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COMPARISON OF HEALTHCARE AND COMMUNITY ASSOCIATED METHCILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* USING PHENOTYPIC AND MOLECULAR ASSAYS IN HOSPITALS OF DUHOK PROVINCE, IRAQ

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ABSTRACT

Infection of healthcare workers with methicillin resistant *Staphylococcus aureus* (HA-MRSA) occurs by direct contact with infected wounds, through hand contamination from asymptomatic people or medical devices. While Community-associated MRSA (CA-MRSA) infections arise in healthy non-hospitalized people or in those having medical procedure within the past year. The study was conducted from April 2021 to March 2022 to determine the rates of *S. aureus* and MRSA isolates, their antibiotic resistance and virulence factors among 712 specimens (308 out-patients and 404 healthcare workers) of Azadi and Bedari hospitals/ Duhok province. *Staphylococcus aureus* isolated at rates of 28.57% and 16.83%, among out-patients and healthcare workers, respectively. Male outpatients', with ages of >40-50 years showed higher isolate rate than healthcare worker (46.15% vs 35.38% and 85.71% vs 25.36 %) respectively. CA-MRSA showed higher resistance to beta lactam antibiotics, while HA-MRSA showed higher multidrug resistance. Males of both MRSA types (CA-MRSA and HA-MRSA) carried higher isolate rates than females (38.46% and 15.87%) respectively. The higher rates of CA-MRSA and HC-MRSA isolates were 64.29% and 22.38% at ages of >40-50 years, respectively. PCR analysis detected *nuc* and *mecA* genes in 100% in both MRSA types. The distribution of virulence genes *pvl, arcA* and *lukE-lukD* among CA-MRSA of both hospitals were 41.03%, 28.02 % and 30.77%, respectively. While the distribution of these genes among HA-MRSA of both hospitals were 42%, 46.15% and 11.52%, respectively.

KEYWORD: *HA-MRSA, CA-MRSA, mec A, pvl, lukE-lukD, arcA*

1.INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen that causes a wide range of infectious illnesses. The clinical importance of *S. aureus* stems from its capacity to thrive in a variety of conditions, produce a wide range of virulence factors, spread quickly, and acquire antibiotic resistance (Cheung *et al*., 2021). Methicillin-resistant *S. aureus* (MRSA) which emerged in 1961, caused different types of infections in hospitalized patients and was referred to as healthcare-associated MRSA (HA-MRSA). In the 1990s, MRSA was observed to cause skin and soft tissue infections (SSTI) in previously healthy individuals in the community and was referred to as community-associated MRSA (CA-MRSA). MRSA infections occurring among healthy people in the community include any infection diagnosed in patients lacking health care-associated MRSA risk factors such as hospitalization, hemodialysis, surgery, presence of indwelling catheters, and other medical devices (Paitoonpong *et al*., 2013). HA-MRSA and CA-MRSA differ in the epidemiological pattern of infection, virulence properties and antibiotic resistance (Choo, 2017). HA-MRSA normally exhibits multidrug resistance whereas CA-MRSA is mostly susceptible to non-β-lactam antibiotics. Vancomycin is the drug of choice for treatment of invasive MRSA infection this drug is expensive, and its missuses may result in emergence of resistant isolates, this led to the use of other antibiotics such as macrolide and Lincosamides (Lakhundi and Zhang, 2018).

Different virulence factors of *S. aureus* encoded by different genes play a major role during pathogenesis. Such factors include Panton Valentine Leukocidin (PVL) is a toxin composed of two components, LukS-PV and LukF-P encoded by two genes, *lukS- PV* and

lukF-PV. They cause 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 (Cheung *et al*., 2021). PVL has been implicated as participating to the invasiveness of the organism by suppressing phagocytosis and is generated by relatively few strains of *S. aureus*, and it is presents in only 2-4 % of all contemporary *S. aureus* strains. However, the role of this toxin in the pathogenesis of CA-MRSA has been highly controversial. Roughly, 60 to 100% of CA-MRSA strains have been shown to involve *pvl* genes (Dayan *et al*., 2016). Studies published by Nawrotek *et al*. (2018) indicated that PVL is associated with fatal *S. aureus* necrotizing pneumonia associated with antecedent influenza. Part of a bi-component leukotoxin that acts by forming pores in the membrane of the target cells. LukE-LukD is as effective as the Panton-Valentine leucocidin (*PVL*) for inducing dermo necrosis when injected in the rabbit skin, but not hemolytic and poorly leucotoxic on human blood cells compared to other leukotoxins expressed by *S. aureus* (Afroz *et al*., 2008).

