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ARCHIVES OF CLINICAL MICROBIOLOGY

2014
Vol. 5 No. 5:1
doi: 10.3823/284

Plasmid-carried macrolides target site modification *erm* and efflux *msr* genes in some *Staphylococcus* spp. From lower respiratory tract infected patients

Shaker, Amr, Aboshanab, Khaled*, Mabrouk Aboulwafa, Mohammad, Hassouna, Nadia

Department of Microbiology and immunology, Faculty of Pharmacy, Ain Shams University (ASU), Cairo, Egypt. Organization of African Unity St., POB: 11566, Abbassia, Cairo, Egypt.

Corresponding author:

Aboshanab2003@yahoo.com

Tel: (202)25082595.
Fax: (202)24051107

Khaled Aboshanab, PhD

Address: Department of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University, Al Khalifa Al Maamoun St., Abbassia, Cairo, Egypt.

Abstract

Background: *Staphylococcus aureus* and coagulase-negative staphylococci are considered to be among the major causes of nosocomial and community-acquired infections. Macrolides, lincosamides and streptogramin type B (MLS) antibiotics are widely indicated for the treatment of staphylococcal infections. A great concern is the increase in the prevalence of macrolide resistance among *Staphylococcus* spp. The aim of our study was to detect plasmid-harbored genes responsible for macrolide resistance in staphylococci involved in lower respiratory tract infections (LRIs) in Egypt.

Methods and findings: A total of 189 bacterial isolates were recovered from 180 sputum and bronchoalveolar lavage specimens obtained from patients with LRIs. The specimens were collected during the period of 2010 to 2012 from Microbiological diagnostic laboratories of two governmental hospitals specialized in the treatment of chest infections, Al-Sadr (Abbassia) and Al-Demerdash hospitals. The antibiogram analysis revealed that 64, out of 189 isolates (33.9%) were resistant to the tested antibiotics (Erythromycin, Clarithromycin, Azithromycin and Clindamycin). Among the resistant isolates, 12 isolates (18.7%) were found to be Gram-positive, including 8 isolates (12.5%) which were identified to be *Staphylococcus* spp. Among these isolates, four showed constitutive (cMLS) phenotype, while the other

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isolates exhibited inducible (iMLS) phenotype. The molecular analysis of resistant isolates revealed the presence of plasmids in the eight test isolates. By using PCR, two genes suspected to be responsible for plasmid-mediated resistance could be detected using two specific primer pairs for erythromycin ribosomal methylase (*erm*) and erythromycin resistance ATP-binding protein (coded as *msr*). The *erm* gene was detected in four isolates (50%), while the prevalence of *msr* gene constituted 25% (detected in two isolates only). Further verification of *msr* gene was performed by DNA sequencing of the PCR product obtained by using the plasmid extract of one isolate as a template. The nucleotide sequence of *msr* gene was submitted to the GenBank database, and given the accession code KJ710361.

Conclusion: Plasmid-harbored genes (*erm* and *msr*) prevail among *Staphylococcus* isolates involved in LRIs as the 8 resistant isolates of this genus was shown to harbor plasmids, with prevalence of 50% (*erm* gene) and 25% (*msr* gene). Therefore, the reasons behind the prevalence of these plasmid-mediated genes among staphylococci have to be studied in order to limit the dissemination of macrolide resistance.

Key words: Macrolide antibiotics; staphylococci; *erm* gene; *msr* gene.

Introduction

Macrolide antibiotics such as erythromycin, clarithromycin and azithromycin have been widely used in treatment of respiratory tract infections caused by Gram-positive pathogens including *Staphylococcus* spp. [1-2]. MLS antibiotics exert their antimicrobial action by inhibition of bacterial protein synthesis which is achieved by reversible binding of these agents to the P-site of the 50S subunit of the bacterial ribosome [1-3].

