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INCIDENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) RECOVERED FROM PATIENTS WITH URINARY TRACT INFECTIONS IN ZAKHO CITY/ KURDISTAN-IRAQ

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ABSTRACT

The prevalence of *Staphylococcus aureus* (*S. aureus*) among patients with urinary tract infections (UTIs) has become a significant cause of morbidity in all age groups. The aim of the current study was to identify *S. aureus* with methicillin-resistant *S. aureus* (MRSA). Moreover, it aimed to determine their antimicrobial susceptibility patterns and to detect the presence of the *mecA, lukPV* and *icaA* genes in isolated S. *aureus* among UTIs patients. A total of 402 urine samples were collected from patients diagnosed with UTIs aged (less than 80 years) at Zakho General Hospital in Zakho City, from August 2021 to March 2022. From these samples, a total of 37 (12.6%) *S. aureus* was identified and molecularly confirmed by the *nuc* gene. A high prevalence of MRSA 28 (75.7%) was identified from these isolates *S. aureus* by Oxacillin sensitive test and confirmed by *mecA* gene. Females were highly infected with UTIs than males, and most cases were in chronic condition and married. In addition, 18 (64.3%) and 21 (75%) of these isolates *S. aureus* were harbored *luk-PV* and *icaA* genes, respectively. There were widespread of antimicrobial resistance patterns for these bacteria. Isolated MRSA were highly sensitive to Imipenem. Interestingly, a significant positive correlation between *mecA* and both virulence marker genes (*luk-PV* and *icaA*) found in MRSA with UTIs. Conclusion, the data demonstrated, for the first time, alarming emergence of multidrug-resistant MRSA isolated from UTIs of community-acquired in Kurdistan Region-Iraq. Further studies are required in other cities to report whether or not MRSA is the cause of UTI patients in Iraqi Kurdistan.

KEYWORDS: Urinary tract infections, chronic, MRSA, Kurdistan, Iraq.

1. INTRODUCTION

Urinary tract infections (UTIs) are one of the most prevalent infectious diseases observed in both outpatients and hospitalized patients (Hernandez *et al.,* 2021; Morado and Wong, 2022; Petca *et al.,* 2021). They are a major health problem affecting 150 million people globally each year and one of the most common reasons for adults seeking medical advice (Onanuga & Awhowho, 2012; Shigemura *et al.*, 2005). It is common knowledge that *Staphylococcus spp.*, from Gram-positive bacteria, are the second most common bacteria after *Escherichia coli* which cause UTI among inpatient and outpatients (Balamurugan *et al.,* 2015; Onanuga & Awhowho, 2012). Generally, *Staphylococcus aureus* (*S. aureus*) is a commensal as well as pathogenic bacterium that it did not only cause a wide range of infections in clinical cases (Baraboutis *et al.*, 2010), but also contributed about 13% to cause series bacteremic UTIs in a large community hospital that predominantly affected older patients (Baraboutis *et al.,* 2010). The thermostable nuclease of *S. aureus* is encoded by the *nuc* gene, and the PCR for amplification of this gene has the potential to rapid the diagnosis of *S. aureus* (Brakstad, *et al.,* 1992). Besides, *S. aureus* that is resistant to methicillin was first identified in the 1950s, and its prevalence has significantly increased over the past several decades (David & Daum, 2010; Grundmann *et al.*, 2006). Studies reported that MRSA strains are a major problem in hospitals, geriatric nursing homes, and other healthcare (Archer & Pennell, 1990; Grundmann *et al.*, 2006; Unal *et al*., 1994a). According to WHO (WHO, 2021), 64% of infected patients with MRSA are more likely to die than those infected with *S. aureus*, are sensitive to antibiotics (WHO, 2021). In 1990, a study noted that the *mecA* gene was not found in methicillin-susceptible isolates of staphylococci (Archer & Pennell, 1990; Louie *et al*., 2000a). However, later a study demonstrated that the *mecA* gene was regarded as the benchmark for identifying methicillin-resistant *Staphylococcus aureus* (MRSA) (Monsen *et al.*, 2003; Unal *et al.*, 1994b). Further studies reported that MRSA strains bearing the genes encoding for Panton-Valentine leucocidin (*PVL*), a highly powerful toxin, have been accountable for a serious threat to public health (Holmes *et al.*, 2005; Sina *et al.*, 2018). In addition to *ica* genes, it is known that the intercellular adhesion (*ica*) locus genes present in *Staphylococcus spp*. (Cramton *et al.,* 1999) and the expression of these genes will activate the capsular polysaccharide of *S. aureus* (Namvar *et al.*, 2013). Gad (Gad *et al.*, 2009) reported that *icaA* genes play a significant role in biofilm formation in *S. aureus.* The creation of bacterial biofilm-like communities within the urinary bladder complicates treatment because their adhesion to uroepithelial tissues is crucial for ascending infection (Balamurugan *et al.,* 2015). Besides, these genes are also responsible for protecting the bacteria from the host immune system and antibiotic therapy (Ribeiro *et al.*, 2012).

