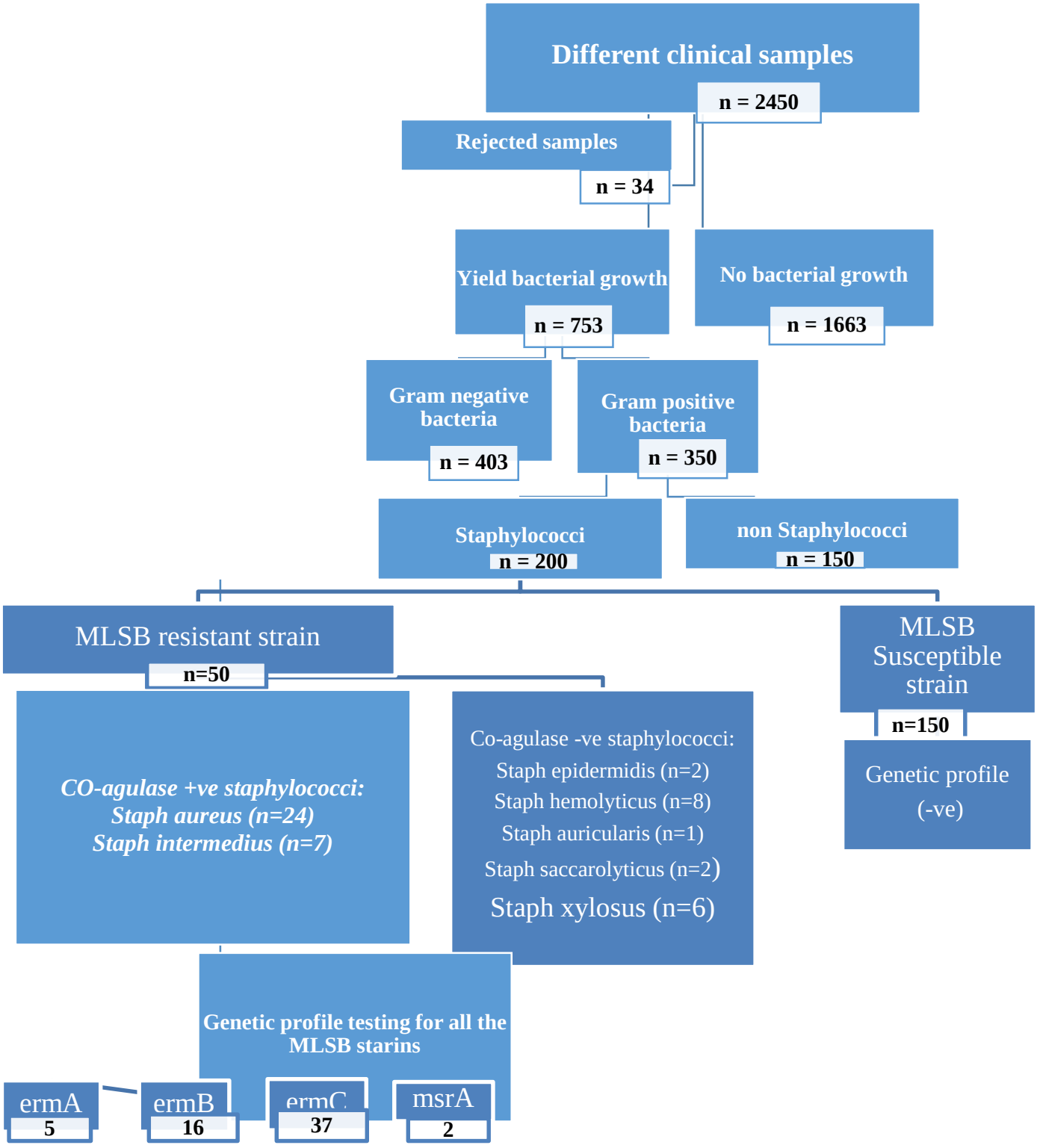

Figure (3): Flowchart shows sample collection and classification.

Specimens were blood cultures (n=151), swabs (n=32), urine (n=11), body fluid (n=3) and sputum (n=3). Biochemical reaction and antibiotic susceptibility testing were performed by automated Vitek2 system and interpreted according to CLSI, 2020.

