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Phenotypic and genotypic analysis of macrolide-lincosamide-streptogramin B resistance in methicillin-resistant *Staphylococcus aureus* and methicillin-susceptible *Staphylococcus aureus* isolates

Metisiline dirençli *Staphylococcus aureus* ve metisiline duyarlı *Staphylococcus aureus* izolatlarında makrolid-lincosamide-streptogramin B direncinin fenotipik ve genotipik analizi

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ABSTRACT

Objective: In this study, we aimed to characterize the MLSB phenotype and genotypic resistance genes of MRSA and MSSA isolates from Baskent University Hospital, and resistance rates of MSSA and MRSA isolates were compared.

Methods: The study included 50 MSSA and 50 MRSA isolates collected between 2016 and 2022 from the hospital of Baskent University located in Ankara and Adana. First, *S. aureus* isolates were confirmed by catalase and coagulase tests, and the MRSA isolates were confirmed by cefoxitin disc diffusion test. To determine the MLSB resistance, isolates were tested for clindamycin and erythromycin resistance using the disk diffusion test(D-test). According to D-test, resistance phenotypes were detected and resistant phenotypes were screened for resistance genes using PCR. PCR amplification was made using primers specific for *ermA*,*ermB*,*ermC*, and *msrA* genes.

Results: Among 50 MRSA isolates, 25 (50%) were resistant to erythromycin, susceptible to clindamycin, and

ÖZET

Amaç: Bu çalışmada Başkent Üniversitesi Hastanesi'nde izole edilen MRSA ve MSSA izolatlarının MLSB fenotipi ve genotipik direnç genlerinin karakterize edilmesi amaçlanmış ve MSSA ve MRSA izolatlarının direnç oranları karşılaştırılmıştır.

Yöntem: Çalışmaya Başkent Üniversitesi Ankara ve Adana Hastanelerinden 2016-2022 yılları arasında toplanan 50 MSSA ve 50 MRSA izolatı dahil edilmiştir. İlk olarak *S. aureus* izolatları katalaz ve koagülaz testleri ile, MRSA izolatları ise sefoksitin disk difüzyon testi ile doğrulanmıştır. MLSB direncini belirlemek için izolatlar, disk difüzyon testi (D-test) kullanılarak klindamisin ve eritromisin direnci açısından çalışılmıştır. D-test sonuçlarına göre direnç fenotipleri saptanmış ve dirençli fenotipler polimeraz zincir reaksiyonu (PZR) kullanılarak direnç genleri açısından taranmıştır. *ermA*, *ermB*, *ermC* ve *msrA* genlerine spesifik primerler kullanılarak PZR amplifikasyonu yapılmıştır.

Bulgular: Elli MRSA izolatından 25'i (%50) eritromisine dirençli, klindamisine duyarlı ve D-zonu

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showed D-zone positivity, indicating an inducible resistance phenotype (iMLSB). The two (4%) isolates were resistant to clindamycin and erythromycin without the D zone indicating constitutive cMLSB phenotype. Twenty-two(44%) isolates were susceptible to clindamycin and erythromycin with D-zone negative indicating S phenotype. Only one isolate(2%) was susceptible to clindamycin and has erythromycin resistance with absence of D-zone indicating MSB phenotype. While 8(16%) of the fifty MSSA isolates were found to have the D-zone positive iMLSB phenotype resistant to erythromycin, two(4%) isolates were found to have the cMLSB phenotype resistant to both antibiotics without the D-zone. There were 40(80%) isolates that were susceptible to clindamycin and erythromycin without D zone. Resistance genes were examined in a total of 38 samples, including 28 MRSA and 10 MSSA samples. Among the MLSB resistance genes, *ermC* was found positive in 25 MRSA(89.3%) and 4 MSSA(40%) samples. The second most commonly detected gene was ermA, which was detected in 4(40%) MSSA isolates but not in any MRSA isolates.The *msrA* gene was confirmed positive in one MRSA sample with the MSB phenotype. All samples were negative for the presence of the *ermB* gene.

Conclusion: In conclusion, iMLSB phenotype was the most common resistance pattern, consistent with previous studies conducted in Turkey, and was more frequently detected among MRSA isolates in this study, which included samples from Ankara and Adana. Among resistance genes for MLSB resistance, the most frequent gene was *ermC* in line with the literature. *ermA* positivity was very less and *ermB* was not detected. Therefore, we can say that the *ermC* gene is more common in this region.