Furthermore, other virulence factors such as arginine catabolic mobile element (*ACME*, *arcA*) that is a genomic island in Staphylococci may contribute to enhanced pathogenicity (Bae *et al.*, 2021). Community acquired MRSA carried *pvl* and *ACME* genes (Senok *et al*., 2020). The *ACME* gene of *S. aureus* is important for bacterial survival in acidic environments (Otto, 2013). The objectives of the present study were to compare the profile of antibiotic resistance and virulence factors of HA-MRSA and CA-MRSA.

2. MATERIALS AND METHODS

2.1. Study area and sample collection

The current study was performed in Azadi and Bedari teaching hospitals in Duhok province, from April 2021 to March 2022. Out of 712 clinical specimens; 308 from healthcare workers of both genders and different ages (20-50 years) and 404 from outpatients (patient who attends a hospital for treatment) of both genders and different ages (one-50 years) were investigated. The samples were collected by rotating cotton swabs over the skin, nares, surgical sites infection. 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.

2.2. Isolation and identification of *Staphylococcus aureus* and MRSA strains

All collected specimens were tested for gram staining. Then within 1-2 hours of collection, all specimens 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 broth of Brain Heart Infusion and then incubated at 37°C for 24 hours (Dilnessa and Bitew, 2016). 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. Formation of yellow color on mannitol salt agar indicates mannitol fermentation by *S. aureus*. *Staphylococcus* isolates were further confirmed by using catalase and coagulase tests (Yimana and Tesfaye, 2022). While the suspected *S. aureus* isolates were confirmed by using API® Staph Kit (Biomerieux, France). Cefoxitin disk diffusion method (30 mg) was used to detect methicillin resistance *S. aureus* isolates (Koupahi *et al.*, 2016).

2.3. Antibiotic sensitivity test

The *S. aureus* from the clinical specimen were tested for their antibiotic susceptibility using the Kirby–Bauer disc diffusion method, the isolates were sub-cultured in nutrient broth and incubated for 24 hours at 37°C. The inoculums were streaked over Mueller-Hinton agar plates, then were subjected to thirteen antibiotics on four plates, each plate contained maximum 4 antibiotic discs placed at equal distance by using sterile forceps. The used antibiotics included: Penicillin (10 µg), Amoxicillin (30 µg), Ampicillin (30 µg), Cefoxitin (30 µg), Cephalexin (30 µg), Cefotaxime (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Erythromycin (5 µg), Fusidic acid (10 µg), Lincomycin (2 µg), Rifampicin (5 µg) and Vancomycin (30 µg) disc and incubated for 18 to 24 hours at 37°C. The zones of inhibition were measured and documented according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2021).

2.4. DNA extraction and PCR amplification

The strains that exhibited phenotypic resistance to antimicrobial agents (Penicillin, Amoxicillin and Ampicillin) were used for DNA extraction using commercial DNA extraction Kit (Favorgen, Taiwan) according to the manufacturer's recommendations. NanoDrop spectrophotometer (Thermo 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, lukE-lukD, arcA* genes using specific primers as shown in Table 1.

Table (1): Molecular weights of the genes and sequences of the primers used

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)
lukE-lukD	F:(5'-TGAAAAAGGTTCAAAGTTGATACGAG-3') R: (5'-TGTATTCGATAGCAAAAGCAGTGCA-3')	269	(Jarraud *et al.,* 2002)
arc A	F: (5'-GCAGCAGAATCTATTACTGAGCC-3') R: (5'-TGCTAACTTTTCTATTGCTTGAGC-3')	513	(Zhang *et al.,* 2005)

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 and 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 2.

PCR products were run electrophoretically on 1.2 % agarose gel stained with RedSafe dye in Tris-Borate EDTA buffer (1X). 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 bps were 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 and Touch down PCR amplification conditions for *nuc, mec A, pvl, lukE-lukD, arcA* genes of *S. aureus*

Genes	Temperature (^{o}C)/ Time				
	Initial denaturation	Denaturation	Annealing	Extension	Final Extension
nuc	94/5 min	94/1 min	58/1 min	72/1 min	72/10 min
Cycles No	1		35		1
mec A	94/5min	94/45 sec	60/45 sec	72/90sec	72/6min
Cycles No	1		30		1
lukS/F-pvl	95/5min	94/30 sec	55/30 sec	72/1 min	72/10 min
Cycles No	1		35		1
LukE-lukD	94/30s	94/30s	55/30s	72/30s	72/2min
Cycles No	1		30		1
arcA	94 ^{o}C/4min	94^{o}C/30s	60 ^{o}C/30s	72 ^{o}C /45s	---------
	1		10		
	----------	94 ^{o}C /30s	52 ^{o}C/30s	72 ^{o}C /45s	72 ^{o}C /6 min.
			25		1

2.5. Sequences analysis

PCR products (40 µl) of twenty MRSA isolates were send to Macrogen Company (Seoul, South Korea) for sequencing using all primers. The DNA sequences were analyzed and edited using BLAST and BioEdit software. The obtained sequences were deposited in GenBank database.