S. aureus was the predominant staphylococcal isolate (Figure 4).

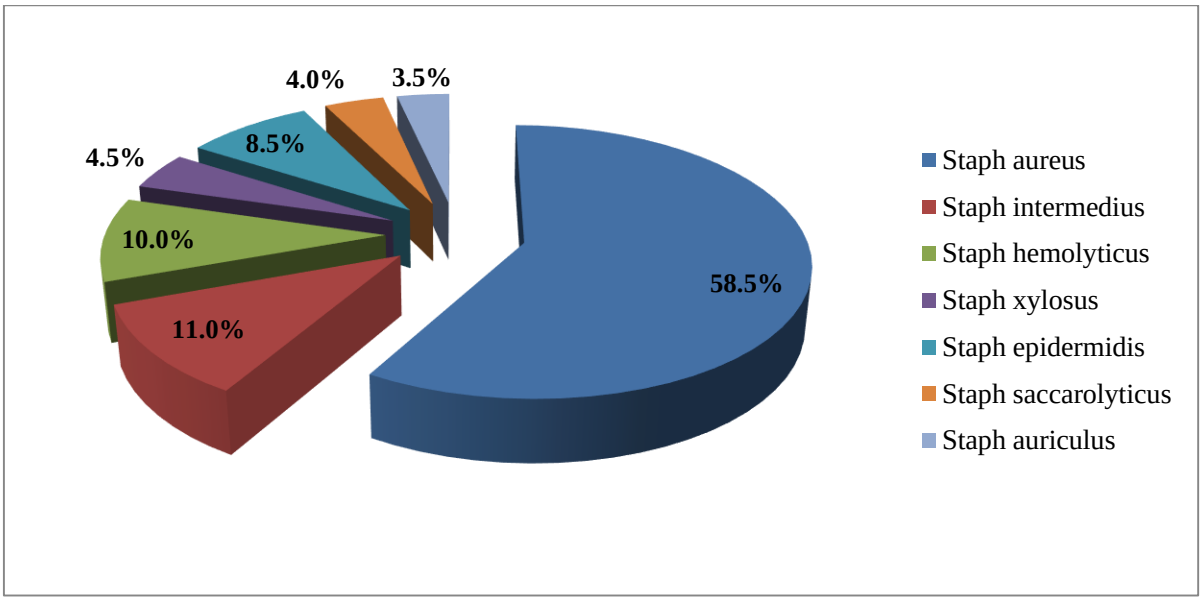

Figure (4): Isolates distribution in our study.

Significant resistance was observed against erythromycin, azithromycin, clindamycin and quinupristin in 200 staphylococcal isolates (Table 1).

Table (1): MLS-B antibiotics sensitivity in 200 studied staphylococci:

Antibiotic	*Staph aureus (n=117)*	*Staph intermedius (n=22)*	*Staph hemolyticus (n=20)*	*Staph xylosus (n=9)*	*Staph epidermidis (n=17)*	*Staph saccharolyticus (n=8)*	*Staph auricularis (n=7)*	P value
Erythromycin	13 (11.1%)	6 (27.3%)	4 (20.0%)	5 (55.6%)	0 (0.0%)	2 (25.0%)	1 (14.3%)	**0.004**
Azithromycin	17 (14.5%)	2 (9.1%)	5 (25.0%)	6 (66.7%)	2 (11.8%)	2 (25.0%)	0 (0.0%)	**0.002**
Lincomycin	6 (5.1%)	0 (0.0%)	2 (10.0%)	0 (0.0%)	2 (11.8%)	2 (25.0%)	0 (0.0%)	0.159
Clindamycin	8 (6.8%)	2 (9.1%)	2 (10.0%)	5 (55.6%)	0 (0.0%)	2 (25.0%)	0 (0.0%)	**<0.001**
Quinupristin	4 (3.4%)	2 (9.1%)	2 (10.0%)	5 (55.6%)	0 (0.0%)	1 (12.5%)	0 (0.0%)	**<0.001**

cMLS-B was the predominant phenotype among 50 MLS-B resistant *staphylococci* (Table 2).