Key Words: *Staphylococcus aureus*, macrolide, lincosamide, streptogramin B, *erm* gene, *msr* gene

pozitif olacak şekilde indüklenebilir direnç fenotipini (iMLSB) göstermiştir. İki (%4) izolat, yapısal direnç fenotipini (cMLSB) gösteren D-zonu negatif, klindamisin ve eritromisin dirençli saptanmıştır. Yirmiiki (%44) izolat ise S fenotipini gösteren D-zonu negatif, klindamisin ve eritromisin duyarlı saptanmıştır. Sadece bir izolat (%2) klindamisine duyarlı, eritromisin dirençli ve MSB fenotipine uygun olarak D-zonu negatif bulunmuştur. Elli MSSA izolatının 8'inin (%16) eritromisine dirençli D-zonu pozitif iMLSB fenotipine sahip olduğu saptanırken, iki (%4) izolatın ise D-zonu olmaksızın her iki antibiyotiğe de dirençli cMLSB fenotipine sahip olduğu bulunmuştur. D-zonu olmayan klindamisin ve eritromisin duyarlı olarak ise, 40 (%80) izolat saptanmıştır. Direnç genleri 28 MRSA ve 10 MSSA örneği olmak üzere toplamda 38 örnekte incelenmiştir. MLSB direnç genlerinden, *ermC*, 25 MRSA (%89,3) ve 4 MSSA örneğinde (%40) pozitif bulunmuştur. İkinci en sık saptanan gen ise ermA geni olmuştur; 4 (%40) MSSA izolatında saptanırken, MRSA izolatlarında ise saptanmamıştır. MSB fenotipli bir MRSA örneğinde *msrA* geni pozitif olarak doğrulanmıştır. Tüm örnekler, *ermB* geni varlığı açısından negatif bulunmuştur.

Sonuç: Sonuç olarak, Ankara ve Adana'dan izole edilen izolatlarda gerçekleştirilen bu çalışmada, ülkemizde daha önce yapılan çalışmalarla benzer şekilde, iMLSB fenotipi en yaygın fenotip olarak bulunmuş ve MRSA izolatlarında daha yaygın olduğu görülmüştür. MLSB direnci için direnç genleri arasında en sık görülen gen literatürle uyumlu olarak *ermC* olmuştur. *ermA* pozitifliği ise az sayıda saptanmış ve *ermB* geni hiç saptanmamıştır. Dolayısıyla *ermC* geninin bu bölgede daha yaygın olduğunu söyleyebiliriz.

Anahtar Kelimeler: *Staphylococcus aureus*, makrolid, linkozamid, streptogramin B, *erm* geni, *msr* geni

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen which causes various infections, such as skin infections, abscess, wound infections, and also fatal diseases, such as endocarditis, osteomyelitis, or septicemia (1). Since 1960s, methicillin-resistant *S. aureus* (MRSA) is one of the global reasons of

healthcare-associated outbreaks (2). The MRSA rates are increasing in the hospital and community settings in the USA, Asia, and some parts of Europe. MRSA has also been found in Turkish hospitals as an important problem (3). *S. aureus* can cause community or nosocomial infections such as mild skin, soft tissue infections and even fulminant sepsis (4). A cut or a wound on the skin, or any other reason might result in *S. aureus* infection. Transition to the blood circulation is one of the most common reasons for septicemia, endocarditis, toxic shock syndrome, osteomyelitis and pneumonia (5). The treatment of invasive *S. aureus* infections is difficult, therefore serious spread control precautions are needed and a long period of antimicrobial treatment (4).