In the last few decades, *S. aureus* has emerged as the most prevalent Methicillin-resistant bacterium in the globe and it has become the most often isolated species among Staphylococcus in various clinical samples (Grundmann *et al.*, 2006; Harkins *et al.,* 2017; Louie *et al.*, 2000b). Although studies reported that MRSA is significantly associated with urinary catheterization

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(Gad *et al*., 2009; Ibtissem *et al.*, 2013; Muder *et al.*, 2006; Walker *et al.*, 2017) and antibiotic use (Nandhini *et al.,* 2022), recent incidents recorded that MRSA occurs among UTIs out-patients as well (Ahmed *et al.,* 2014; Mitiku *et al.,* 2021b). Since there is not a thorough surveillance program of community-acquired UTIs caused by MSRA in Kurdistan Region-Iraq, this study was undertaken to assess the prevalence and antimicrobial susceptibility pattern of MRSA in Zakho Hospital. It is believed that this is the first article from Kurdistan Region-Iraq that describes the *mecA, icaA* and *PVL* of MRSA and causes of community-onset UTIs.

2. Materials and Methods

2.1 Patients and samples collection

This current study was conducted at Zakho General Hospital in Zakho City, Kurdistan Region, Iraq, from August 2021 and March 2022. A total of 402 midstream urine specimens of symptomatic outpatient UTIs (less than 80 years) and they had some symptoms of the following; burning, dysuria, as well as discomfort, pain in the pelvic and back region, and polyuria and confirming that patients did not receive antimicrobials treatment.

2.2 *S. aureus* isolation and Identification

All urine samples were directly cultured on Mannitol Salt Agar (MSA) and Blood Agar (BA) (5% of sheep blood) and incubated aerobically for 24 hrs. at 37°C. A specimen was considered positive for UTI if a single organism was cultured at a concentration of 10^4-10^5 CFU/ml (A. Silva *et al.*, 2022a). Then sub-cultured on Mannitol Salt Agar and incubated aerobically at 37°C for 24hrs. The identification of *S. aureus* based on the standard microbiological protocols and biochemical characteristics of these pure colonies includes Gram-staining (Atom Scientific Ltd, UK), MSA (Neogen Ltd, UK), and catalase and coagulase tests (Oxoid Ltd, England) (Bale *et al.*, 2021; Selim *et al.,* 2022).

