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Research Article

Prevalence and molecular analysis of virulence genes of Methicillin-resistant *Staphylococcus aureus* colonized among inpatients in hospitals of Duhok Province, Iraq

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Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) is considered a serious public health problem. This study aimed to estimate the rate of MRSA among Staphylococcus aureus isolates of inpatients and to determine their susceptibility to antibiotics and some of the virulence genes. The study was conducted from April 2021 to March 2022 and involved specimens from 302 inpatients admitted to Azadi and Bedari teaching hospitals in Duhok province. Staphylococcus aureus was detected in 20.53% (62/302) of the examined specimens with the highest rate (37,50%) from surgical swabs. Antibiotics sensitivity tests showed that all isolates were resistant to penicillin G. Methicillin-resistant *S. aureus* was detected in 77.42% (48/62) of *S. aureus* isolates with a higher rate from skin swabs (85.71%). According to gender, a significantly (P<=0.005) higher rate of S. aureus and MRSA isolates was recorded in Males (21.52%). As regards age, ages > 40-50 years showed the highest rate of MRSA isolate (24.62%). Among MRSA, 77.08% (37/48) were multidrug resistant. Molecular analysis revealed the presence of nuc, mecA, pvl and eta genes at rates of 100%, 66.6%, 31.2% and 22.9%, respectively among the analyzed isolates.

Keywords: inpatients, MRSA, Panton-Valentine Leucocidin, ETA

Introduction

Staphylococcus aureus is considered an important pathogenic bacterium causing nosocomial infections. It causes a variable range of infections including skin, tissues, toxic shock syndrome,

pneumonia, and bacteremia (Hidron et al., 2009). Infection can be transmitted by direct contact or through droplet spread among both hospital and community patients or through the consumption of contaminated food causing food poisoning (Ajayi, and Oluwoye, 2015). It has been estimated that the rate of colonization of the human skin and mucosal surfaces by S. aureus is about 20-40%, which increases the chances of infection (Lee et al.,2018). The overuse and misuse of antibiotics for any reason in treating infections caused by *S. aureus*, can rapidly enhance the appearance of multidrug-resistant isolates which are difficult to treat, especially with strains producing extended-spectrum β-lactamases (ESBLs) (Fernández et al.,2016). *Staphylococcus aureus* can survive in hospitals in some places such as floors, door handles, bed linen and inanimate objects as well as some fomites and inanimate objects and can transmit from these sites directly or indirectly causing infections (Pacheco et al.,2021). A recent study stated that more than 40% of hospital-acquired or nosocomial infections are caused by cross-infection through the contaminated hands of hospital staff with patients or indirectly through touching contaminated environmental surfaces (Hillier,2020). Notably, the appearance and dissemination of strains that resist antibiotics in hospitals have increased the risk of infection and posed a great threat to community health (Baptista et al.,2017).

Staphylococcus aureus strains produce many virulence factors such as epidermolysis toxins including exfoliative toxins (ETs) and toxic shock syndrome toxins (TSST) which cause staphylococcal scalded skin syndrome. Other toxins produced by S. aureus strains are Panton-Valentine leucocidin (PVL), hemolysins and epidermal cell differentiation inhibitors (EDINs), all of these toxins are extracellular protein toxins that increase the pathogenicity and the majority of them were mostly detected in MRSA strains. Also, some strains can form heat-stable enterotoxins (SEs) that are responsible for food poisoning (Iwatsuki et al.,2006). Methicillin-resistant S. aureus (MRSA) is the major cause of nosocomial infection globally because it's difficult to treat, moreover, it is highly resistance to antibiotics, especially methicillin (Tarai et al.,2013). These infections can range from superficial skin and soft tissue infections to life-threatening infections such as bacteremia, endocarditis, necrotizing pneumonia, necrotizing fasciitis, and osteomyelitis (Burillo, and Bouza,2014). Some of S. aureus exotoxins like Panton-Valentine Leucocidin (pvl) and exfoliative toxins (eta) contribute in tissue invasion and evasion of host defence.

The cytotoxin, Panton-Valentine Leucocidin (pvl) is encoded by lukS-pvl and lukF-pvl genes it causes lysis of leukocyte and tissue necrosis. This toxin has been detected in 5% of S. aureus isolated from patients with severe necrotizing pneumonia in Europe (Rasheed et al.,2020).