Methicillin-resistant *S. aureus* strains are resistant to every antibiotic with ß-lactam ring, such as carbapenems and cephalosporins. Also, MRSA gains resistance against antibiotics without ß-lactam ring such as erythromycin (ERY) and clindamycin (CLI). Resistance to CLI and ERY in staphylococci, happens via ribosomal target site methylation and it is usually encoded by erm genes. When strains are resistant to ERY and CLI, this resistance is called constitutive (cMLSB), when strains are ERY resistant and susceptible to CLI in vitro, this resistance is inducible (iMLSB). Inducible resistance can cause difficulties in clinical treatments (6). Beside nowel antimicrobials have been presented, clindamycin supports treatment choices with its great pharmacologic features for MSSA and MRSA. Also, for penicillin-allergic patients, clindamycin is a good alternative. However, drug inactivation mechanisms, efflux or target site modification can cause resistance to MLSB antibiotics (7). In the 1980s, MRSA became a major clinical problem in Turkish hospitals. MRSA resistance varied between 7% and 55% across centers. Recently, data from various centers in Turkey for the past 5 years showed that the percentage of MRSA strains was 65.5%. All strains were reported to be susceptible to vancomycin and teicoplanin, but resistance to these drugs may develop (8).

The aim of this study is to detect phenotypic and genotypic MLSB resistance in MRSA and MSSA isolates in Baskent University Hospitals and to compare the resistance rates of MSSA and MRSA isolates.

MATERIAL and METHOD

Phenotypic Tests

Fifty MSSA and 50 MRSA isolates from Baskent University Ankara and Adana Hospitals that were collected between 2016-2022 were included in the study. Firstly, *S. aureus* isolates were confirmed by catalase and coagulase tests. MRSA and MSSA isolates were confirmed by disc diffusion test via cefoxitin disc (30 µg). To determine the MLSB resistance, isolates were tested for clindamycin (2 µgr) and erythromycin (15 µgr) resistance using the disk diffusion (D-test) test (Bioanalyse, Ankara/ Turkey).

Genotypic Tests

Clindamycin and/or erythromycin resistant MSSA and MRSA isolates were taken to the genotypic tests. DNA isolation was made by boiling method that described before (9). Resistant phenotypes were screened for resistance genes by using polymerase chain reaction (PCR). PCR amplification (7) was performed by using *ermA*, *ermB*, *ermC* (10), *msrA* primers (11) and PCR Mastermix (Solis Biodyne, G*erm*Any). *S. aureus* RN1551, *S. aureus* 6520, *S. aureus* FPR3757, *S. aureus* 15114 reference strains were used as positive controls for *ermA*, *ermB*, *ermC* and *msrA* genes, respectively. Amplicons were loaded on to the 1.5% agarose gel with ethidium bromide and observed via UV light.

Statistical analysis

Since the resistance rates of the MSSA and MRSA isolate groups in the study was compared, "Significance test of the difference between two percentages in independent groups" or "2x2 Chi-square tests in independent groups" or "Fisher's exact test" was used. Type I error probability was taken as α=0.05 in all hypothesis tests and SPSS v25.0 package program was used for statistical evaluations. G*Power

3.1.9 program was used while calculating the required sample size to test the research hypothesis.

This study was approved by Baskent University Institutional Review Board (Project no: KA22/361) and supported by Baskent University Research Fund.

RESULTS

The clinical samples were collected and sent to Microbiology Laboratories between March 2016 and October 2022. A hundred *S. aureus* isolates were included in the study. Number of sample types were 33 blood, 35 tissue biopsy, 13 body fluid, 12 wound/pus, 4 sputum, 2 deep tracheal aspirate and 1 respiratory secretion. All samples were sent from different departments such as cardiovascular surgery, nephrology, cardiology, chest diseases, emergency, anesthesia, general surgery, orthopedics and traumatology, pediatric nephrology, plastic surgery, neonatal, obstetrics and gynecology, d*erm*Atology, pediatric cardiology, burn treatment, neurology, infectious diseases, otolaryngology, geriatrics, general internal medicine, oncology, neurosurgery, urology, pediatric infectious diseases, endocrinology.

Phenotypic Results

Among all included isolates, 50 of them were methicillin-susceptible *S. aureus* (MSSA) and the other 50 were methicillin-resistant *S. aureus* (MRSA), all of them were confirmed by catalase and coagulase tests. We also confirmed methicillin resistance by using cefoxitin discs according to CLSI standards. Both MSSA and MRSA isolates were tested for erythromycin and clindamycin susceptibility for the MLSB resistance. Among 50 MRSA isolates 25 (50%) isolates were resistant to erythromycin with D-zone positive and susceptible to clindamycin indicating iMLSB phenotype. Two (4%) isolates were resistant to clindamycin and erythromycin without the D-zone indicating cMLSB phenotype. Twenty-two (44%) isolates were susceptible to clindamycin and erythromycin with D-zone negativity. Only one isolate (2%) was susceptible to clindamycin and has erythromycin resistance with absence of D-zone indicating MSB phenotype. Among all 50 MSSA isolates; 8 (16%) isolates were iMLSB phenotype and 2 (4%) isolates were cMLSB phenotype. There were 40 (80%) isolates that were susceptible to both erythromycin and clindamycin without D-zone.