Table (2): Phenotypic identification using D-test:

Organism	cMLSb	iMLSb	MS	Total
Staph aureus	6 (18.8%)	5 (15.6%)	3 (9.3%)	14 (43.8%)
Staph intermedius	2 (6.3%)	2 (6.3%)	2 (6.3%)	6 (18.7%)
Staph hemolyticus	2 (6.3%)	2 (6.3%)	0 (0.0%)	4 (12.5%)
Staph epidermidis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Staph xylosus	5 (15.6%)	0 (0.0%)	0 (0.0%)	5 (15.6%)
Staph saccharolyticus	2 (6.3%)	0 (0.0%)	0 (0.0%)	2 (6.3%)
Staph auricularis	0 (0.0%)	0 (0.0%)	1 (3.1%)	1 (3.1%)
Total	17 (53.2%)	9 (28.2%)	6 (18.6%)	32 (64.0%)

ermC was the predominant gene followed by *ermB* among MLS-B resistant isolates (Figure 5).

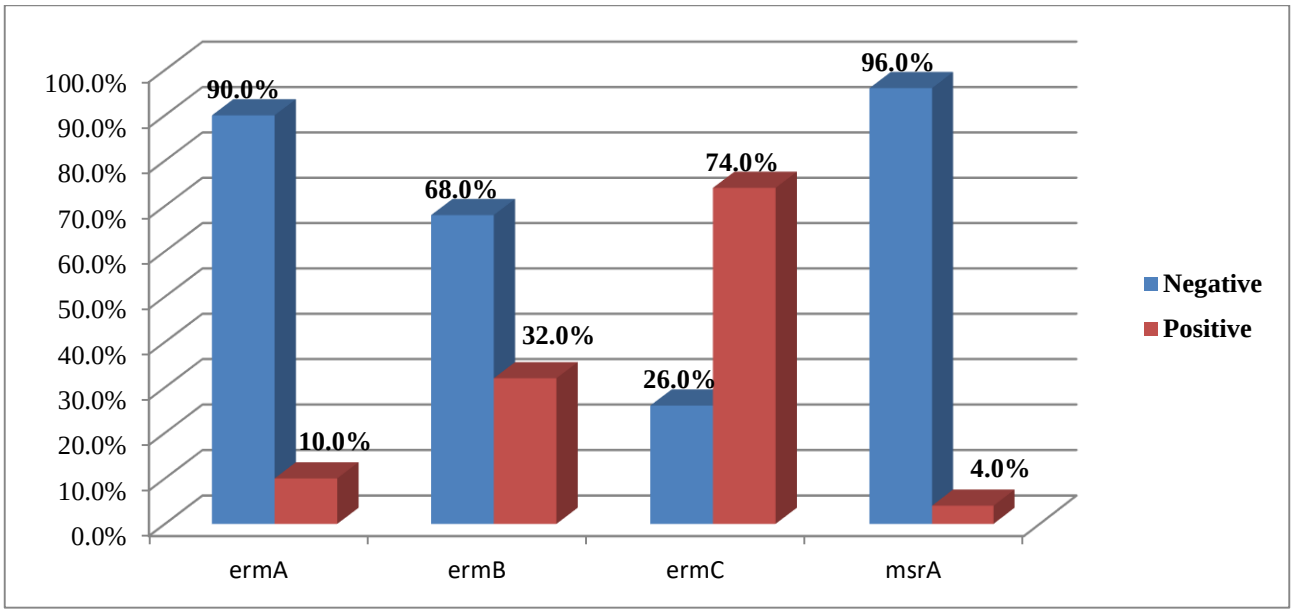

Figure (5): Distribution of genes in our study

Among ***S. aureus*** with cMLSB resistance phenotype, the predominant gene was ermC in 2 isolates followed by ermB in 1 isolate and ermA in 1 isolate. Among S. aureus with iMLSb and MS resistance phenotypes, the predominant gene was ermC in 4 isolates and 2 isolates respectively. Among Staph intermedius isolates with cMLSb resistance, ermC was the predominant gene followed by ermB like iMLSb and MS phenotype

Among CoNs with cMLSb resistance phenotype, the predominant gene was ermC found in 9 isolates followed by ermB in 5 isolates in which 3 isolates of them carried combination of (ermB+ermC), while in iMLSb the only detected gene was ermC in 2 isolates.