Staphylococcus aureus produce exfoliative toxins (ETA) which are responsible for scalded skin syndrome, this toxin is a specific serine protease encoded by eta gene that target the superficial skin layers by cleaving the desmosomal cadherins (Mariutti et al., 2017). Hence, it is vital to estimate the carriage of S. aureus in various clinical specimens, and to determine their susceptibility to the commonly used antibiotics. Furthermore, to estimate the rates of MRSA and MDR isolates among them. Moreover, it's vital to determine the virulence genes using molecular techniques among these isolates from inpatients of two major hospitals, the Azadi (Duhok city) and Bedari (Zakho city) both are in Duhok province, Iraq.

Materials and methods

Study area and sample collection

The current study was performed in Azadi and Bedari teaching hospitals at Duhok province, during April 2021 to March 2022. In which 302 clinical specimens from inpatients of both genders and different ages (one-50 years) were investigated. These specimens included, 68 nasals, 59 skin; 44 Ear, 32 pus and 39 throat swabs in addition to 60 Urine specimens. The samples were collected by rotating cotton swabs over the throat, ear and nasal vestibule as well as skin, while for urine, the midstream urine specimens were collected from each participant in a fully labelled leak-proof, screw-cupped sterile plastic container. All swabs, were placed in labelled sterile containers containing Brain Heart Infusion broth with full label. Then they were transferred within one hour to the Microbiology Laboratory, Biology Department, Zakho University for processing.

Isolation and identification of *Staphylococcus aureus* and *MRSA* strain

All collected specimens were tested for gram staining. Then within 1-2 hours of collection, they were cultured onto blood and Mannitol salt agar plates and incubated for 24 hours at 37 ^{o}C for colony identification. While the urine specimens were inoculated aseptically into Brain Heart Infusion broth and then incubated at 37°C for 24 hours. After that they were sub-cultured onto Mannitol salt agar (MSA) plates using a sterile loop and incubated at 37°C for another 24 hrs. yellow color on Mannitol salt agar indicates Mannitol fermentation by *S. aureus*. *Staphylococcus* isolates were further confirmed by using catalase and coagulase tests. While the suspected *S. aureus* isolates were confirmed by using API® Staph Kit (Biomerieux, France). Cefoxitin disk diffusion method was used to detect methicillin resistance among *S. aureus* isolates and Vitek 2 system (bioMerieux-Vitek)

Antibiotic sensitivity test

All isolated *S. aureus* from the clinical specimen were tested for their antibiotic susceptibility using the Kirby–Bauer disc diffusion method according to the guidelines of the Clinical Laboratory Standards Institutes (2021) in addition to Vitek 2 system (bioMerieux-Vitek). The *S. aureus* strains were sub-cultured in nutrient broth and incubated for 24 hours at 37°C, then were subjected to 13 antibiotics. The inoculums were streaked over Mueller-Hinton agar plates, to each plate 4-5 antibiotic discs were placed using a sterile forceps at equal distance. Plates were incubated for 18 to 24 hours at 37°C(Alexander et al.,2013).

DNA extraction and PCR amplification

The strains that exhibited phenotypic resistance to antimicrobial agents were used for DNA extraction using commercial DNA Extracted Kit (Favorgen, Taiwan) according to the manufacturer's recommendations. NanoDrop spectrophotometer (Thermo Fisher Scientific) was used for measuring the concentration and the purity of the extracted DNA. While single plex polymerase chain reaction was used for targeting the *nuc*, *mecA, pvl* and *eta* genes using specific primers as shown in table 1.

Table 1. Molecular weights of the genes and sequences of the primers used

Toxin genes	Sequences 5′-3	Product size (bp)	References
Nuc	F: GCGATTGATGGTGATACGGTT R: AGCCAAGCCTTGACGAACTAAAGC	279	(Karimzadeh, and Ghassab,2022).
mec A	F: GTAGAAATGACTGAACGTCCGATAA R: CCAATTCCACATTGTTTCGGTCTAA	310	(Elhassan et al.,2015).
lukS/F-pvl	F: ATCATTAGGTAAAATGTCTGGACATGATCCA R: GCATCAAGTGTATTGGATAGCAAAAGC	433	(McClure et al.,2006).
Eta	F: GCAGGTGTTGATTTAGCATT R: AGATGTCCCTATTTTTGCTG	93	(Saleem et al.,2017).