2.3 Antimicrobial susceptibility testing and Oxacillin test

All *S. aureus* was tested for antibiotic sensitivity patterns using the Kirby-Bauer method (Disc Diffusion Method) (Biemer, 1973; Omar, 2014), including MRSA detection by Oxacillin disc test (Velasco *et al.*, 2005). This was performed on Mueller–Hinton Agar with the following antibiotic discs (Bioanalyse Antimicrobial Susceptibility Testing Discs, Turkey); Imipenem (IPM; 10µg), Rifampin (RA; 5µg), Gentamicin (CN; 10µg), Ciprofloxacin (CIP; 10µg), Amikacin (AK; 10µg), Norfloxacin (NOR; 30µg), Meropenem (MEM; 10µg), Amoxicillin/clavulanic acid (AMC; 20/10µg), Levofloxacin (LEV; 5µg), Cephalexin (CL; 30µg), Oxacillin (OX; 5µg), Trimethoprim (TMP; 10µg), Tetracycline (TE; 10µg), Cloxacillin (CX; 10µg), Cefotaxime (CTX; 30µg), Methicillin (ME; 10µg), Erythromycin (E; 10µg), and Ampicillin (AM; 10µg). The antibiotic discs were then placed on Muller-Hinton Agar and the inhibition zones were measured using a ruler. The sensitivity pattern was scored simply as whether resistant or sensitive according to the Clinical and Laboratory Standards Institute (CLSI, 2007).

2.4 Bacteria DNA extractions

Bacterial DNA was isolated from overnight cultures on nutrient broth at 37°C. Genomic DNA was extracted by using the commercially available kit (Addprep Bacterial Genomic DNA Extraction kit, INC Daejeon, Korea) following the manufacturer's protocol. The high-quality of extracted bacterial DNA, DNA concentration and purity, was measured by NanoDrop (Thermo Scientific NanoDrop One, United States) and then stored at −20 °C for further investigation.

2.5 Molecular Identification of the specific-species gene of *S. aureus* and detection of MARSA genes

After phylogenetic identification of isolated *S. aureus*, all of them were confirmed by PCR amplification using the specific-gene primer (*nuc*) size (267bp) according to Brakstad (Brakstad *et al.,* 1992) (Table 1 & Figure 1). Then, all *S. aureus* isolates were tested for the presence of three marker genes of MRSA by Multiplex-PCR amplification of the *mecA* (310bp), *luk-PV* (432bp), and *icaA* (188bp) (Strommenger *et al.,* 2008). Details of the four primer sequences (Macrogen, Seoul, Korea), PCR product sizes and thermocycler conditions are illustrated in Table 1.

2.6 PCR and Multiplex amplification

PCR and Multiplex-PCR was performed using (GeneAmp PCR system 9700 Thermocycler PCR machine). Regarding the *nuc* gene, the reaction was carried out in a 20µl containing 10µl of 2X Taq PCR Master Mix polymerase (Guangzhou Dongsheng Biotech Co., Ltd.)), 1µl (10 pmol) of each forward and reverse primers and a 2µl of DNA template (100ng/µl), and then added 6µl free nuclease water. In addition, 20µl of the reaction was prepared for the Multiplex PCR, and the tubes of PCR contains 10µl of 2XTaq PCR Master Mix polymerase and 0.5µl for each forward and reverse of three primers, 2µl DNA and then added 5µl of free-nuclease water. The thermocycle condition of PCR amplifications is illustrated in (Table 1).

2.7. Gel electrophoresis for visualization the PCR products

The PCR products for those genes were visualized 1.5% agarose gel in TAE buffer and staining with RedSafe™ Nucleic Acid Staining Solution (20,000x) (iNtRON Biotechnology Co., Ltd. Korea). On the gel electrophoresis, the amplified PCR products were separated (80V, 45mins) and compared to a DNA marker ladder (GeNet Bio, Korea). The gel was exposed to UV light to visualize the bands under UV illumination (Cleaver Scientific Ltd, UK) and expected amplicon sizes are shown in Table (1).

Table 1. Primer sequences, PCR product sizes and thermocycle conditions in this study.