Comparison of the MLSB phenotypes with the methicillin resistance were given in Table 1. The phenotypic difference between methicillin-susceptible and resistant groups is statistically significant with 95% confidence due to the iMLSB and ERY/CLI susceptible phenotypes ($p<0.05$).

Genotypic Results

We studied the resistance genes *ermA*, *ermB*, *ermC* and *msrA* in 28 MLSB resistant samples of MRSA and 10 MLSB resistant samples of MSSA from a total of 38 samples. The most common gene was *ermC* due to 25 positive samples (89.3%) for MRSA samples and positive for 4 MSSA samples (40%). The difference in the number of *ermC* gene region positive isolates between the methicillin-susceptible and resistant groups is statistically significant with 95% confidence ($p<0.05$).

Table 1. Comparison of the MLSB phenotypes distribution according to methicillin resistance status

No (%)	iMLSB	cMLSB	MSB	ERY/CLI Susceptible	Total
MRSA	25 (75.8%)	2 (50%)	1 (100%)	22 (35.5%)	50 (50%)
MSSA	8 (24.2%)	2 (50%)	-	40 (64.5%)	50 (50%)
Total	33 (100%)	4 (100%)	1 (100%)	62 (100%)	100 (100%)

The second common gene was *ermA*; it was detected in 4 MSSA samples (40%) and was not detected in MRSA samples. The difference in the number of *ermA* gene region positive isolates between the methicillin susceptible and resistant groups is not statistically significant with 95% confidence (p=0.117). *ErmB* gene was not detected in any of the samples. In addition, *msrA* gene was confirmed in one MSB phenotype sample. The difference in the number of *msrA* gene region positive isolates between the methicillin-susceptible and resistant groups is not statistically significant with 95% confidence (p=1.000).

DISCUSSION

We studied a hundred samples of *S. aureus* isolates that were tested for susceptibility to erythromycin and clindamycin. Fifty of them were MRSA and the other 50 were MSSA and also confirmed in our study by using cefoxitin disc. In the 50 MRSA isolates, the inducible phenotype was detected in 25 isolates (50%), the constitutive phenotype in 2 isolates (4%), the MSB phenotype in 1 isolate (2%) and 22 isolates (44%) were susceptible to erythromycin and clindamycin without D zone. In 50 MSSA isolates tested 8 (16%) isolates showed iMLSB phenotype which is resistant to erythromycin with D zone positive. And 2 (4%) isolates are cMLSB that give resistance to both antibiotics with D zone negative. There are 40 (80%) isolates that are susceptible to clindamycin and erythromycin without D zone. In a recent study by Nahar et al. in 2023 iMLSB resistant MRSA isolates were detected more than iMLSB resistant MSSA (58.6%, 23.5%) respectively, these results are similar to our results (12). Mahesh et al. found out in 2022 that of 140 *S. aureus* isolates, 33.6% were MSSA and 66.4% were MRSA. iMLSB phenotype rate was 29.3%, and cMLSB phenotype rate was 26.4%. And only 8 (17%) iMLSB isolates were found in MSSA strains similar to our results (13). In the study of Assefa in 2022, 605 of 3064 *S. aureus* isolates were iMLSB resistant (19.8%) (14). iMLSB phenotypes in MRSA strains were highest in Egypt, Nigeria and Libya as 77.8%, 75.0%, 66.2%, respectively. The lowest occurrence of the phenotypes iMLSB among MRSA isolates was reported in Coteˆd'Ivoire's study with 3.9%. The iMLSB phenotype was not detected in MSSA strains in 2007 in Libya and in 2017 in Cote d'Ivoire, cMLSB phenotypes was showed in MRSA and MSSA strains as 0–75% 0–60%, respectively (14).