The constituent of the PCR reaction (30 µL) included 15 µl of PCR master mix (ADDBIO.INC, South Korea), 4 µL of DNA sample, 7 µl of deionized distilled water, 2 µl of each primer including the forward and reverse primers (10 pmol/µl) to each reaction. Thermocycles PCR was used for amplifying the extracted DNA as indicated in table 3.

PCR products were run electrophoretically on 1.2 % agarose gel stained with RedSafe dye in Tris-Borate EDTA buffer (10X). The submerged gel was run for 15 minutes at 45 Voltage then the voltage was raised to 85 Volt for one hour. DNA ladders with 100-1000 was used as a reference to determine the molecular weights of the PCR products. The gel was examined under UV Transilluminator to confirm PCR amplification (Ibrahim et al.,2020).

Table 2. Single plex PCR amplification conditions for *nuc, mec A, pvl* and *eta* genes of *S. aureus*

Genes	Temperature (ºC)/ Time					Cycles No
	Initial denaturation	Denaturation	Annealing	Extension	Final Extension	
Nuc	94/5 min	94/1 min	58/1 min	72/1 min	72/10 min	35
mec A	94/5min	94/45 sec	60/45 sec	72/90sec	72/6min	30
lukS/F-pvl	95/5min	94/30 sec	55/30 sec	72/1 min	72/10 min	35
Eta	94/5 Min	94/2min	57/2min	72/1min	72/7 min	35

Sequences analysis

PCR products (30 µl) of twenty MRSA isolates were sent to Macrogen Company (Seoul, South Korea) for sequencing using *mecA,* pvl and *eta* primers. The DNA sequences were analyzed and edited using BLAST and BioEdit software. The obtained sequences were deposited in the GenBank database**.** The collected data were statistically analyzed using SPSS version 25 software, while the Chi-square (X2) test was used to determine the differences between qualitative variables. *P* value $\leq$ 0.05 was considered significant, and more than this value was considered non-significant. Consent was taken from each patient for using his/her sample in this research. The study was approved by the Scientific Committee of the General Directorate of Health, Duhok (Ref No:299/5, in 29/3/2021).

Results

The results of this study showed that 20.53% (62/302) of the patients' specimens had positive bacteria growth of *S. aureus* with the highest prevalence in surgical infection swabs (pus swabs) 37.50% (12/32), followed by Nasal swabs 26.47% (18/68)**,** while the lowest rate 6.67% (4/60) was observed in urine specimens (table 3).

Table 3. The Distribution of *S. aureus* among clinical specimens

Clinical Samples	**Number of tested samples**	***S. aureus* positive No. %**
Nasal swabs	68	18/68 (26.47)
Skin swabs	59	14/59 (23.73)
Ear swabs	44	8/44 (18.18)
Throat swabs	39	6/39 (15.38)
Urine	60	4/60 (6.67)
Surgical infection swabs (Pus swabs)	32	12/32 (37.50)
Total	302	62/302 (20.53)
P value = 0.88 X^2 = 1.768		

All of the 62 isolates of *S. aureus* were tested for their susceptibility pattern to 13 of the commonly used antibiotics and they showed the highest level of resistance to penicillin G (100%), followed by ampicillin (93.55%), amoxicillin (90.32%) and cephalexin (83.87%). On the other hand, most of the isolates were susceptible to vancomycin and rifampicin at an equal rate (98.39%). MRSA isolates were found in 77.42% (48/62) of these isolates as shown in table 4.

Table 4. Antimicrobial susceptibility pattern of *S. aureus* isolates**.**

Antibiotics	**R (%)**	**S (%)**
Penicillin (PG)	62 (100)	0(0)
Ampicillin (AMP)	58(93.55)	4(6.45)
Amoxicillin (AX)	56 (90.32)	6(9.68)
Cephalexin (CL)	52(83.87)	10(16.13)
Cefoxitin (CX)	48(77.42)	14(22.58)
Cefotaxime (CTX)	41(66.13)	21(33.87)
Lincomycin(L)	40 (64.52)	22(35.48)
Erythromycin(E)	10 (16.13)	52(83.87)
Ciprofloxacin (CIP)	8 (12.90)	54(87.10)
Trimethoprim (TMP)	5(8.06)	57(91.94)
Fusidic Acid (FA)	2 (3.23)	60(96.77)
Vancomycin (VA)	1 (1.61)	61(98.39)
Rifampicin (RA)	1 (1.61)	61(98.39)
Total No. Tested	62	

