Iraqi Journal of Medical and Health Sciences, ISSN: 3078-8722, Vol. 2, No. 1, 2025, Pages 23-30
DOI: https://doi.org/10.51173/ijmhs.v2i1.26

Iraqi Journal of Medical and Health Sciences
Journal Homepage
https://ijmhs.mtu.edu.iq/ijmhs/index.php/home

RESEARCH ARTICLE – GENERAL BIOCHEMISTRY, GENETIC AND MOLECULAR BIOLOGY

Molecular Detection of Methicillin-Resistant Staphylococcus aureus and Multidrug Resistance in Patients with Urinary Tract Infection

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Article Info.

Article history:

Received
13 Dec. 2024

Accepted
20 Feb. 2025

Publishing
10 May 2025

Abstract

Background: methicillin resistant *staphylococcus aureus* is global problem which cause urinary tract infections and this affecting approximately 150 million people worldwide annually, in addition its high resistant to most of antibiotic.

Objective of study: Amid of this study diagnose and determine methicillin resistant *S. aureus* in patient which urinary tract infections, and detect virulence gene, multidrug resistance by PCR technique.

Materials and Methods: This study included 100 urine samples collected from urinary tract infection (UTI) patients. These samples were first cultured on standard media, then confirmed on HiCrome MeReSa Agar selective medium for the isolation of MRSA. Then (PCR) was performed to identify *mec A* gene. All isolates were subjected to susceptibility testing against a range of antibiotics to determine their resistance profiles and multi-resistance.

Results: Twenty samples (44%) of the total 45 samples were diagnosed as methicillin-resistant *S. aureus* (MRSA) based on a set of phenotypic characteristics, the most important of which was growth on mannitol salt agar. Then diagnosis on Hi-Chrome MeReSa media, which is a selective medium dedicated to identifying MRSA based on the colorimetric indicator, as colonies appear blue after incubation for 18-24 hours. Then, the diagnosis of MRSA isolates was confirmed using polymerase chain reaction (PCR) to detect the *mec*A gene, which showed that all isolates that showed positive growth on Hi-Chrome MeReSa agar possessed the *mec*A gene with a molecular weight of 147 base pairs, at a rate of 100%. also noted prevalence of MRSA infection its higher in females by 75%, compared to males, which reached 25%.

Conclusion: in this study shed light on prevalence of MRSA in patients with urinary tract infections, revealing that infection in females is 3 times higher than in males. The study also showed that all isolates showed multiple resistance to different antibiotics.

Publisher: Middle Technical University

Keywords: Staphylococcus aureus, MRSA, *mec A*, Urinary Tract Infections, Multidrug Resistance.

1. Introduction

Urinary tract infections (UTI) its serious health problem affecting approximately (150 million) peoples worldwide every year. These are community- or hospital-acquired infections that typically present within 48 hours of hospital admission [1]. *S. aureus* its second most bacteria causes Urinary tract infections after *E.coli*. Several studies have confirmed increasing prevalence of *S.aureus* particularly which methicillin resistant [2] prevalence infections is influenced by several factors including , age , sex catheterization, Long term antibiotic therapy ,and incorrect use of these antibiotic [3]. Females are more susceptible to UTI than males, with one in three females having at least one UTI before the age 24, so that requiring antibiotic treatment, and half of females with UTI required antibiotic treatment throughout their lives [4,5]. *S. aureus* is an opportunistic bacterium that infected elderly, immunocompromised and those which chronic disease. Since 1961 (MRSA) infections have been major public health concern and hospital acquired infections have become important due to their high mortality rates [6]. MRSA is notorious to acquiring drug resistance and continues to defy attempts at medical control, and persistent exposure to methicillin has led to emergence of MRSA strains capable of resisting multiple drugs [7]. Methicillin-resistant *Staphylococcus aureus* is referred to as MDR if it is resistant to at least three drugs [8].