Genes	Primer Sequence (5'_3')	Product size (bp)	PCR Condition for all genes		References
nuc-F *nuc-R*	5'-GCGATTGATGGTGATACGGTI-3' 5'-AGCCAAGCCTTGACGAACTAAAGC-3'	267bp	Initial denaturation= 95°C for 5 mins,		(Brakstad *et al.*, 1992)
mecA-F *mecA-R*	5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3' 5'-CCA ATT CCA CAT TGT TTC GGT CTAA-3'	310 bp	Denaturation= 94°C for 35 Sec. Annealing= 57°C for 90 Sec. Extension= 72°C for 60 Sec.	35 Cycles	(Strommenger *et al.,* 2008)
luk-PV-F *luk-PV-R*	5'-ATC ATT AGGTAA AAT GTC TGG ACA TGA TCC A-3' 5'-GCA TCA AGT GTA TTG GAT AGC AAA AGC -3'	432 bp			
icaA-F *icaA-R*	5'-CGAGAAAAAGAATATGGCTG-3' 5'-ACCATGTTGCGTAACCACCT-3'	188pb	Final extension= 68 °C for 10 mins.		

F: forward, R: reverse.

2.8 Statistical analysis

The Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) was carried out online to analyze the distribution of MRSA marker genes among isolates with UTIs. GraphPad Prism version 9.1.4 was used to calculate the Spearman's correlation coefficient was used for nonparametric correlation between these marker genes of MRSA and antibiotics resistance patterns depending on age with UTIs and the significance was established if $p < 0.05$.

2.9 Ethical approval

The approval for conducting this study was given by the Ethical Committee of Duhok Directorate General of Health (ethical code n 18082021-8-27) and the Ethical and Protocol Review Committee of the Biological Sciences Committee (BSCZ) at the University of Zakho (ID: "BSCZ/28/7/2021").

3. RESULTS

3.1. *S. aureus* isolation with phenotypic and genotypic detection

A total of 402 specimens of (midstream) urine were collected from adult outpatients of both genders with UTI symptoms. The growth bacterial cultures were 293 (72.9%) and from these, 37 (12.6%) were phenotypic identification of *S. aureus*. Gram-positive cocci, golden yellow colonies on BA and mannitol fermenting yellow color on MSA and positive for both catalase and coagulase tests were done for phenotypic identification. Then, all isolated *S. aureus* were molecularly confirmed by PCR amplification of the specific-species gene (*nuc*) and the electrophoresis gel is as shown in Figure 1.

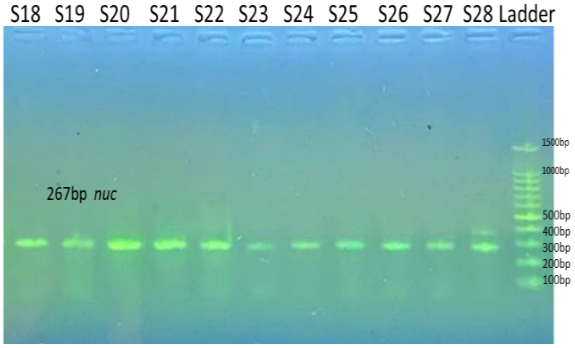
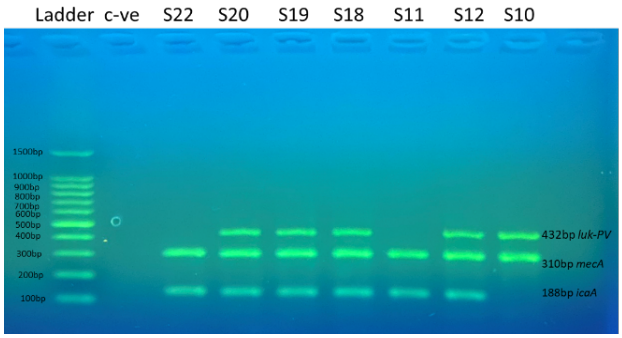


Figure 1. Gel electrophoresis of PCR amplification of the specific-species gene (*nuc*) of *S. aureus* isolates

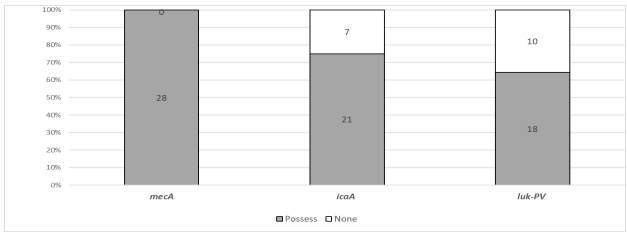
The amplified DNA fragments specific primers *nuc* gene; lanes for isolated bacteria samples S20-S28 and lane Ladder for 100bp (GDSBio Marker). These amplified DNA fragments were pipetted into a prepared 1.5% agarose gel stained with 5µl of RedSafe™ Nucleic Acid Staining Solution.