There are three different mechanisms responsible for bacterial resistance to MACs in staphylococci: (1) Target site modification mediated by *erm* gene; (2) active efflux mediated by *msr* A gene; and (3) enzymatic inactivation of antibiotic mediated by esterases (encoded by *ere* gene) or phosphotransferases (encoded by *mph* gene) [4-5]. The first mechanism confers resistance not only to MACs, but also to lincosamides and streptogramin type B (MLS phenotype), which is achieved by dimethylation of specific adenine residue in 23S rRNA molecule in the ribosome [4, 6-7]. The expression of *erm* gene can be either constitutive (cMLS phenotype), or inducible (iMLS phenotype) [4]. The second mechanism confers resistance to either MAC antibiotics only (M phenotype) or MACs and streptogramin type B (MS phenotype) [4]. The MAC resistance genes as *erm* and *msr* genes are widely distributed in staphylo-

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cocci of humans, and located on small multicopy plasmids [8-12] *erm* genes are mostly responsible for erythromycin resistance in different *Staphylococcus* spp. strains and are borne by plasmids. Also, to date, the only efflux proteins responsible for acquired macrolide resistance characterized in *Staphylococccus* spp. are ABC transporters encoded by plasmid borne *msr* genes [13].

The emergence of antibiotic resistance among pathogenic bacteria, either in hospital or community-acquired infections is considered to be a major public health problem. Plasmids, which are an extrachromosomal pieces of DNA, can replicate independently of the genome, and are considered of great importance in the spread of antibiotic resistance genes, which result in the acquisition of resistance to many antibiotics [14]. Due to their capability of horizontal gene transfer between different species and genera, plasmid-mediated resistance is considered to be a significant problem in the spread of antibiotic resistance among pathogenic bacteria [14-15].

The aim of this study was to detect macrolide target site modification gene (*erm* gene) and macrolide active efflux gene (*msr* gene) on plasmids extracted from *Staphylococcus* spp recovered from patients with lower respiratory tract infections (LRIs) in Egypt. Specific PCR primers were used to detect target site modification gene *erm* (erythromycin ribosomal methylase), and active-efflux gene *msr*.

Materials and Methods

A total of 189 clinical bacterial isolates, recovered from patients with LRIs from the Microbiology diagnostic laboratories of Al-Sadr (Abbassia) and Al-Demerdash Hospitals in Egypt during the period of 2010 to 2012, were studied. The history sheets of all patients were examined, and all patients read and signed an "informed consent" form before the beginning of the study verifying their acceptance for using their data for research purpose.

Microbiological cultures

The collected isolates were obtained from sputum and bronchoalveolar lavage specimens of both pediatric and adult patients by culture on 5% sheep blood agar plates and incubating them overnight at 37°C. Mannitol salt agar plates and coagulase test were used for identification of *Staphylococcus aureus*. The isolates were preserved in glycerol broth at -20°C till use.

Antimicrobial susceptibility test

The susceptibilities of the collected isolates were determined by disc diffusion method on Mueller hinton agar according to clinical and laboratory standard institute (CLSI). The tested antibiotics (BBL™ Sensi-Disc™, USA) include: erythromycin (15 µg), clarithromycin (15 µg), azithromycin (15 µg) and clindamycin (2 µg). The inducible type of resistance was demonstrated using double-disc diffusion test (D test) by placing clindamycin discs and erythromycin discs at distances of 16-25 mm followed by incubation at 37°C for 24 hrs [16].

Plasmid profile analysis

Analysis of plasmid-DNA for the resistant bacterial isolates was performed by plasmid DNA extraction followed by direct agarose gel electrophoresis of the extracted plasmids [17]. Plasmids were extracted by alkaline lysis method as described by Birnboim and Doly [18] using GeneJet Plasmid Miniprep Kit (Fermentas, USA) according to the manufacturer's manual.

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Table 1. List of primers used in this study.

Primer	Target gene	Primer sequence (5'à3')	Ta (°C)	PCR size (kb)	Reference
ERM-f	erm	CGAGTGAAAAAGTACTCAACC	52	617	[19]
ERM-r		GGCGTGTTTCATTGCTTGATG			
MSR-f	msr	GCGTTTAAGATAAGCTGGCA	53	1664	[19]
MSR-r		CCTGCACCATTTGCTCCTAC			

Conventional Polymerase Chain Reaction (PCR)

Amplification of the selected resistance genes was performed through PCR, using plasmid-DNA of each resistant strain as a template and the primers outlined in **Table 1**.