2.6. Statistical analysis:

The collected data were statistically analyzed using SPSS version 25 software, while Chi-square (X^2) test was used for determining the differences between qualitative variables. *P* value <0.05 was considered significant, and more than this value was considered non-significant.

3.RESULT

3.1. Isolate of *Staphylococcus aureus*:

The results of this study demonstrated that 21.91% (156/712) of *S. aureus* were isolated from the enrolled participants, constituting 28.57% (88/308) from out-patients and 16.83% (68/404) from healthcare workers with statistically significant difference (*P*< 0.05) between both groups (Table 3).

Table (3): frequency of *S. aureus* among categories

Categories	No. Examined	*S. aureus*	
		No.	%
Out-patients	308	88	28.57
healthcare workers	404	68	16.83
Total	712	156	21.91
P value <0.05 X^2=14.078			

Regarding to the relationships between the gender and age of the out- patients and healthcare workers; the rate of positive cases with *S. aureus* was higher among outpatient males than healthcare worker males (46.15% vs 35.38%), with statistically significant difference (*P*< 0.05) between both groups. In addition, *S. aureus* associated with age group indicated the higher percentages among both groups were isolated from ages of > 40-50 years, which were 85.71% and 25.36%, respectively. Statistically the difference between both groups was highly significants (*P*=0.001) as shown in Table 4

Table (4): frequency of *S. aureus* among both genders and different ages of outpatients and healthcare workers.

Variable	Examined No.	*S .aureus* among out-patients	Examined No.	*S. aureus* among healthcare workers	
		Gender			
Females	178	28 15.73	215	22 10.23	P value = <0.001 X^2 = 14.297
Males	130	60 46.15	189	46 35.38	
		Age			
>1-10	88	11(12.5)	-	-	P value =0.001 X^2 = 16.215
>10-20	74	15 (20.27)	-	-	
>20-30	66	17(25.76)	205	20(9.76)	
>30-40	52	21(40.38)	61	13(21.31)	
>40-50	28	24(85.71)	138	35(25.36)	
Total	308	88 (28.57)	404	68 (16.83)	

The rates of *S. aureus* carriage among out-patients and healthcare workers were higher in specimens of Azadi hospital than those of Bedari hospital which were 41.03% and 29.27% vs 15.79% and 8.33%, respectively. Statistically this difference non-significant (*P*=0.76) as shown in Table 5.

Table (5): Distribution of S. *aureus* among both hospitals in Duhok Province

Hospitals	NO. examined	*S. aureus* out-patients No. %	No. examined	*S aureus* healthcare worker No. %
Bedari Hospital (Zakho)	152	24/152(15.79)	240	20/240(8.33)
Azadi Hospital (Duhok)	156	64/156(41.03)	164	48/164(29.27)
Total	308	88/308(25.32)	404	68/404(12.87)
P value = 0.768 X^2 = 0.087				

3.2. Determination of the rate of methicillin resistant *Staphylococcus aureus*

The 156 isolates of *S. aureus* were tested for 13 antibiotics. The results of resistance among out-patients and healthcare workers are demonstrated in Tables 6. Most of the isolate in out-patients and healthcare workers were resistance to penicillin 88(100%) and 58(85.29) respectively. On the other hand, the most effective antibiotic for outpatients and healthcare workers was vancomycin as they showed very low resistance to it (2,27% and 0%), respectively.