Phenotypic analysis showed that MRSA was detected at a rate of 77.42% (48/62) with the highest rate (85.71%) in-skin swabs, while the lowest rate (62.5%) was in ear swabs as shown in table 5

Table 5. The Distribution of MRSA among *S. aureus* isolates from clinical specimens

Clinical Samples	**Number of tested samples**	**MRSA No. %**
Nasal swabs	18	15/18(83.33)
Skin swabs	14	12/14(85.71)
Ear swabs	8	5/8(62.5)
Throat swabs	6	4/6(66.67)
Urine	4	3/4(75)
surgical infection swabs (Pus swabs)	12	9/12(75)
Total	62	48/62(77.42)
P value = 0.794 ; X^2 = 2.38		

Regarding to the relationships between the gender and age of the patients; the rate of positive cases with *S. aureus* and MRSA was significantly (*P*= 0.005) higher among males 21.52% (34/158) and 16.46% (26/158) than that in females 19.44 (28/144) and 15.28% (22/144), respectively. In addition, *S. aureus* and MRSA associated with age indicated higher percentages (27.69% and 24.62%), respectively, among ages >40-50 years, with statistically non-significant differences between different ages (P=0.078) as shown in Table 6.

Table 6: Distribution of *S. aureus* and MRSA isolates associated with gender and age among inpatients**.**

Variable	Examined No	*S. aureus* No (%)	MRSA No (%)	P-value X^2
Gender				
Female	144	28/144(19.44)	22/144(15.28)	P value = 0.005
Male	158	34/158(21.52)	26/158(16.46)	X^2 = 0.944
Age				
>1-10	26	4/26(15.38)	2/26(7.69)	
>10-20	58	12/58(20.69)	8/58(13.79)	P value = 0.078
>20-30	82	13/82(15.85)	9/82(10.98)	X^2 = 0.878
>30-40	71	15/71(21.13)	13/71(18.31)	
>40-50	65	18/65(27.69)	16/65(24.62)	
Total	302	62/302(20.53%)	48/302(15.89%)	

The rate of *S. aureus* carriage was slightly higher (21.05%) in specimens of Azadi hospitals, while the rate of MRSA among the isolates was somewhat similar and statistically the differences between these rates were non-significant (*P*=0.06) as indicated in table 7**.**

Table 7. Distribution of *S. aureus* among both hospitals in Duhok Province

Hospitals	**Examined**	***Staph. aureus*** **No (%)**	**MRSA** **No (%)**
Azadi Hospital (Duhok)	114	24/114(21.05)	16/114(14.04)
Bedari Hospital (Zakho)	188	38/188(20.21)	28/188(14.89)
Total	302	62/302(20.53)	48/302(15.89)
P value = 0.06 X^2 = 0.806			

Multi-drug resistant isolates among MRSA

A large number of the tested isolates showed resistance to at least three groups of antibiotics and were considered multidrug resistance (MDR) as obvious in Table 9. From 48 MRSA isolates, 77.08% (37) were MRD. The most common resistance among MDR-MRSA isolates was to β-lactam antibiotics, which included Trimethoprim, Erythromycin, Lincomycin, Ciprofloxacin and Vancomycin and Rifamycin as shown in Table 8.

Table 8. Resistance patterns of MRSA isolates from different clinical specimens

Resistance Patterns	**The number of Antibiotic resistance seen**	**Number of MDRSA Strains %**	**Numbers of MDRSA among specimens**
PG, AX, AMP	3	15 /48(31.25)	5 pus swabs, 3 nasal swabs, 2 skin swabs one throat swab, one ear swab and 3 urine specimens.
PG, AMP, AX, CL	4	9/48(18.75)	3 pus swabs, 2 nasal swabs, one skin swab one throat swab, one ear swab and one urine specimen.
PG, AMP, AX, CL ,CTX	5	7/48(14.58)	3 pus swabs, 2 nasal swabs, one throat swab and one urine specimen.
PG, AMP, AX, CL, CTX,CX	6	6/48	3 pus swabs**,** two nasal swabs., one urine
Total		37/48(77.08%)	

Molecular detection and identification of virulence genes encoding toxins

PCR amplification of *nuc, mecA, pvl* and *eta* genes was done to detect *S. aureus* Methicillin resistance (MRSA) and toxin-encoding genes. The size of the amplified PCR product for *nuc* gene

was 279 bps, while that for *mec A* gene was 310 bps and has been recorded in GenBank under accession number (ON960230) for *mecA* gene (figure 1) *Staphylococcus aureus* strain HSP 147 Penicillin -binding protein. Regarding *pvl* and *eta* genes; the sizes of PCR products were 433 and 93 bps, respectively (figures2 and 3). The amplified sequences were recorded in GenBank under accession numbers OP018997 for the *lukS-pvl* gene (*staph aureus* strain TPS3156 panton valentine leukocidin subunit S) and OP856944 for *eta* gene.