3.2 MRSA detection

Isolated *S. aureus* were subjected to detect whether they are MRSA or not, by Oxacillin Disc test and confirmed by PCR amplification of *mecA* gene. The amplicon size is 310bp. From these isolated *S. aureus*, a total of 28 (75.7%) of *S. aureus* were resistant to Oxacillin and have *mecA* gene and considered as MRSA. The total number of UTIs with MRSA was 26 (92.9%) and 25 (89.3%) in married and suffered from UTIs chronic cases, respectively. In addition, the prevalence rate of infected females 24 (85.7%) was higher than males. In addition to *mecA* gene, these isolates MRSA were tested to get both *icaA* and *luk-PV* genes. The prevalence rate of both *icaA* and *luk-PV* genes was found in 21 (75%) and 18 (64.3%) of isolates MRSA with amplicon sizes 188bp and 432bp, respectively (Figure 1b).



(a)



(**b**)

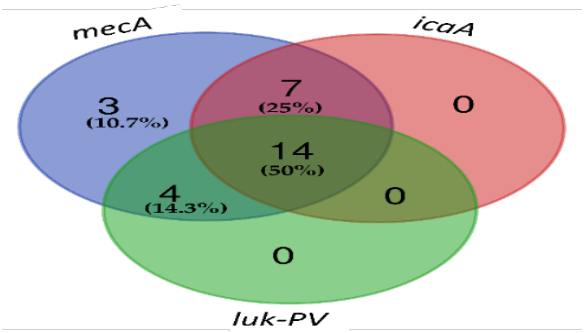
Figure 2. Gel electrophoresis for the multiplex PCR assay for detecting *mecA*, *icaA* and *luk-PV*, genes of isolates MRSA (a) and the prevalence rate and total number of these marker genes (b).

The amplified DNA fragments by multiplex PCR assay for the marker genes (a); lane control negative (Control -ve), lane 3-7 for the amplified DNA fragments produced (S22 and S11, possess both *mecA* and *icaA* genes, S10 possess both *mecA* and *luk-PV,* S20, S19, S18 and S12 have three genes; *luk-PV, mecA* and *icaA* genes). These amplified DNA fragments were pipetted into a prepared 1.5% agarose gel stained with 5µl of RedSafe™ Nucleic Acid Staining Solution. The prevalence rate and total number of *mecA*, *icaA* and *luk-PV* genes.

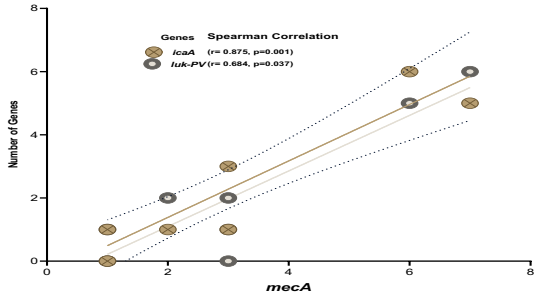
3.3 Distribution and relationship of these marker genes in isolated MRSA

Figure (3a) demonstrates the details of MRSA marker genes harbored in all of the isolated *S. aureus* samples. A total of 14 (50.0%) of isolated samples possess of *mecA, icaA*, and *luk-PV* genes while 7 (25%) of them harbored two genes: *mecA,* and *icaA*. Furthermore, 4 (14.3%) of isolated have both genes; *mecA and luk-PV*, and only 3 (10.7%) have *mecA*, respectively. In addition, one bacterium has only *luk-PV* gene. In addition, the Spearman's correlation coefficient of these data indicated a significant positive correlation between *mecA* and both virulence marker genes of *luk-PV*, and *icaA* in MRSA with UTIs (Figure 3b).