Table (6): Antimicrobial resistance pattern of *S. aureus* isolates

Antibiotics	*S. aureus* out-patients (resistance number)	*S. aureus healthcare workers* (resistance number)
Penicillin (PG)	88(100)	58(85.29)
Ampicillin (AMP)	86(97.73)	56(82.35)
Amoxicillin (AX)	84(95.45)	55(80.88)
Cephalexin (CL)	80(90.91)	54(79.41)
Cefoxitin (CX)	78(88.64)	52(76.47)
Cefotaxime (CTX)	51(57.95)	21(30.88)
Lincomycin(L)	48 (54.55)	20(29.41)
Erythromycin(E)	22 (25.0)	18(26.47)
Ciprofloxacin (CIP)	18 (20.45)	14(20.59)
Trimethoprim (TMP)	15(17.05)	12(17.65)
Fusidic Acid (FA)	6 (6.82)	4(5.88)
Rifampicin (RA)	4(4.55)	3(4.41)
Vancomycin (VA)	2 (2.27)	0(0)

Table 7 shows the rates of *S. aureus* and the number and rates of MRSA strains among each group. Which showed a high rate of 83.33% (130/156) for MRSA among the tested *S. aureus* with the highest rate 88.64% (78/88) among outpatients rather than healthcare workers 76.47% (52/68), but this difference was statistically non-significant (*P*=0.54).

Table (7): Frequency of *S. aureus* and *MRSA* among tested isolates of both of healthcare workers and out-patients.

Category	No. Examined	Positive *S. aureus* No.	%	Positive MRSA No.	%
Healthcare workers	404	68	16.83	52/68	76.47
Out-patients	308	88	28.57	78/88	88.64
total	712	156/712	(21.91)	130 /156	83.33
P value =0.54 X^2 = 0.375					

The results of this study showed that 28.57% of the out-patients' specimens and 16.83% of healthcare workers showed positive bacterial growth of *S. aureus* with the highest prevalence in skin swabs followed by Nasal swabs among both groups as indicated in Table (8).

The distribution of HA-MRSA and CA-MRSA among all of the examined clinical

specimens showed the highest rate of MRSA was isolated from outpatients which was 88.64 % with the highest of 95.83% for CA-MRSA from the skin swabs. On the other hand, the lowest rate was 25% in HA-MRSA from the throat swabs.

Table (8): The Distribution of *S. aureus* and Methicillin resistant *S. aureus* isolates among all examined clinical specimens

Clinical specimens	No. Examined	*S. aureus* in out-patient	CA-MRSA No. %	No. Examined	*S. aureus* in healthcare workers	HA-MRSA No.%
Skin	72	41(56.94)	23/24(95.83)	74	31(41.89)	24/28(85.71)
Nares	58	32(55.17)	19/21(90.48)	92	21(22.83)	22/24(91.67)
Ear	54	6(11.11)	10/12(83.33)	71	7(9.86)	3/5(60.0)
Throat	33	4(12.12)	11/14(78.57)	68	5(7.35)	1/4 (25.0)
Surgical infection site	29	2(6.89)	8/9(88.89)	0	0	0
Urine specimens	62	3(4.84)	7/8(87.50)	99	4(4.04)	2/7(28.57)
Total	308	88(28.57)	78/88(88.64)	404	68(16.83)	52/68(76.47)
P value = 0.277 X^2 = 1.182						

The distribution of CA-MRSA and HA-MRSA among both genders and different ages is demonstrated in Table 9. Which showed the highest rates among males for both groups, for CA-MRSA and HA-MRSA (38.46% and 15.87%), respectively, with statistically highly significant difference (*P*<0.001) between both groups. With respect to age, for both groups ages above 40-50 years showed the highest rates of MRSA isolated, for CA-MRSA, the rate was 64.29% (18/28) while for HA-MRSA, it was 22.38%. Statistically the differences between different ages were highly significant (*P*= 0.001).

Table (9): Distribution of HA-MRSA and CA-MRSA isolates among both genders and different ages.

Variable	**Examined No.**	**CA-MRSA No. positive %**		**Examined No.**	**HA-MRSA No. positive %**		
	Gender						
Females	178	28	15.73	215	22	10.23	P value <0.001 X^2 = 13.297
Males	130	50	38.46	189	30	15.87	
	Age						
>1-10	88	6	6.82	-	-	-	P value =0.001 X^2 = 15.215
>10-20	74	12	16.22	-	-	-	
>20-30	66	14	21.21	205	12	5.85	
>30-40	52	16	30.77	132	25	18.94	
>40-50	28	18	64.29	67	15	22.38	
Total	308	78	25.32	404	52	12.87	

Table 10, illustrates the distribution of MRSA isolates among CA-MRSA and HA-MRSA of Azadi and Bedari hospitals. As indicated the rate of CA-MRSA and HA-MRSA were higher in specimens of Azadi hospital than Bedari, but these differences were statistically non-significant (*P*= 0.364)

Table (10): Distribution of MR*SA. aureus* among both hospitals in Duhok Province