In 2015, Ozansoy et al. from Turkey reported that 39.1% of *S. aureus* isolates were MRSA and 60.9% were MSSA. In MRSA samples cMLSB and iMLSB resistances were 7.6% and 56.3%, respectively. 1.3% of isolates were MS phenotype and 34% of isolates belonged to erythromycin/clindamycin susceptible phenotype. Among the MSSA samples iMLSB was 8.9% and cMLSB was 2.9%, MSB was 1.2% and erythromycin/clindamycin susceptibility was 87% (15). Özbek et al found in 2021 that cMLSB resistance (49%) was higher than iMLSB (19%) and MSB phenotype was not detected (16). In the study of Uyar Gulec et al. iMLSB resistance was determined in 25%, structural resistance in 42.9% and MSB phenotype resistance in 3.5% of MRSA strains in 2010. This rate was 15.3% structural resistance and 7.7% inducible resistance in MSSA. No resistance was observed in the MSB phenotype in MSSA strains (7).

There are differences between our study and Modukuru et al's study which reported that 165 *S. aureus* isolates were susceptible to erythromycin and clindamycin in a total of 339 isolates. Rest of them (174 isolates, 56.3% MRSA and 43.7% MSSA) had resistance to erythromycin or clindamycin, or both. Among MRSA strains, 76.6% were cMLSB, 64.5% were iMLSB, and 43.75% were MSB phenotype. Among 76 MSSA isolates; 23.40% was cMLSB, 35.48% iMLSB and 56.25% was MS phenotype. This study shows that iMLSB phenotype had higher rate in MRSA isolates comparing to MSSA isolates (17). In the study of Nagarkoti et al., 60 isolates were found as erythromycin-resistant in 312 Staphylococcal isolates comprising 65% *S. aureus* and 71% of them were representing MRSA, among them cMLSB, iMLSB, MS phenotypes were 12%, 44%, 44%, respectively. Among MSSA isolates cMLSB resistance was 35.7%, iMLSB 7.2% and MS 57.1% (18). There are differences between our results and Pereira et al's study in 2016

which reported that 22 (21.4%) MRSA and 37 (35.9%) MSSA were detected. It was detected that among MRSA isolates 22.7% of them belonged to erythromycin/clindamycin susceptible phenotype, 68.2% cMLSB, 4.5% iMLSB, 4.5% MSB phenotype. Among MSSA isolates erythromycin/clindamycin susceptible phenotype was 67.6%, cMLSB 10.8%, iMLSB 10.8%, MSB 4.5% (19). Abouelnour et al.'s study from Egypt obtained that in MRSA isolates iMLSB rate was 25.2%, cMLSB 30.8% and MS 4.7%. About MSSA results iMLSB phenotype was 18.7%, cMLSB 12.4%, MS 8.4% (20). In the study of Tandon et al. in 2018; among 604 isolates, 36.4% were MRSA and 11.4% of them were reported as iMLSB phenotype (21). Zeki et al. in 2015 reported that in a total of 63 *S. aureus* isolates 32 (50.8%) were MRSA and 31 (49.2%) were MSSA. They reported that among MSSA isolates, 29 strains had resistance to erythromycin and 18 strains had resistance to clindamycin and 10 strains had resistance to both erythromycin and clindamycin (22). Timsina et al. in 2020 showed that in 64 *S. aureus* isolates, 17 (26.6%) were MRSA and 15 (23.4%) of them have iMLSB resistance. iMLSB resistance were higher in MRSA isolates (76.4%) than MSSA isolates (4.2%) (23). Khodabandeh et al. in 2019, among 106 MRSA isolates the rate of cMLSB resistance was 56.2%, iMLSB resistance was 22.9%, and MSB resistance was 16.6% (24). Goudarzi et al. reported in 2020 that in MRSA and MSSA isolates, cMLSB phenotype was found (30.2%, 24.4%), however iMLSB and MS phenotypes were detected only in MRSA isolates (25). In the study of Antonio et al. between 1990 and 2019, 3544 MSSA and 819 MRSA isolates were detected in blood stream infections (26). Aetrugh et al. in 2022 reported that the distribution of isolates showing iMLSB phenotype was 19.4% for MRSA and 6.4% for MSSA isolates (27). The studies from literature show that according to the region iMLSB and cMLSB resistance rates are variable. In general, iMLSB resistance is higher than cMLSB resistance.