PCR amplification was carried with the cycling parameters as follows: After an initial denaturation step at 95°C for 4 min, 30 cycles of amplification were performed as follows: Denaturation at 95°C for 30s, annealing temperature at 53°C for 45 sand extension temperature at 72°C for 1 min, this was followed by I cycle of final extension at 72°C for 5 min, and finally the reaction was hold at 4°C for 10 min. The sizes of PCR products of these genes were analyzed by 0.8% agarose gel electrophoresis containing ethidium bromide (0.5µg/ml).

Sequencing of PCR products

The obtained PCR products were purified using GeneJET™ PCR Purification kit (Fermentas, USA) at Sigma Scientific Services Company, Egypt. Finally, sequencing was done at GATC Biotech Company (Germany), through Sigma Scientific Services Company (Egypt) by the use of ABI 3730xl DNA Sequencer.

Computer programs used for sequence analysis

The obtained sequence files (forward and reverse) were analyzed using the following programs: Staden-package program version 3 [20] was used for sequence assembly and formation of final contigs; Frameplot [21] for detection of the ORFs in the final contigs; Clustal W [22] for amino acid alignment. Finally DNA search in the GenBank database using BLAST (Basal Local Alignment Research tool) to visualize fully and/or partially conserved domains or regions within the macrolide resistance proteins products.

Results

Collection of bacterial isolates from clinical specimens

A total of 189 bacterial isolates were recovered from sputum and bronchoalveolar lavage specimens from patients with LRIs.

Antimicrobial susceptibility tests and detection of *Staphylococcus* spp. resistant isolates

Using Gram-stain, 47 isolates, out of 189 collected isolates, were found to be Gram-positive (24.9%),

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Table 2. Overall antibiotic sensitivity test results of the resistant bacterial isolates by disc diffusion method.

Antimicrobial class	Tested antimicrobial agents	No. of resistant isolates	Percentage (%)
Macrolides	Erythromycin	64	100
	Clarithromycin	64	100
	Azithromycin	57	89
Lincosamides	Clindamycin	60	93.8

Total number of resistant isolates was 64.

64 isolates (33.9%) were found to be resistant to the tested antibiotics. Among the resistant isolates, 12 isolates (18.7%) were found to be Gram-positive, including 8 isolates (n=8/64; 12.5%) which were identified to be *Staphylococcus* spp. Out of these 8 isolates, 7 isolates (S34b, S56, S63, S67, S77, S78 and R26) were confirmed to be *Staphylococcus aureus* based on yellowish growth on mannitol salt agar and positive coagulase test, while one isolate (S26) was identified as coagulase-negative *Staphylococcus* spp. The results of antibiotic sensitivity test were summarized in **Table 2.**

Among the 8 resistant *Staphylococcus* spp isolates, 4 isolates (S26, S34b, S77 and R26) showed complete resistance to all tested antibiotics. While the other 4 isolates (S56, S63, S67 and S78) showed D-shaped inhibition zone around clindamycin discs.

Plasmid profile analysis and PCR

Plasmid extraction, carried out on *Staphylococcus* spp resistant isolates, revealed the presence of plasmids in the 8 resistant isolates. The extracted plasmids were used as template for PCR using the