Hospitals	Examined of CA-MRSA	*CA-MRSA* No. %	Examined of HA-MRSA	HA-MRSA No %
Bedari Hospital (Zakho)	152	24/152(15.79)	240	20/240(8.33)
Azadi Hospital (Duhok)	156	54/156(34.62)	164	32/164(19.51)
Total	308	78/308(25.32)	404	52/404(12.87)
P value = 0.364				
$X^2 = 0.825$				

3.3. Multi-drug resistant isolates among methicillin resistant *Staphylococcus aureus*

Table 11 shows the rates of multidrug resistance among MRSA isolates from the clinical specimens. The total rate of multidrug resistance was 51.92% (27/52) of the HA-MRSA isolates, with the highest rate (13.46 %) for four antibiotics. CA-MRSA showed less resistance (47.44 %). Furthermore, 7.69% of HA-MRSA were resistant to β-lactam antibiotics (PG, AX and AMP) and 19.23% of CA-MRSA were resistant to three antibiotics.

Table (11): The rates of multidrug resistance MRSA among 130 MRSA isolates

Resistance to multiple antibiotics	CA- MRSA	HA-MRSA
PG, AMP, AX,	15 /78(19.23)	14/52(7.69)
PG, AMP, AX, CL	11/78(14.10)	7/52(13.46)
PG, AMP, AX, CL, CX	9/78(11.54)	6/52(11.54)
PG, AMP, AX, CL, CX, CTX	2/78(2.56)	0/52(0)
Total	37/78(47.44)	27/52(51.92)

3.4. Molecular identification and virulence genes encoding toxins

PCR amplification of *nuc*, *mecA*, *pvl* and *arc A g*enes were done to detect *S. aureus* methicillin resistance (MRSA) and toxin encoding genes. All tested isolates for both *nuc* and *mec A* genes were 100% positive for both genes. The size of the amplified PCR product for *nuc* gene was 279 bps (Figure 1), while the size of the amplified PCR product for *mec A* gene was 310 bps (Figure 2).

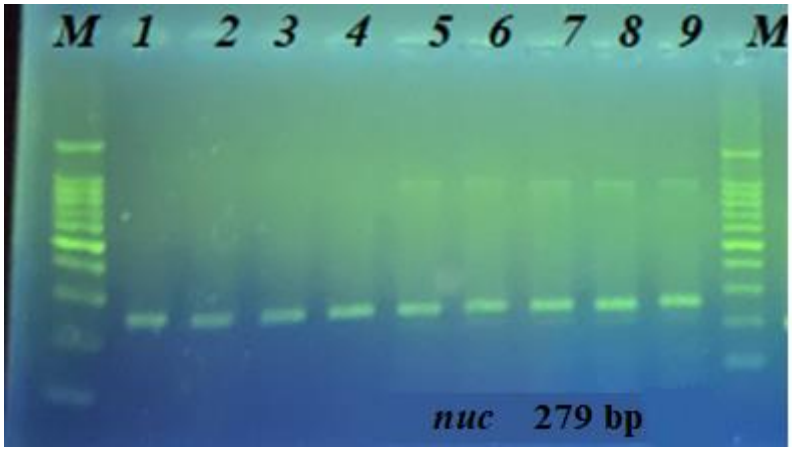

Fig. (1): The PCR amplification of *nuc* gene,using 1.2% agarose gel electrophoresis. Lane M contains a DNA ladder of 100-1000 bps, Lanes 1-9 show positive bands of 279bp for *nuc* gene.