Figure 1. The PCR amplification of *mec*A and *nuc* genes, using 1.2% agarose gel electrophoresis. Lane M contains a DNA ladder of 100-1000 bps, Lanes 1-5 show positive bands of 310 bp for *mec A* gene; Lanes 6-11 show positive bands of 279 bps for *nuc* gene

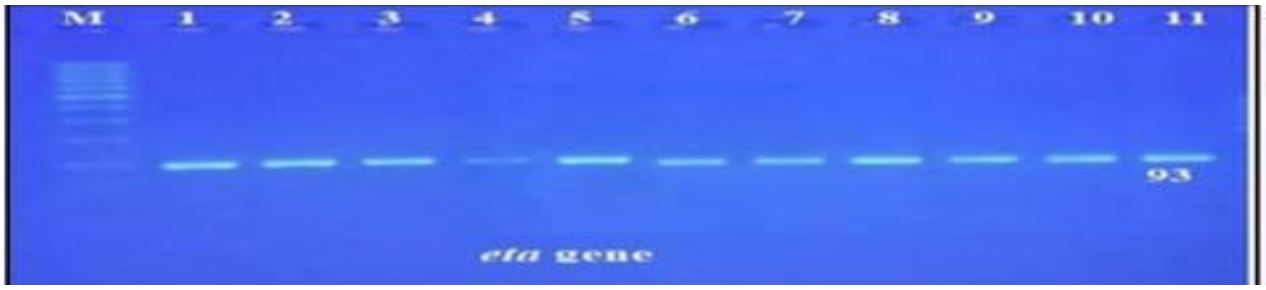

Figure 2. The PCR amplification of *eta* gene, using 1.2% agarose gel electrophoresis. Lane M contains a DNA ladder of 100-1000 bps; Lanes 1-11 show positive bands of 93 bps.

Table 9, shows the distribution of virulence genes among the tested isolates. *nuc* gene was detected in 100% (48/48) of MRSA isolates with different clinical sources, while *mecA* gene was detected in 66.67% (32/48) of the isolates. Among the tested isolates, 31.25 (15/48) carried *pvl* gene and 22.92 (11/48) carried the *eta* gene.

Table 9. Distribution of virulence encoded genes among 48 methicillin-resistant *Staphylococcus aureus* isolates from inpatients of Azadi and Bedari Hospitals in Duhok Province/Iraq.

Clinical sources	No of tested samples	Detected genes			
		nuc	*mec A*	*Pvl*	*eta*
		No. %	*No. %*	*No. %*	*No.%*
skin swabs	12	12/12(100)	11(91.67)	7(58.33)	6(50.0)
Nasal swabs	15	15/15(100)	9(60.0)	5(33.33)	0(0)
Ear swabs	5	5/5(100)	3(60.0)	1(20.0)	0(0)
Throat swabs	4	4/4(100)	3(75.0)	0(0)	0(0)
urine sample	3	3/3(100)	1(33.33)	1(33.33)	0(0)
pus swabs	9	9/9(100)	5(55.56)	1(11.11)	5(55.56)
Total	48	48/48 (100)	32/48 (66.6)	15/48(31.2)	11/48(22.9)
P value = 0.333 X^2 = 16.772					

Discussion

Staphylococcus aureus is one of the oldest known pathogens and is the most common cause of pyogenic infections in humans as well as in hospital environments (Algammal et al., 2020). The majority of the worldwide published studies on the prevalence of this bacterium focused on its carriage among patients, at specific age groups or health conditions (Boncompain et al., 2017).

In this study, the overall prevalence of *S. aureus* among different clinical specimens of inpatients was 20.53%, which is much lower than the rate reported previously in Duhok city which was 41.66% (Al Zebary et al.,2017). However, lower rates have been reported in Nepal, which were 14.4% and 19.96%, respectively (Shahi et al.,2018; Maharjan et al.,2021). The highest rate (37.50%) of *S. aureus* was isolated from pus swabs, indicating their important role in pyogenic soft tissue. High prevalence of *S. aureus* in pus samples compared to other specimens have been reported in Trinidad and Tobago (Akpaka et al., 2006).