We studied the resistance genes in 28 samples of MRSA and 10 samples of MSSA isolates with MLSB phenotypic resistance from a total of 100 samples. In our study, it was found that the *ermC* gene is the most common gene (89.3%) in MRSA isolates and positive for 2 iMLSB MSSA isolates and *ermC* gene was detected in 2 cMLSB MRSA isolates and 2 MSSA isolates. The second common gene was *ermA* because it was positive in 4 MSSA samples with iMLSB resistance. And *ermB* gene was negative for all isolates. The other detected gene was the *msrA* gene which was positive for one isolate in MRSA samples. The results of Nagarkoti et al. in 2019 agreed with our results due to in 39 *S. aureus* isolates, *ermC* gene was found as 36% which was the most common gene in the study and *ermB* gene was detected as 5%. However, the *ermA* gene was not detected. Also *msrA* and *msrB* genes were detected in 2.6% and 5.1% of *S. aureus* isolates (18). Pereira et al. reported in 2016 that among 44 *S. aureus* isolates with cMLSB and iMLSB phenotypes had 38.6% *ermC* gene and 9.1% had *ermA* gene (19). In the study of Assefa in 2022, the rate of *ermC* gene was 70% and the *ermA* gene was detected as 67.9% in Egypt, which is very different from our result. They also reported that another common gene was *msrA* in Egypt with 70% detection rate (14). Nahar et al. in 2023 found *ermC* gene 14.3% in MSSA and 11.5% in MRSA isolates. The *ermA* gene predominated in both MSSA (70.1%) and MRSA (86.9%) isolates, different from our study (12). This result found higher *ermC* rate in MSSA isolates. Mazloumi et al. in 2021 detected the *ermC* gene as the frequent gene in *S. aureus* isolates (43.5%) and *ermA* gene had the lowest frequency among MRSA and MSSA isolates and 7% of these strains were positive for the *msrA* gene. They detected the *ermB* gene as the most frequent gene among *S. aureus* (44.6%) isolates. In addition, *ermB* (57.1%) and *ermC* (53.1%) genes were found to have a high frequency in MRSA isolates (28). Abouelnour et al. obtained in 2020 that *ermA* (29%) and *ermC* (18.7%) were widespread genes carried by the isolates, however *ermB* (4.7%) was carried by a few isolates (20). Tandon et al. in 2018 found among inducible resistant isolates, 25 *ermC* (84%) isolates and *ermA* and *ermB* genes were not detected (21). Timsina et al. reported in 2020 that 15.6% were *ermA* positive, 3.1% were *ermB* and 18.7% were *ermC* positive (23).

Khodabandeh et al. reported in 2019 that 81.8%, 63.6% and 54.5% of 11 isolates with iMLSB phenotype, *ermC*, *ermB* and *ermA* genes were detected, respectively. The rates of *ermA*, *ermB*, *ermC*, *msrA* and *msrB* genes were 25.9%, 18.5%, 44.4%, 0.0% and 0.0%, respectively in cMLSB phenotype isolates (24). In the study of Goudarzi et al. in 2020, the results showed that the *ermC* gene was detected as 40.7%, *ermB* gene rate was 14% and *ermA* gene rate was 8.1% among all gene regions studied (25). In the study of Antonio et al. in 2019 MLSB resistance was detected in 35 isolates, related with genes *ermA* and *ermC* (26). Uyar Gulec et al. detected *ermA* as the most common gene in *S. aureus* isolates (7). Yildiz et al. in 2014 reported that in 225 erythromycin-resistant isolates, 48 had *ermA*, 20 had *ermC*, and among MRSA isolates 64 had erythromycin intermediate resistance. Of which these isolates, 36 were positive for *ermA*, so the most common resistance gene was *ermA* (29). In the study of Gulaydin et al. in 2023, iMLSB, cMLSB, MS and erythromycin and clindamycin susceptible phenotypes were 10%, 0%, 6.66% and 83.33%, respectively. Also, the *ermC* gene with a positive D-zone was detected in one isolate (30). In the literature, *ermC* and *ermA* genes are the most common genes. Most of the studies found *ermC* gene higher than *ermA*, in some studies *ermA* gene is higher than *ermC*. The least common genes are *ermB* and msr genes. So our results are compatible with the literature.