The distribution of MRSA marker genes among isolates with UTIs by Venn Diagram software. A total 14 of MRSA possess three genes; *mecA and luk-PV*, and *icaA and* followed by 7, 4, and 3 isolated MRSA possess two genes (*mecA,* and *icaA),* and (*mecA* and *luk-PV*), and *luk-PV,* respectively. The non-parametric Spearman correlation analysis indicated that the numbers of MRSA increased with increased both marker genes and the marker denote a sum observation of these genes for a particular age group of UTIs. The significance was considered when $p<0.05$.



(a)



(b)

Figure 3. The Venn Diagram for the distribution of marker genes among (a) and correlation analysis between the specific-gene (*mecA*) and both the marker genes of (*icaA* and *luk-PV*) (b) of isolates MRSA with UTIs.

3.5 Antimicrobial susceptibility patterns of MRSA

Isolated MRSA were subjected to the antimicrobial susceptibility to determine patterns for the 18 antibiotics disks, and the results are shown in Figure (2).

Isolated MRSA exhibited total resistance to Ampicillin and approximately 96%, 85%, 78%, 78 and 74% for Cefotaxime, Methicillin, Norfloxacin, Cloxacillin, and Amikacin, respectively. Nearly the same percentage 70% was resistant to Trimethoprim, Tetracycline Erythromycin, and Cephalexin. Additionally, around 63% of MRSA isolates were resistant Meropenem and the same percentage of both 56% and 52% were resistant to both Levofloxacin and Gentamicin, and Ciprofloxacin and Rifampin, respectively. By contrast, these isolates MRSA were sensitive to Imipenem 92.6%.

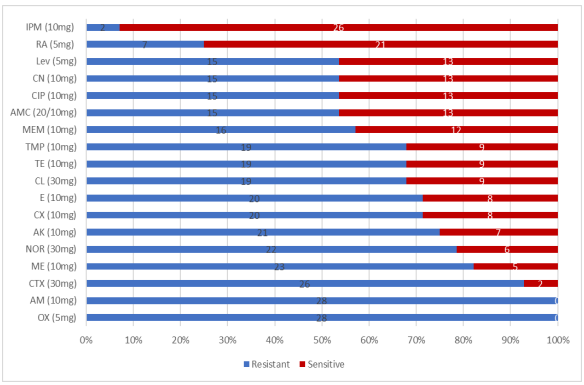


Figure 2. The antibiotics sensitivity patterns of MRSA isolates

Keys: Antibiotic; IPM; Imipenem, RA; Rifampin, CN; Gentamicin, CIP; Ciprofloxacin, AK; Amikacin, NoR; Norfloxacin, MEM; Meropenem, AMC; Amoxicillin/clavulanic acid, LEV; Levofloxacin, CL; Cephalexin, OX; Oxacillin, TMP; Trimethoprim, TE; Tetracycline, CX; Cloxacillin, CTX; Cefotaxime, ME; Methicillin, E; Erythromycin, AM; Ampicillin.

4. DISCUSSION

Uropathogenic bacteria have been identified as a major cause of UTIs, with highly significant morbidity and mortality rates, worldwide (Tula *et al.,* 2016). Generally, Gram-positive bacteria, particularly *S. aureus*, have emerged as significant contributors to hospital and community-acquired infections and almost are resistant to antibiotics and easily spread (Lunacek *et al*., 2014). In this study, *S. aureus* accounted for only 37 (12.6%) of isolates from urine samples submitted from the community. This finding was approximately similar with laboratory-based studies conducted in Iraq; 11.1% in Tikrit City (Al-Jebouri & Mdish, 2013) and 13.5% in Thi-Qar City (Abbas & Hamim, 2019). In addition, this finding was supported by earlier studies that reported that *S. aureus* was the second most common pathogen in UTIs, and it is more common in women (Onanuga & Awhowho, 2012; Silva *et al.,* 2022a). However, their studies did not show whether *S. aureus* was MRSA or not.