Moreover, patients at ages over 40-50 years showed the highest carriage rate (27.69%) with a higher prevalence among males this could be attributed to the aggressive inflammatory reaction of their immune system to microbial agents as compared with females who possess high immunity against microbes, hence male patients had a higher mortality rate (Tarzi et al., 2021).

All *S. aureus* isolates were subjected to 13 commonly prescribed antibiotics in this area. They showed different rates of susceptibility against them. Vancomycin and Rifampicin were the most effective antibiotic against *S. aureus* with a sensitivity rate of 98.39% for each of them. Similarly,

in India a study found *S. aureus* isolates were 100% sensitive to Vancomycin and Rifampin (Sarita et al.,2021). On the other hand, all isolates were 100% resistant to Penicillin G, the current result is in line with a study in Duhok, Iraq, in which also *S. aureus* isolates showed a high resistance to Penicillin G, and they attributed it to the ability of *S. aureus* isolates to produce β- lactamase which hydrolysis the amide bond of Beta-lactam antibiotics (Al Zebary et al.,2017).

In the present study, MRSA was detected in 77.42% (48/62) of *S. aureus* isolates and as a whole of the total tested specimens, the rate of MRSA was 15.89% (48/302). Variable rates of MRSA were reported in this Province, very low rate (4.2%) of MRSA was reported among university students (Assafi et al.,2018). While, a higher rate (50.4%) of MRSA was reported among hospital staff in Duhok (Hussein et al.,2019). Similarly, in some other Iraqi cities high rates of MRSA were reported, in Baghdad a rate of 31.4% was reported among healthy second-stage pharmacy students (Saeed et al.,2014) in Mosul, a rate of 44.11% was reported among healthcare workers and patients (AL-Mola et al.,2019). and in Muthanna, a rate of 24% was reported among students (Hantoosh,2022). MRSA strains are considered a main health problem in developing countries owing to their ability to cause serious infections. Therefore, their high occurrence in hospitals of Duhok province highlights a significant threat among both community and healthcare settings.

Moreover, the current results finding are higher than the rates reported in countries neighboring Iraq, in Turkey, 47.9% of the patients attended intensive care units carried MRSA (Cikman et al.,2019). in Saudi Arabia, 39.47% of patients who attended Turaif general hospital carried MRSA (Taha et al.,2022), in Jordan, 38.6% of patients in Prince Hamzah Hospital carried MRSA(Khasawneh et al.,2020), and in Iran, 41.9% of the patients from Motahari Burns Hospital carried MRSA (Tajik et al.,2019). These data are concerning because patients and healthy people may be at risk of frequent and direct transmission when they come into contact with other members of the community.

Several factors might be associated with MRSA carriage such as; geographical area, the immune status of the host, the associated environmental risk factors and the degree of bacterial virulence (Lee et al.,2018). The degree of influence of some factors in increasing the rate of MRSA carriage was studied such as gender, in which males significantly showed a higher rate of carriage (16.4%) than females (15.2). This might be due to several reasons, including levels of vitamin D, elevated smoking habits, sharing shaving tools, sports clothing and poor practice of hands hygiene (Assafi et al.,2015).

As regards to age, older ages (above 40 -50 years) had the highest MRSA rate (24.6%). Since infections in older patients are commonly considered as a serious medical issue, moreover it is known that the humoral and cellular immune responses are declined as a consequence of aging especially in old individuals, making them more susceptible to infections as compared to younger ones**.** Older patients visit hospitals more frequently because they require care and treatment, hence they have a greater chance of acquiring multidrug resistance MRSA from hospital environments (Humphreys et al., 2015). Regarding specimen sources, in this study, the highest rate (85.71%) of MRSA was detected among skin swabs. This finding is higher than the result obtained in India in which 71% of MRSA was carried by hand swab (Tambekar et al.,2007). The endogenous microflora among healthcare individuals and inpatients can be considered as the principal source for spreading bacteria. There is a demand for performing studies on the mechanism of resistance and the molecular identification of highly resistant strains harboured by individuals in the community (Neopane et al., 2018). The analysis of antimicrobial resistance profiles of *S. aureus* can help in the treatment of bacterial infection (Hussein et al.,2019).