To conclude, methicillin-resistant *Staphylococcus aureus* strains produce an important healthcare problem since they might have multi-drug resistance, and MLSB resistance is related with methicillin resistance. The transition of the erm genes between bacteria causes MLSB resistance and restricts the usage of macrolides. Therefore, phenotypic and genetic analysis to detect the frequency of resistance genes should be done for the epidemiological information.

ETHICS COMMITTEE APPROVAL

* This study was approved by Baskent University Institutional Review Board (Project no: KA22/361) and supported by Baskent University Research Fund.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Silva V, Hermenegildo S, Ferreira C, Manaia CM, Capita R, Alonso-Calleja C, et al. Genetic Characterization of Methicillin-Resistant Staphylococcus aureus Isolates from Human Bloodstream Infections: Detection of MLSB Resistance. Antibiotics (Basel), 2020; 9(7): 1-7.
2. Miklasińska-Majdanik M. Mechanisms of Resistance to Macrolide Antibiotics among Staphylococcus aureus. Antibiotics (Basel), 2021; 10(11):1-23.
3. Gul HC, Kilic A, Guclu AU, Bedir O, Orhon M, Basustaoglu AC, et al. Macrolide-lincosamide-streptogramin B resistant phenotypes and genotypes for methicillin-resistant Staphylococcus aureus in Turkey, from 2003 to 2006. Pol J Microbiol, 2008; 57(4): 307-12.
4. Camp J, Glaubitz L, Filla T, Kaasch AJ, Fuchs F, Scarborough M, et al. Impact of Immunosuppressive Agents on Clinical Manifestations and Outcome of Staphylococcus aureus Bloodstream Infection: A Propensity Score-Matched Analysis in 2 Large, Prospectively Evaluated Cohorts. Clin Infect Dis, 2021; 73(7): 1239-47.
5. Alalem AM. Antibiotics resistance staphylococcus aureus infection studies in hospital. ph.D Thesis, Middle East Technical University, Institute of Science, 2008.
6. Wang H, Zhuang H, Ji S, Sun L, Zhao F, Wu D, et al. Distribution of erm genes among MRSA isolates with resistance to clindamycin in a Chinese teaching hospital. Infect Genet Evol, 2021; 96: 1-5.