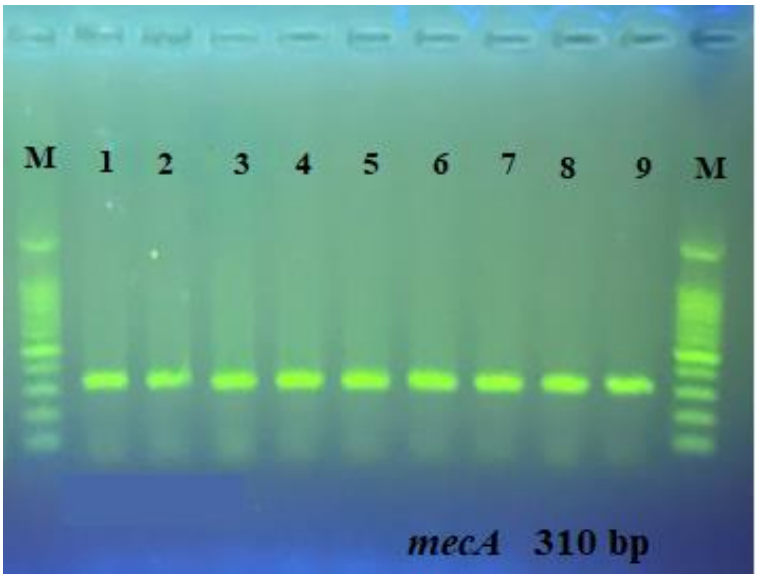

Fig. (2): The PCR amplification of *mecA* gene, using 1.2% agarose gel electrophoresis. Lane M contains a DNA ladder of 100-1000 bps: Lanes 1-9 show positive bands of 310 bps of *mecA* gene.

3.5. The virulence genes

The virulence genes investigated included *pvl*, *lukE-lukD* and *arcA* as illustrated in Figures 3, 4 and 5, respectively.

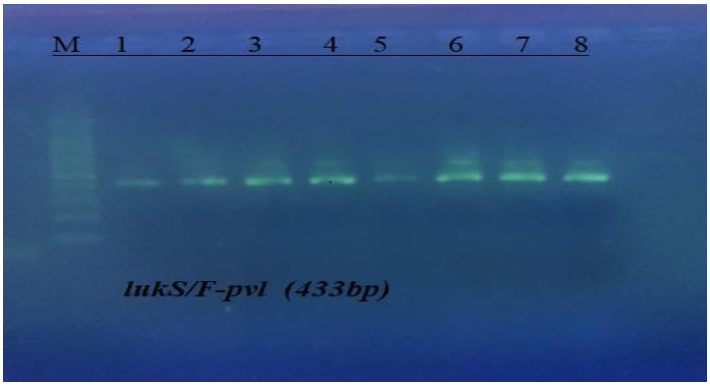

Fig. (3): The PCR amplification of *pvl* gene, using 1.2% agarose gel electrophoresis. Lane M contains a DNA ladder of 100-1000 bps: lanes 1-8 show positive bands of 433 bps for *pvl* gene.

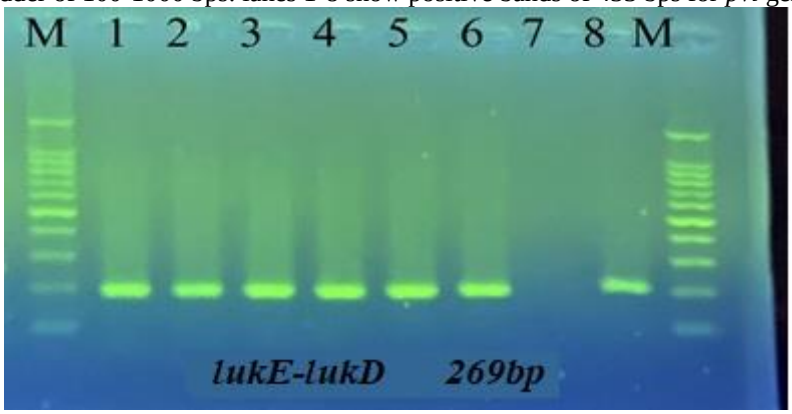

Fig. (4): The PCR amplification of *LukE-LukD* gene, using 1.2% agarose gel electrophoresis. Lane M contains a DNA ladder of 100-1000 bps, Lanes 1-8 show positive bands of 269bp for *LukE-LukD* gene.

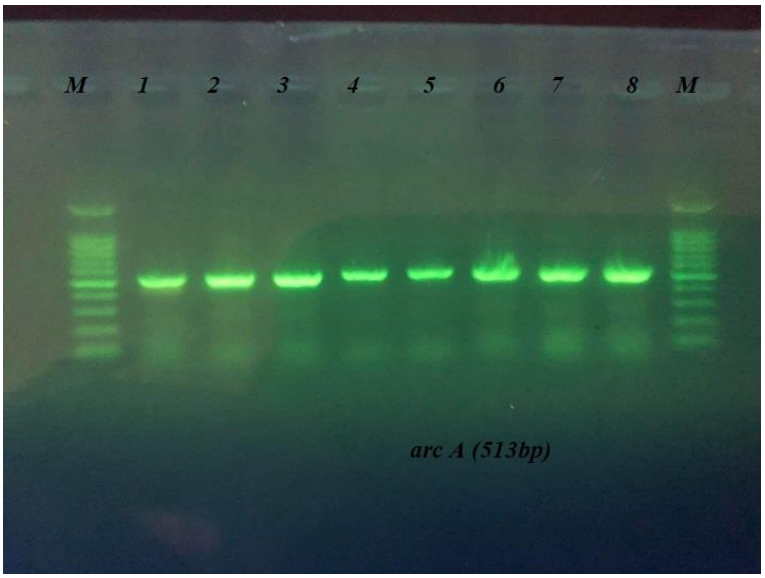

Fig.(5): The PCR amplification of *arcA* gene, using 1.2% agarose gel electrophoresis. Lane M contains a DNA ladder of 100-1000 bps : lanes 1-8 show positive bands of 513 bps for *arc A* gene.