MRSA can be a severe problem for both the hospital and the community environments and resistance to broad spectrum of antibiotics could limit treatment options of infectious diseases leading to high levels of mortality (Admi et al.,2015). In the current study, 77.8% of tested MRSA isolates were MDR and showed resistance to all β-lactam antibiotics. This is in line with a study conducted in Ethiopia, in which 82.3% of MRSA isolates were resistant to 3 or more antimicrobial agents, and they attributed the high rate of MDR in MRSA isolates obtained from pus of surgical site infection compared to other clinical samples, might be these samples were taken from hospitalized patients who overuse and misuses antimicrobial agents either during the surgical operation or after it (Sari et al.,2019). The possible reason of the higher susceptibility to antimicrobial agents of isolates from urine, nasal and ear swabs as compared to those from surgical wounds could be due to infrequent previous exposure to antimicrobial agents (Polse et al.,2016). It is known that *S. aureus* is considered one of the nosocomial pathogens surviving on inanimate objects and surfaces for several weeks and can thereby act as a main source for transmission especially if these surfaces are not disinfected regularly(Ababneh et al.,2022). Several studies on the rate of infection among inpatients considered the spread of MRSA in the hospital environment is significantly correlated with the rates of occupancy (Borg et al.,2008).

The regular disinfection of beds, linen, chairs and door handles might reduce the rate of contamination with pathogenic bacteria, although some disinfectants and detergents cannot kill all of these bacterial pathogens (Dancer,2008). Therefore, disinfecting contaminated fomites and regular cleaning of environmental surfaces with effective disinfections will reduce the spread of infections acquired from hospitals (Maryam et al., 2014). Using the conventional PCR technique for 48 isolates revealed the presence of *nuc gene* in all of them (100%). *nuc* gene is specialized for the identification of *S. aureus* bacterium and this method can be used to diagnose *S. aureus* and dispense with other methods (Strommenger et al.,2006). The present study is in agreement with the findings of another study performed in Iraq demonstrated the molecular detection of selected *S. aureus* isolates to the species level by amplifying the *nuc* gene, which is specific and accurate identifying technique for *S. aureus* (Mazaal et al.,2009). In addition, a study in Saudi Arabia, demonstrated the amplification of *nuc* gene in 100% of *S. aureus* isolates (Moussa, , and Shibl, 2009). From the current results, the *mecA* gene, which lies in the SCCmec resistance island was carried by 66.67% of MRSA and all of the multidrug resistant isolates detected by genotype analysis. However, in Tahran, Iran, a higher rate (87.3%) of MRSA isolates had the *mec A* gene (Koosha et al.,2016).. *Staphylococcus aureus* can adapt to environments and hosts efficiently by the help of various virulence factors and resistance genes that play the major role in pathogenicity. Panton-Valentine leucocidin (*pvl*) is a toxin encoded by *lukS-PV* and *lukF-PV* genes and is responsible for severe infections caused by community-acquired MRSA (CA-MRSA). The *pvl* toxin destroys leukocytes by creating lytic pores in the cell membrane. The current results showed that the rate of *pvl-positive* isolates was 31.2% among patients, while some studies reported variable rates of this gene such as, in Iraq (29.5%), in Turkey (6.9%) and in China (47.8%), respectively (Koosha et al.,2016; Mazaal et al.,2013).

The exfoliative toxin is encoded by the *eta* gene, which is responsible for skin and cutaneous tissue infections as well as scalded skin syndrome (Rolle et al.,2015).. In the current study, the rate of *eta-positive* isolates was 22.9 % among patients. Other studies in Indonesia and India reported lower rates of this gene among analyzed MRSA isolates, which were 13.8% and 11.65 %, respectively (Santosaningsih et al.,2018; The inadequate hospital's sanitary conditions and the lack of frequent surveillance of antibiotic susceptibility of circulating bacterial strains might lead to the emergence of resistant bacteria.

Conclusion

This study provides valuable information regarding the prevalence of Methicillin-resistant *S. aureus* from different clinical sources. The alarming number of Methicillin-Resistant *S. aureus* is a worrisome finding. Antibiotics like Vancomycin which has not developed resistance should be cautiously used only in Methicillin-Resistant *S. aureus* cases. MRSA is a serious health threat and possesses remarkably high rates of various virulence factors which were detected through genotypic study in inpatient specimens.

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