7. Güleç GU, Öncü SE, Bozdoğan B, Barcin O, Bulent E, Serhan S, et al. Phenotypic and Molecular Detection of Macrolide Lincosamide Streptogramin B Resistance in Clinical Isolates of Staphylococci. Flora, 2020; 25(2): 190-6.
8. Boyce JM, Cookson B, Christiansen K, Hori S, Vuopio-Varkila J, Kocagöz S, et al. Meticillin-resistant Staphylococcus aureus. Lancet Infect Dis, 2005; 5(10): 653-63.
9. Guclu A, Guney M, Sig A. Arising Prevalence of OXA-48 producer Escherichia coli and OXA-48 with NDM co-producer Klebsiella pneumoniae Strains. Revista Romana de Medicina de Laborator, 2019; (27): 319-26.
10. Sutcliffe J, Grebe T, Tait-Kamradt A. Detection of Rythromycin-Resistant Determinants by PCR. Antimicrob. Agents Chemother, 1996; 40: 2562-6.
11. Wondrack L, Massa M, Yang BV. Clinical strain of Staphylococcus aureus inactivates and causes efflux of macrolides. Antimicrob. Agents Chemother, 1996; 40: 992-8.
12. Nahar L, Hagiya H, Nada T. Prevalence of Inducible Macrolide Lincosamide, and Streptogramin B (inducible MLSB) Resistance in Clindamycin-Susceptible Staphylococcus aureus at Okayama University Hospital. Acta medica Okayama, 2023; 77(1): 1-9.
13. Mahesh S, Kalyan R, Gupta P. MecA and ermA Gene Discrepancy from Their Phenotypic Profile in Staphylococcus aureus Isolates. J Microbiol Infect Dis, 2022; 12(01): 6-11.
14. Assefa M. Inducible Clindamycin-Resistant Staphylococcus aureus Strains in Africa: A Systematic Review. Int J Microbiol, 2022: 1-9.
15. Ozansoy F, Cevahir N, Kaleli İ. Staphylococcus aureus suşlarında makrolid, linkozamid ve streptogramin B direncinin fenotipik ve genotipik yöntemlerle araştırılması. Mikrobiyol Bul, 2015; 49(1): 1-14.
16. Özbek E, Temiz H, Özcan N. The Antibiotic Susceptibilities of Methicillin - Resistant Staphylococcus aureus Strains Isolated from Various Clinical Samples. Med Sci Discov (Internet), 2021; 8(4): 266-70.
17. Modukuru GK, Surya PMS, Kakumanu VR. Phenotypic characterization of Macrolide-Lincosamide-Streptogramin B resistance in Staphylococcus aureus. J Pure Appl Microbiol, 2021; 15(2): 689-94.
18. Nagarkoti D, Prajapati K, Sharma AN. Distribution of Macrolide-Lincosamide-Streptogramin B antibiotics resistance genes in clinical isolates of Staphylococci. Preprint (Version 1) available at Research Square, 2019; 1: 1-15.
19. Pereira JN, Rabelo MA, Lima JL. Phenotypic and molecular characterization of resistance to macrolides, lincosamides, and type B streptogramin of clinical isolates of Staphylococcus spp. of a university hospital in Recife, Pernambuco, Brazil. Braz J Infect Dis, 2016; 20(3): 276-81.
20. Abouelnour A, Zaki ME, Hassan R. Phenotypic and genotypic identification of Staphylococcus aureus resistant to clindamycin in Mansoura University Children Hospital, Egypt. Afr J Clin Exper Microbiol, 2020; 21(1): 30-5.
21. Tandon M, Kulkarni M, GS S. Prevalence of inducible clindamycin resistance in clinical isolates of methicillin-resistance Staphylococcus aureus mediated through gene erm C Expression. Asian J Pharm Clin Res, 2018; 11(9): 106-9.
22. Zeki C, Murat K, Osman A. "Prevalence and Antimicrobial-Resistance of Staphylococcus aureus Isolated from Blood Culture in University Hospital, Turkey. Glob J Infect Dis Clin Res, 2015; 1(1): 10-3.
23. Timsina R, Shrestha U, Singh A. Inducible clindamycin resistance and erm genes in Staphylococcus aureus in school children in Kathmandu, Nepal. Future Sci OA, 2020; 7(1): 2020-92.
24. Khodabandeh M, Mahmoud M, Abdolsalehi MR. Analysis of resistance to macrolide-lincosamide-Streptogramin B among mecA-positive Staphylococcus aureus isolates. Osong public health and research perspectives, 2019; 10(1): 25-31.
25. Goudarzi M, Tayebi Z, Fazeli M. Molecular Characterization, Drug Resistance and Virulence Analysis of Constitutive and Inducible Clindamycin Resistance Staphylococcus aureus Strains Recovered from Clinical Samples, Tehran - Iran. Infect Drug Resist, 2020; 13: 1155-62.
26. Vázquez-Sánchez DA, Grillo S, Carrera-Salinas A. Molecular Epidemiology, Antimicrobial Susceptibility, and Clinical Features of Methicillin-Resistant Staphylococcus aureus Bloodstream Infections over 30 Years in Barcelona, Spain (1990-2019). Microorganisms, 2022; 10(12): 2401.
27. Aetrugh S, Aboshkiwa M, Tawil K. Inducible clindamycin resistance among Staphylococcal clinical isolates from Tripoli Central Hospital, Libya. Libyan International Medical University Journal, 2017; 2(20): 128-33.
28. Mazloumi MJ, Akbari R, Yousefi S. Detection of inducible clindamycin resistance genes (ermA, ermB, and ermC) in Staphylococcus aureus and Staphylococcus epidermidis. Microbiology and Biotechnology Letters, 2021; 49: 449-57.
29. Yıldız Ö, Çoban AY, Şener AG. Antimicrobial susceptibility and resistance mechanisms of methicillin-resistant Staphylococcus aureus isolated from 12 Hospitals in Turkey. Ann Clin Microbiol Antimicrob, 2014; 13(44): 1-6.
30. Gulaydin O, Gurturk K, Ekin IH. Phenotypic and Genotypic Characterization of Macrolide-Lincosamide-Streptogramin Resistance in Staphylococcus aureus Isolates from Bovine and Human. Acta Veterinaria, 2023; 73(1): 102-18.