It is interesting to note that in this study, out of 28 (75.7%) isolates were Oxacillin resistant from the total of 37 clinical isolates of *S. aureus*, which are considered as MRSA. The incidence of UTIs with MRSA is much higher than what has been reported in recent studies in Iraq; in Baghdad City Khaleel *et al.* (2021) reported that 7.7% of isolates were positive for MRSA when using Oxacillin and Cefoxitin resistances as a marker for detecting MRSA, In contrast, in Thi-Qar City a study condcuted by Abbas and Hamim (Abbas & Hamim, 2019) reported that all isolated *S. aureus* were Oxacillin-resistant without mentioning whether they are MRSA or not. Furthermore, the high frequency of MRSA is resembled to those recently found in Khartoum, Sudan (Omar, 2014), where 72% of the detected isolates were MRSA. However, their study methods of identification were different from this study. On the other hand, this finding was much higher than that found in recent studies in South Ethiopia by Mitiku *et al.,* (2021b) and in India by Mendem *et al.*, (2016), lower prevalence of 42.6% and 55.3% among outpatients' community-acquired UTIs, respectively.

It is emphasized that *mecA* gene is the specific-genes for the identification of MRSA from clinical samples (Maes *et al.,* 2002; Metri & Jyothi, 2021). In the current study, the genotypic detection of MRSA in UTIs by *mecA* gene was confirmed for all isolates. Females were highly significant infected than males, particularly among married and chronic UTIs cases. This high frequency of detecting the *mecA* gene is comparable to this found in Sudan (Ahmed *et al.,* 2014). The incidence of detecting *mecA* is almost double than that illustrated in a recent study in India (Jyothi & Metri, 2021), with a lower prevalence of 44 % from catheterized patients with UTIs. This variant rate could be explained by identification methods, time and condition of collecting sample and geographical differences (Mitiku *et al*., 2021b). Indeed, MRSA has become not only a global nosocomial disease and rapid dissemination to healthcare and the community but also it is extremely antibiotic resistant, with variations between institutions and countries (Grundmann *et al.*, 2006; Louie *et al.,* 2000b; Mitiku *et al.,* 2021b). The UTIs with MRSA are probably because of a number of clinical factors, including anatomical variations, hormonal impacts (hormonal changes during pregnancy favor UTIs in females), behavioral tendencies, and physiological causes (Silva *et al.,* 2022b). Because of their smaller urethral length and closer vaginal cavity and rectal entrance (where possible uropathogens reside), females are more likely than males to have germs enter the urethra and climb to the bladder (Silva *et al.,* 2022b). Additionally, it is believed that chronic cases highly sexually active individuals, and child-bearing age groups are the key areas where *S. aureus* is prevalent (Akortha & Ibadin, 2008; Ramasamy *et al.,* 2019). Other factors associated with urinary tract infection as mentioned above such as gender, age and marriage may play a significant role in