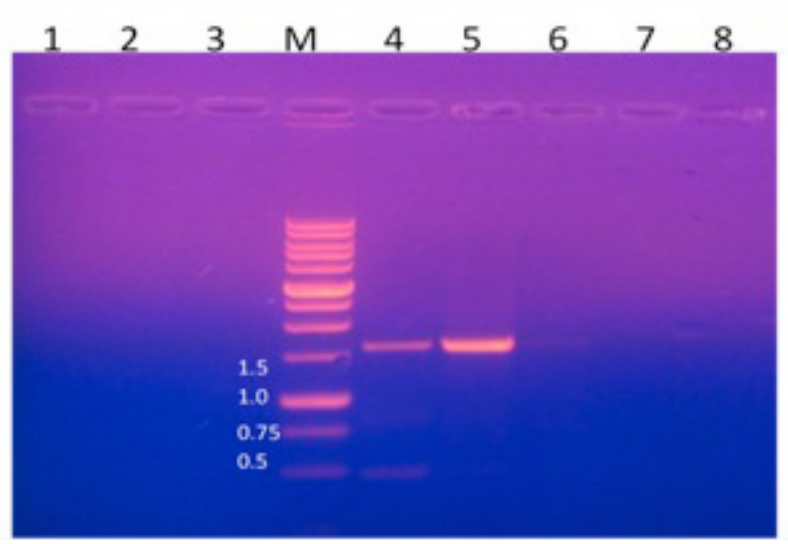

Figure 2. Agarose gel electrophoresis of the PCR products of two positive erythromycin resistance ATP-binding protein-coding genes (***msr***). Lanes: 1, PCR product of isolate S26; 2, PCR product of isolate S34b; 3, PCR product of isolate S56; 4, 1664 bp PCR product of *msr* gene in isolate S63; 5, PCR product of isolate S78; 6, PCR product of isolate S67; 7, PCR product of isolate S77; 8, PCR product of isolate R26 and M, 1 Kb size marker (Fermentas, USA).

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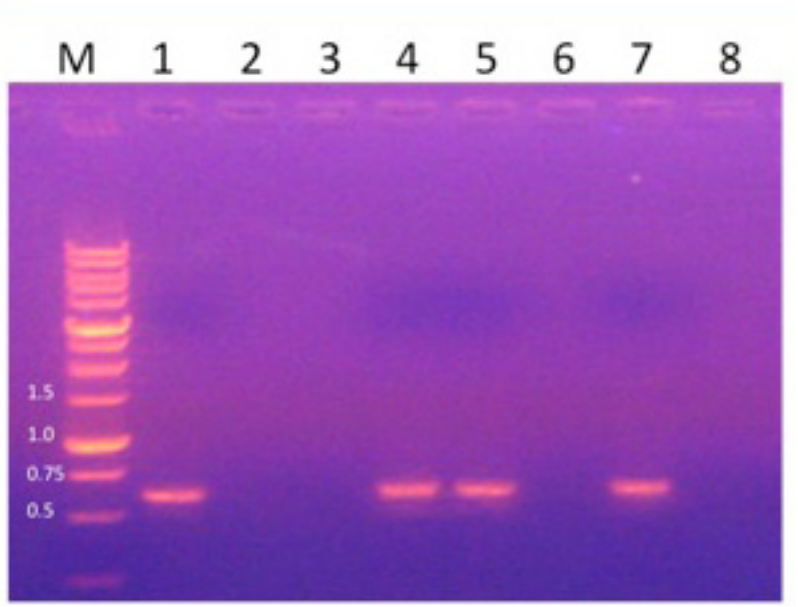

Figure 3. Agarose gel electrophoresis of the PCR products of four positive erythromycin ribosomal methylase coding genes (*erm*). Lanes: 1, 617 bp PCR product of *erm* gene in isolate S26; 2, PCR product of isolate S34b; 3, PCR product of isolate S56; 4, PCR product of isolate S63; 5, PCR product of isolate S67; 6, PCR product of isolate S77; 7, PCR product of isolate S78; 8, PCR product of isolate R26 and M, 1 Kb size marker (Fermentas, USA).

Table 3. The prevalence of the resistance genes among the tested isolates.

Isolate number	Presence of plasmids	erm	msr
S26	+	+	-
S34b	+	-	-
S56	+	-	-
S63	+	+	+
S67	+	+	-
S77	+	-	-
S78	+	+	+
R26	+	-	-

erm (erythromycin ribosomal methylase) and *msr* (erythromycin resistance ATP-binding protein).

primers listed in **Table 1**. The gel electrophoresis of PCR products showed the presence of *msr* and *erm* genes **(figure 2 and figure 3)**. The prevalence of the resistance genes among the tested isolates were summarized in **Table 3**. The correlation between the genotype and phenotype of resistant isolates was shown in **Table 4**.

Sequencing of PCR products

Further verification of the *msr* gene was performed by DNA sequencing of the PCR product obtained by using the plasmid extract from isolate S78 as a template for PCR. The sequence analysis revealed the presence of *msr* gene. The nucleotide sequence of *msr* gene was submitted to the nucleotide Gen-

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Table 4. Correlation between the genotype and phenotype of resistant *S. aureus* isolates.