The frequencies of virulence factors were illustrated in Table 12. Among the tested specimens, *pvl, arcA,* and *lukE-lukD* genes showed high percentages associated with community MRSA (CA-MRSA) by 41.03%, 28.20%, and 30.77% respectively.

Table (12): The distribution of virulence encoded genes among 78 CA-methicillin out-patients of Azadi and Bedari Hospitals in Duhok Province/Iraq.

Clinical sources	**No. of tested specimens**	**Detected genes**		
		Pvl	*arc A*	*lukE-lukD*
		No. *%*	*No.* *%*	*No.* *%*
Skin	23	9(39.13)	7(30.43)	7(30.43)
Nares	17	7(36.84)	6(31.58)	4(21.05)
Ear	12	4(40.0)	3(30.0)	5(50.0)
Throat	17	7(63.64)	4(36.36)	6(54.55)
Surgical infection site	3	2(25.0)	0(0)	1(33.33)
Urine specimens	6	3(42.86)	2(28.57)	1(14.29)
Total	78	32/78(41.03)	22/78 (28.20)	24/78(30.77)

Regarding the specimens of HA-methicillin from healthcare workers, Table 13 highlights the frequencies of virulence genes, *pvl, arcA, lukE-lukD,* among the tested specimens of the healthcare worker that showed high percentages associated with these genes at rates of 42.30%, 46.15% and 11.54%, respectively.

Table (13): Distribution of virulence encoded genes among 52 HA-methicillin from healthcare workers of Azadi and Bedari Hospitals in Duhok Province/Iraq.

Clinical sources	No. of tested isolates	Detected genes		
		Pvl	*arc A*	*lukE-luk*D
skin	26	9(37.50)	13(54.17)	4(16.67)
Nares	20	8(36.36)	10(45.45)	2(10.0)
Ear	3	2(66.67)	1(33.33)	0
Throat	1	1(100)	0	0
Urine specimens	2	2(100)	0	0
Total	52	22/52(42.30)	24/52(46.15)	6/52(11.54)

3.6. Sequence Analysis:

PCR amplification of *mecA* and *nuc* genes was done to detect *Staphylococcus aureus* Methicillin resistance (MRSA) from positive bacterial cultures. The amplified sequence of PCR product of *mecA* and *nuc* genes have been deposited in GenBank under the accession numbers (OP654148) and (OR326965), respectively and they showed very similar homology with MRSA sequences deposited in GenBank.

4. DISCUSSION

Staphylococcus aureus is one of the earliest known bacteria, that most frequently cause pyogenic infections in humans and the hospital environment (Algammal *et al*., 2020). The majority of the worldwide published studies on the prevalence of this bacterium focused on its carriage among out-patients and healthcare workers, at specific age groups or health conditions (Boncompain *et al*., 2017).

In this study, the overall prevalence of *S. aureus* among the specimens of out-patients and healthcare workers were 28.57% and 16.83% respectively, which is in accordance with the rate reported previously in Duhok city which was for healthcare workers 22.5% and for out-patients 18.7% (Hussein *et al.*, 2017). However, lower rates have been reported in Tanzania, which was 13.9% for healthcare workers and 10.3% for out-patients (Moremi *et al.*, 2019)

The highest rate (46.23 %) of *S. aureus* was isolated from skin swabs, indicating their important role in soft tissue infection. High prevalence of *S. aureus* in skin specimens compared to other specimens have been reported in Trinidad and Tobago (Akpaka *et al*., 2006). Moreover, ages over 40-50 years showed the highest carriage rate (85.71% and 46.42%) in out-patient and healthcare workers respectively, with a higher prevalence among males. The high rate in males 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 morbidity rate (Tarzi *et al*., 2001). As regards to older ages some study in Malaysia showed a high rate of 20.9% for *Staphylococcus aureus* and attributed this rate to multiple factors including epidemiological conditions, concurrent medical illness, increased use of invasive devices, longer hospital stay, malnutrition, as well as physiologic and anatomical age-dependent changes, such as immune-senescence, all these factors increase the vulnerability of older persons to infections and lead to significantly higher mortality among them (Hasmukharay *et al*., 2023)