contributing to the increased incidence of MRSA in the community. In addition to those factors, the existence of a chronic underlying condition was statistically related with the frequency of MRSA, who has a history of UTIs. This finding is in agreement with a study condcuted in Southern Ethiopia (Mitiku *et al*., 2021a). They reported that patients with a history of UTI were more likely to have MRSA than those without a history of UTIs. Besides, another reason for the high frequency might be possible MRSA patients who have been discharged from the hospital spreading the infection throughout the community (Lunacek *et al.*, 2014).
In this current study, the high frequency of *icaA* (75%) and *luk-PV* (64.3%) in all isolated MRSA, is comparable to a study done in Sudan (Ahmed *et al.*, 2014) where 64% of MRSA possess the *icaA* gene while it was *luk-PV* (25%). According to an epidemiological study by Bhatta (Bhatta *et al.*, 2016), *PVL* genes were linked to increased virulence of community-acquired MRSA from various samples. Thus, the *PVL* gene has been identified as a reliable marker of community-acquired MRSA strains in various clinical samples (Amin *et al*., 2020; Holmes *et al.,* 2005; Motamedi *et al.,* 2015). It is noted that a few studies have established antibiotics susceptibility patterns of MRSA with UTIs. In this study, all MRSA was resistant to Ampicillin. This finding was similar to a recent study conducted in Ethiopia (Mitiku *et al.,* 2021b). Furthermore, MRSA was highly resistant to Cefotaxime (92%) and this finding was higher than that found in recent studies (71%) conducted in Sudan (Ahmed *et al*., 2014), and 74.2% in Egypt (Ibrahim *et al.*, 2020), while MRSA were around 75% resistant to Norfloxacin, and Amikacin and they are nearly close to the finding reported in the recent studies in Ethiopia (Mitiku *et al*., 2021b) and in Austria (Lunacek *et al.,* 2014). In addition, around 67.8% of MRSA were resistant to Trimethoprim, Tetracycline, Erythromycin, and Cephalexin and these findings were roughly similar to a study done in Iraq (Hamad *et al.,* 2016) and in Khartoum State (Ahmed *et al.,* 2013). By contrast, these isolates MRSA were sensitive to Imipenem 92.8% and this finding was a bit higher than that arrived at in the study conducted in Afghanistan 81.4% (Naimi *et al.,* 2017). The high prevalence resistance rate of MARSA to antibiotics is due to several factors ,namely its ability to form biofilms, by *icaA*, might be a significant factor in chronic UTIs and antimicrobial drug resistance (Silva *et al.*, 2021; Yousefi *et al.,* 2016). The development of multidrug resistance may be maintained by the slow diffusion of antibiotics through the biofilm matrix, conceivably by selecting highly tolerant strains that are briefly exposed to sub-inhibitory doses of antimicrobial therapy. Di Domenico *et al.* (2017) reported that the creation of biofilms may give colonizing bacteria important virulence traits ,such as immunity to the host immune system protection and increased general antibiotic tolerance, non-biofilm producers. In addition, The overuse of antibiotics, especially imposes selection pressure on the generation of resistant strains, may potentially contribute to the high prevalence of PVL together with the virulence factor (Amin *et al.*, 2020; Kaur *et al.,* 2012; Motamedi *et al.,* 2015).

This is not suppressing that MRSA strains are typically resistant to multiple antibiotics (Grundmann *et al.*, 2006; Onanuga & Awhowho, 2012; Petca *et al.,* 2021), and it may transmit among people by physical contact and rarely by air according to WHO (WHO, 2021). The role of biofilm formation in these bacteria might also associated with multidrug resistance (Balamurugan *et al.,* 2015). In fact, the community strains' resistance to multiple therapies indicates that they may have originated from the hospital. In addition to these factors, in Iraqi Kurdistan, over the counter and both general practitioners and many nurses have prescribed antibiotics for patients without obtaining the antibiotic sensitivity test from the microbiological laboratory clinic. Therefore, the emergence of MRSA resistant to antibiotics is needed to be well documented and creating strategies for empirical treatment and in assessing the current guidelines (Chambers & DeLeo, 2009).
The main limitation of the study is the lack of sequencing of the 16s rRNA gene of MRSA. Hence studying the phylogenetic tree and whole gene sequencing is essential for understanding the epidemiology and infections in the urinary tract.

5. CONCLUSIONS

In conclusion, this study demonstrates that MRSA isolates were the common pathogens from Gram-positive bacteria, particularly married and chronic cases with of UTIs from Zakho City in Iraqi-Kurdistan. Females were highly infected than males and acute cases found in the community-acquired community UTIs. Furthermore, the molecular detections of MRSA strain in UTIs and the highly incidence of infections was strongly associated with both *icaA* and *luk-PV* genes as well as multidrug-resistant which were positively associated with these genes. In addition, the most effective antibiotic for treating UTIs, especially with MRSA, is considered to be Imipenem. The recommendations for the use of antibiotics should be monitored by the public health sectors. Further studies of UTIs with *S. aureus* should investigate whether they are MRSA or not in other cities in Kurdistan Regional-Iraq.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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