Isolate number	Genotype		Phenotype			
	erm	msr	EM	CLA	AZM	CLI
S26	+	-	+	+	+	+
S34b	-	-	+	+	+	+
S56	-	-	+	+	+	D-shaped I.Z
S63	+	+	+	+	+	D-shaped I.Z
S67	+	-	+	+	+	D-shaped I.Z
S77	-	-	+	+	+	+
S78	+	+	+	+	+	D-shaped I.Z
R26	-	-	+	+	+	+

I.Z: Inhibition zone, EM: Erythromycin, CLA: Clarithromycin, AZM: Azithromycin, CLI: Clindamycin.

Bank database, and given accession code KJ710361. The results of multiple sequence alignment of *msr* gene with its homologous proteins by using Clustal W software were shown in **figure 4**.

Discussion

This study was concerned with the detection of different determinants of macrolide resistance in plasmids extracted from *Staphylococcus* spp. recovered from sputum and bronchoalveolar lavage specimens from patients with LRIs in Egypt.

There are two main mechanisms of MLS resistance in staphylococci, which are: Target site modification due to *erm* genes, and active-efflux due to *msr A* gene [13, 23-24]. *msr* gene affects only MACs (M phenotype) or MACs and streptogramin type B(MS phenotype). While, The expression of *erm* gene can be constitutive (cMLS phenotype) or inducible (iMLS phenotype) [25].

In this study, the antimicrobial susceptibility testing of the recovered *Staphylococcus* spp was carried out using disc diffusion method according to CLSI. 4 isolates (S26, S34b, S77 and R26) were found to be resistant to MACs as well as clindamycin (linosamide), which indicate cMLS resistance phenotype. The other 4 isolates (S56, S63, S67 and S78) showed flattening of the clindamycin inhibition zone towards an adjacent erythromycin disc by using double-disc diffusion test (D test), while, they are resistant to the other MACs, which indicate iMLS resistance phenotype.

The phenotype of MLS resistance, either constitutive or inducible, may show great variations based on the patient groups in different hospitals, and on geographical region. This variation may be attributed to several factors as: The inconsistent use of MACs in different hospitals, patient age and the origin of the tested isolate [26-27]. In our study, it was found that 4 resistant *Staphylococcus* Spp (50%) showed cMLS resistance. While, the other 4 isolates (50%) showed iMLS resistance. In study carried out in London by Hamilton-Miller et al. iMLS phenotype was the predominant phenotype (43%) followed by cMLS phenotype which was found in 24% of the isolates [28]. In another study conduct-

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msr-2Sa         MEQYTIKFNQINHKLTDLRSLNIGHLYAYQFEKIALIGGNGTGKTTLLNMIAQKTKPESG 60
msr-query       MEQYTIKFNQINHKLTDLRSLNIGHLYAYQFEKIALIGGNGTGKTTLLNMIAQKTKPESG 60
msr-4Sa         MEQYTIKFNQINHKLTDLRSLNIGHLYAYQFEKIALIGGNGTGKTTLLNMIAQKTKPESG 60
msr-3Sa         MEQYTIKFNQINHKLTDLRSLNIGHLYAYQFEKIALIGGNGTGKTTLLNMIAQKTKPESG 60
msr-1Sa         MEQYTIKFNQINHKLTDLRSLNIGHLYAYQFEKIALIGGNGTGKTTLLNMIAQKTKPESG 60
                ************************************************************

msr-2Sa         TVETVGEIQFEQLNMDVENDFNTLDGSLMSELHIPMHTTDSMSGGEKAKYKLANVISNYS 120
msr-query       TVETVGEIQFEQLNMDVENDFNTLDGSLMSELHIPMHTTDSMSGGEKAKYKLANVISNYS 120
msr-4Sa         TVETVGEIQFEQLNMDVENDFNTLDGSLMSELHIPMHTTDSMSGGEKAKYKLANVISNYS 120
msr-3Sa         TVETVGEIQFEQLNMDVENDFNTLDGSLMSELHIPMHTTDSMSGGEKAKYKLANVISNYS 120
msr-1Sa         TVETVGEIQFEQLNMDVENDFNTLDGSLMSELHIPMHTTDSMSGGEKAKYKLANVISNYS 120
                ************************************************************