All *S. aureus* isolates were subjected to thirteen 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 susceptibility rate of 97.73% and 95.45% respectively for each of them. Similarly, in India a study found *S. aureus* isolates were 100% sensitive to vancomycin and rifampin (Tiwari and Sen, 2006) . On the other hand, all isolates were with higher 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)

The genotypic, phenotypic, and clinical characteristics of CA-MRSA and HA-MRSA frequently differ in many parts of the world (Chen *et al.*, 2022). In the current study CA-MRSA and HA-MRSA were detected in 25.32% and 12.87 % of isolates. Variable rates of MRSA were reported in this Province, with a very low

rate (4.2%) among university students (Assafi *et al.,* 2015). While a higher rate (50.4%) of MRSA was reported among healthcare workers 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). While a lower rate (24%) was reported in Muthanna, among students (Hantoosh, 2022). MRSA strains are considered as 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 rates 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 out-patients 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). This data is concerning because apparently patients and healthy people may be at risk of frequent and direct transmission when they meet other members of the community.

The present study shows a significant difference between CA-MRSA and HA-MRSA regarding the types of samples and antibiotic susceptibility pattern including multi drugs resistant showed by HA-MRSA more than CA-MRS. Some of the studies reported a higher degree of resistance to erythromycin and clindamycin among CA-MRSA (Emilda *et al*., 2016). Regarding specimen sources, in this study the highest rate (39.62%) of CA-MRSA was detected among skin swabs. This finding is lower than the result obtained in India in which 71.0% of CA-MRSA was carried by hand swab (Pathare *et al.,* 2015). 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 resistance strains harbored by individuals in the community (Humphreys *et al*., 2015). The analysis of antimicrobial resistance profiles of *S. aureus* can help in the treatment of bacterial infection (Hussein *et al*., 2019). 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. 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 CA-MRSA 38.46% than females. 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) of both groups showed the highest rates of CA-MRSA and HA-MRSA which were 64.29% and 22.38%, respectively. 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 consequences of aging especially in old individuals, making them more susceptible to infections as compared to younger once. Older patients visit hospitals more frequently because they require care and treatment, hence they have a greater chance of acquiring multidrug resistance MRSA from hospitals environments (Neopane *et al*., 2018). MRSA can be a severe problem for both the hospital and the community environments and its resistance to a broad spectrum of antibiotics could limit treatment options of infectious diseases leading to high level of mortality (Admi *et al*., 2015). In the current study, 51.92% of the tested HA-MRSA isolates were MDR and showed resistance to all β-lactam antibiotics which was more than CA-MRSA (47.44%). This rate is lower than a study conducted in Ethiopia, in which 82.3% of MRSA isolates were MDR. 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 these bacterial pathogens (Dancer, 2008). Therefore, disinfecting contaminated fomites and the regular cleaning of environmental surface with effective disinfections will reduce the spread of infections acquired from the hospitals (Aminu *et al*., 2014).

Using conventional PCR technique for 52 and 78 isolates revealed the presence of *nuc* gene in all HA-MRSA and CA-MRSA isolates *nuc* gene is the identification gene of *S. aureus* bacterium and this method can be used to diagnose *S. aureus* and dispense with other methods (Bale, 2021). The present study is consistent 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*., 2021). In addition, a study in Saudi Arabia, demonstrated the amplification of *nuc* gene in 100% of *S. aureus* isolates (Ibab, 2008).

From the current results, the *mecA* gene, which locate in the SCC *mec* resistance island was carried also, by 100 % of HA-MRSA and all the multidrug resistant isolates detected by PCR 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 with 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 42.42 % among out-patients associated with CA-MRSA same situation in *lukE-lukD* gene, 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 (Oksuz *et al*., 2013; Hadyeh *et al*., 2016; Xie *et al*., 2016). This is the first report of *S. aureus* associated with *arcA* genes among CA-MRSA isolates in Iraq and in healthcare workers were 26.67% less rate associated with CA-MRSA.

CONCLUSION

Methicillin resistant *S. aureus* among out-patients is more resistant to β-lactam antibiotics than methicillin resistant of healthcare workers. Molecular assay of MRSA among out-patients showed higher rate for *pvl*, *lukE-lukD* associated with nasal swabs while *arcA* gene showed higher rate associated with the MRSA among healthy hospital staff in skin.

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