Table (3): Correlation between phenotypes and genotypes for MLS-B resistance in staphylococcal tested isolates:

Genes	cMLSb			iMLSb			MS		
	S. aureus	S. intermedius	CoNs	S. aureus	S. intermedius	CoNs	S. aureus	S. intermedius	CoNs
ermA	1	0	0	0	0	0	0	0	0
ermB	1	1	5	0	1	0	1	1	0
ermC	2	1	9	5	1	2	2	2	0
msrA	0	0	0	1	1	0	0	0	0
ermA + ermB	1	0	0	0	0	0	0	0	0
ermA + ermC	0	0	0	0	0	0	0	0	0
ermB + ermC	1	1	3	0	1	0	1	1	0
ermA + ermB + ermC	0	0	0	0	0	0	0	0	0

There were 31 isolates positive phenotype and 40 positive genotypes out of 50 studied isolates. There were 7 isolates with positive phenotype and no detected genes. There were 16 isolates positive genotype and negative phenotype. There were 3 MLSb resistant isolates negative phenotypically and genotypically.

Table (4): Comparison between results of genotype and phenotype:

		Genotype				P
		Negative		Positive		
Phenotype	**Negative**	3	30.0%	16	40.0%	0.722
	Positive	7	70.0%	24	60.0%	

DISCUSSION

Antimicrobial resistance (AMR) is becoming a major clinical issue on a global scale. Despite several measures adopted in recent decades to address this problem, the trends of worldwide AMR show no indications of slowing down. In hospital settings, several antimicrobial drugs are abused and overused [(7)].

In our study, 50 out of 200 staphylococcal isolates were highly resistant to one or more of MLS-b antibiotics in which erythromycin, azithromycin, lincomycin, clindamycin and quinupristin demonstrated resistance with P values (P=0.004, 0.002, **0.159**, <0.001, <0.001 respectively). This is in accordance with **Bishr *et al.*** [(8)] who found 36% of isolates were resistant to one or more antibiotic of macrolides. This can be explained by the fact that MLS-B antibiotics are routinely used to treat infections caused by Gram-positive bacteria. Although their chemical structures differ, their modes of action are similar. As a result, genes that cause resistance to any of these antibiotics may result in cross-resistance to others [(9)].

Phenotypic identification using D-test revealed cMLS-B in 53.2%, iMLS-B in 28.2%, and MS phenotype in 18.6%, however **Nagarkoti *et al.*** [(1)] discovered cMLS-B phenotype in 40%, MS in 37%, and iMLS-B in 23%. In our study, S. aureus had 18.7% cMLS-B, 15.7% iMLS-B, and 9.3% MS phenotype. Such findings were consistent with **Kavitha** [(10)] whereas **Abdelhalim *et al.*** [(11)] found that the cMLS-B phenotype was the prevalent phenotype in 80% of S. aureus isolates. Furthermore, cMLS-B was the predominant phenotype among CoNs in our study, similar to **Abdelhalim *et al.*** [(11)] whereas **Juda *et al.*** [(12)] found MS phenotype predominates in CoNs because they studied a larger sample size with more different types of CoNs that may carry different characters.

In our study, the genetic profile performed for ermA, ermB, ermC and msrA genes yielded (10%, 32%, 74%, 4.0%, respectively). Regarding S. aureus, ermC gene predominates among iMLS-B phenotype (55%), which was in accordance with **Osman *et al.*** [(13)] and **Ghanbari *et al.*** [(14)]. However, in CoNs, the ermC gene predominates among cMLS-B, followed by ermB, which was in accordance with to **Szemraj *et al.*** [(6)], **El Said *et al.*** [(15)], and **Teeraputon *et al.*** [(16)].

The most prevalent mechanism of resistance is target site modification, which is carried out by the enzymes adenylyl-N-methyl transferase erm and results in resistance to all MLS-B. The gene encoding erm methylase synthetase can be expressed constitutively (resistance to all MLS-B) or inducibly (resistance to antibiotics that stimulate methylase production, such as erythromycin and azithromycin). The presence of an inducer, such as erythromycin or another macrolide M14-15, is required for resistance to the other MLS-B.

The location of the research influences the distribution of genes that determine resistance to macrolide antibiotics. Resistance to macrolide antibiotics is most typically indicated by the presence of ermC in the Middle East. The ermB gene is more often isolated in China and Egypt. On the other hand, in South America, the ermA gene is the most common [(2)].

CONCLUSION

We discovered that the dominant genes among MLS-B resistant isolates are ermC and ermB, while cMLS-B is the prevalent phenotype. To prevent therapeutic failure, it is crucial to identify the iMLS-B phenotype by D-test and find the resistance genes prior to clindamycin administration.

- **Sponsoring financially:** Nil.
- **Competing interests:** Nil.

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