msr-2Sa         PILLLDEPTNHLDKIGKDLKNILKYYYGTLIIVSHDRALIDQIADTIWDIQEDGTIRVFK 180
msr-query       PILLLDEPTNHLDKIGKDLKNILKYYYGTLIIVSHDRALIDQIADTIWDIQEDGTIRVFK 180
msr-4Sa         PILLLDEPTNHLDKIGKDLKNILKYYYGTLIIVSHDRALIDQIADTIWDIQEDGTIRVFK 180
msr-3Sa         PILLLDEPTNHLDKIGKDLKNILKYYYGTLIIVSHDRALIDQIADTIWDIQEDGTIRVFK 180
msr-1Sa         PILLLDEPTNHLDKIGKDLKNILKYYYGTLIIVSHDRALIDQIADTIWDIQEDGTIRVFK 180
                ************************************************************

msr-2Sa         GNYTQYQNQYEQEQLEQQRQYEQYISEQRLSQASKAKRNQAQQMAQASSKQKNKSIAPDR 240
msr-query       GNYTQYQNQYEQEQLEQQRQYEQYISEQRLSQASKAKRNQAQQMAQASSKQKNKSIAPDR 240
msr-4Sa         GNYTQYQNQYEQEQLEQQRQYEQYISEQRLSQASKAKRNQAQQMAQASSKQKNKSIAPDR 240
msr-3Sa         GNYTQYQNQYEQEQLEQQRQYEQYISEQRLSQASKAKRNQAQQMAQASSKQKNKSIAPDR 240
msr-1Sa         GNYTQYQNQYEQEQLEQQRQYEQYISEQRLSQASKAKRNQAQQMAQASSKQKNKSIAPDR 240
                ************************************************************

msr-2Sa         LSASKQKGTVEKAAQKQAKHIEKRMEHLEEVEKPQSHEFNFPQNKIYDIHNNYPIIAQNL 300
msr-query       LSASKQKGTVEKAAQKQAKHIEKRMEHLEEVEKPQSHEFNFPQNKIYDIHNNYPIIAQNL 300
msr-4Sa         LSASKQKGTVEKAAQKQAKHIEKRMEHLEEVEKPQSHEFNFPQNKIYDIHNNYPIIAQNL 300
msr-3Sa         LSASKQKGTVEKAAQKQAKHIEKRMEHLEEVEKPQSHEFNFPQNKIYDIHNNYPIIAQNL 300
msr-1Sa         LSASKQKGTVEKAAQKQAKHIEKRMEHLEEVEKPQSHEFNFPQNKIYDIHNNYPIIAQNL 300
                ************************************************************

msr-2Sa         TLVKGSQKLLTQVRFQIPYGKNIALVGANGVGKTTLLEAIYHQIEIDCSPKVQMAYYRQL 360
msr-query       TLVKGSQKLLTQVRFQIPYGKNIALVGANGVGKTTLLEAIYHQIEIDCSPKVQMAYYRQL 360
msr-4Sa         TLVKGSQKLLTQVRFQIPYGKNIALVGANGVGKTTLLEAIYHQIEIDCSPKVQMAYYRQL 360
msr-3Sa         TLVKGSQKLLTQVRFQIPYGKNIALVGANGVGKTTLLEAIYHQIEIDCSPKVQMAYYRQL 360
msr-1Sa         TLVKGSQKLLTQVRFQIPYGKNIALVGANGVGKTTLLEAIYHQIEIDCSPKVQMAYYRQL 360
                ************************************************************

msr-2Sa         AYEDMRDVSLLQYLMDETDSSESFSRAILNNLGLNEALDRSCNVLSGGERTKLSLAVLFS 420
msr-query       AYEDMRDVSLLQYLMDETDSSESFSRAILNNLGLNEALDRSCNVLSGGERTKLSLAVLFS 420
msr-4Sa         AYEDMRDVSLLQYLMDETDSSESFSRAILNNLGLNEALDRSCNVLSGGERTKLSLAVLFS 420
msr-3Sa         AYEDMRDVSLLQYLMDETDSSESFSRAILNNLGLNEALDRSCNVLSGGERTKLSLAVLFS 420
msr-1Sa         AYEDMRDVSLLQYLMDETDSSESFSRAILNNLGLNEALDRSCNVLSGGERTKLSLAVLFS 420
                ************************************************************

msr-2Sa         TKANMLILDEPTNFLDIKTLEALEMFMNKYPGIILFTSHDTRFVKHVSDKKWELTGQSLH 480
msr-query       TKANMLILDEPTNFLDIKTLEALEMFMNKYPGIILFTSHDTRFVKHV------------ 467
msr-4Sa         TKANMLILDEPTNFLDIKTLEALEMFMNKYPGIILFTSHDTRFVKHVS----------- 468
msr-3Sa         TKANMLILDEPTNFLDIKTLEALEMFMNKYPGIILFTSHDTRFVKHVSDKKWELTG---- 476
msr-1Sa         TKANMLILDEPTNFLDIKTLEALEMFMNKYPGIILFTSHDTRFVKHVSDKKWELTGQSIH 480
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Figure 4. Multiple alignment of amino acid sequence of MSR-query Erythromycin resistance ATP-binding protein of *Staphylococcus aureus* isolate (S78) (AC=KJ710361) against its homologous in the GenBank database: **MSRA-1Sa**= Erythromycin resistance protein, *Staphylococcus aureus*, AC= YP_254220.1; **MSRA-2Sa** = Erythromycin resistance protein, *Staphylococcus aureus*; AC= EVR81644.1; **MSRA-3Sa** = Erythromycin resistance protein, *Staphylococcus aureus*, AC= EVR51631.1; **MSRA-4Sa** = Erythromycin resistance protein, *Staphylococcus aureus*, AC= EUR46906.1. The numbers indicate positions within the corresponding proteins. AC = GenBank accession number.

ed by Fiebelkorn et al. in Texas, cMLS phenotype was more prevalent (41.7%) followed by both iMLS and MS phenotypes (3.3% each) [29]. The high incidence of iMLS phenotype in this study may be attributed to the increased concurrent use of MAC and clindamycin antibiotics [25].

In addition to the phenotypic tests used for screening of MLS resistance, genetic tests were also carried out. It was found that the 8 resistant isolates showed plasmid bands upon plasmid extraction and running the extract on 0.8% agarose gel electrophoresis. PCR was done on all 8 resistant isolates, using plasmid extracts as templates, to amplify the two plasmid-mediated genes: *erm* and *msr*. *erm* gene was more prevalent than *msr* gene among the 8 resistant isolates. The results revealed the presence of *erm* gene in 4 isolates (50%), and *msr* gene in only 2 isolates (25%). It was noticed that *erm* gene was more prevalent than *msr* gene. This finding was in accordance with the prevalence results in the study conducted in Turkey by Aktas et al. [26].

In this study, 3 resistant *Staphlococcus* spp isolates (S34b, S77 and R26) showed cMLS phenotype, and 1 isolate (S56) showed iMLS phenotype. However, PCR results, using the primers listed in **table 1**, were negative for *erm* genes. This study was concerned with plasmid-mediated genes and the PCR was carried out on plasmid extracts of the resistant isolates to detect plasmid-harbored genes. The resistance in the *erm*-negative isolates may be due to the presence of either *erm* or *msr* genes or both on the genomic-DNA. 3 isolates (S63, S67 and S78) were positive for *erm* genes, and showed iMLS resistance phenotype. While only 1 isolate (S26) was positive for *erm* gene, and showed cMLS phenotype. In inducible resistance, the bacteria produce inactive mRNA that is unable to encode methylase. The mRNA becomes active only in the presence of a macrolide inducer. While, in constitutive resistance, active methylase mRNA is produced in the absence of an inducer [13]. Induction is related to the presence of an attenuator upstream from the structural *erm* gene coding for the methylase [30].

The *msr* gene, responsible for M phenotype, was found in 2 isolates in this study (S63 and S78). However, these 2 isolates showed iMLS resistance phenotype. This may be due to the presence of *erm* genes in these isolates. So, the M phenotype of *msr* gene was masked by the iMLS phenotype of *erm* gene.

Therefore, results obtained from our study were of great significance regarding the prevalence of plasmid-mediated MAC resistance genes in staphlococci, which may result in horizontal transfer of MAC resistance genes among different gram-positive bacteria causing LRIs. Therefore, the reasons behind the prevalence of these plasmid-mediated genes among staphylococci have to be studied in order to limit the dissemination of macrolide resistance.

List of Abbreviations

erm= Erythromycin ribosomal methylase
LRIs= Lower resoiratory tract infections.
MACs= Macrolides.
MLS= Macrolides, lincosamides and streptogramin type B..

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