Antibiotic resistance occurs through several mechanisms, including degradation or modified antibiotics, alteration bacterial target of antibiotic, and decreased intracellular concentration antibiotic, through decreased in cell wall permeability or efflux of antibiotic from cells [9,10]. *S.*

aureus. Strains which carry wide array of multidrug resistance genes carry in plasmids that help spread resistance even between different species [11]. The *mec*A gene is carried on the bacterial chromosome and is component of the large chromosome region (SCC*mec*) (Staphylococcal Chromosomal Cassette *mec*), which confers resistance to many antibiotics when it encodes the penicillin-binding protein PBP2a. Some key resistance mechanisms contributing to this spread include: Beta-lactam Resistance S. aureus has developed resistance to beta-lactam antibiotics, including methicillin. The production of penicillin-binding proteins (PBPs) with low affinity for beta-lactams also can form biofilms and develop efflux pumps that actively expel antibiotics [1, 12]. The present study aims to detect MRSA bacteria causing UTI with multiple resistance antibiotic in 100 isolated samples of *S. aureus* which isolate from patients infected with urinary tract in Diyala province.

2. Materials and method

2.1. Samples collection

This study was conducted in Baquba teaching hospital, Diyala government in urine specimens of symptomatic UTI patients. Urine specimens collected from males and females in sterile universal container and process according to microbiological techniques [13]. During the period from April to August 2024, 100 urine samples were collected from people suffering with UTI infections who visited Baqubah Teaching Hospital. The samples taken directly from the patients under the supervision of the specialized medical staff and transferred in ideal conditions to the laboratory unit to conduct initial examinations.

2.2. Isolation and identification (MRSA) isolates

All samples were inoculated in mannitol salt agar media then incubated to 24 hours at 37°C, which is considered differential and selective medium for diagnosing *S.aurus* [14]. They were then cultured on HiCrome™ MeReSa agar for the diagnosis of MRSA. Then Isolates identified by phenotypic, microscopic, cultural characteristics, and some biochemical tests, and growth in Mannitol Salt Agar, this medium with a high salt concentration is considered a diagnostic and selective medium is special for isolating *S. aureus*. These bacteria ferment mannitol and then Isolates of (MRSA) bacteria were investigated using HiCrome ™ MeReSa agar base (HIMEDIA- India). This medium considered a highly selective medium for isolating and differentiating strains of (MRSA) by depending on the color.

2.3. Molecular to identification S.aureus methicillin-resistant (MRSA)

Genetic study enhances traditional detection methods using (PCR), which is effective tools in microbiology studies and is commonly use to identify genes of clinical interest [15]. DNA extraction kit (ABIOpure, USA) was efficient in extraction. Extracted DNA was electrophoresed (Microamp ; Applied Biosystems, USA) on 1% agar gel (USA), ethidium bromide (USA), electrophoresed at 70V for 1 h, then visualized using a UV light device. The sequences of *mec* A were amplified using specific primer which shown in Table1. PCR reaction used a total volume of (50 µl) of the mixture as shown in Table 2. PCR schedule for *mec A* gene is shown in Table 3. The PCR results stained with ethidium bromide and visualized after electrophoresis in (2%) agarose gel.

Table 1. Primers used in this study

Gene	Primer	Sequence (5`-3)	Size (bp)	References
Mec A	Forword	GTG AAG ATA TAC CAA GTG ATT	146	[16]
	Reverse	ATG CGC TAT AGA TTG AAA GGA		

2.4. Antibiotic susceptibility test

Bauer and Kerby were used to test the antibiotic susceptibility of the isolates [17]. Five ml of nutrient broth medium was inoculated with 5 pure colonies of 24-h-old bacterial cultures, in clear tubes and incubated at 37°C for18- 24 h. Turbidity of the growth then compared with standard turbidity constant solution 0.5 McFarland, to give an approximate count of 1.5 × 10 cells/ml and 0.1 ml of the bacterial suspension transferred and spread in Mueller-Hinton agar, and the plate left for 3 minutes at room temperature to drying. Then, the antibiotic discs transferred to the surface of culture medium by sterile forceps at a rate of 5 discs per plate, and these plates incubated at 37°C to 18-24 h. Diameters of inhibition zones around each disc which measured and the isolates classified in to susceptible (S), intermediate (I), and resistant (R) according to the standard specifications mentioned in [18]. Antibiotic tablets were procured from Hi Media, India, which included; Amikacin (30 mg), Azithromycin (15 mg), Cefepime (30 mg), Cefoxitin (30 mg), Chloramphenicol (30 mg), Ciprofloxacin (5mg), Clindamycin (10mg), Erythromycin (15 mg), Gentamicin (10 mg), Imipenem(10 mg), Levofloxacin (5 mg), Penicillin(10 U), Rifampin(5 mg), and Tetracycline (30 mg).

Table 2. Materials of Polymerase Chain Reaction used in study

Master mix	Volume
One Sample	
Master Mix	12.5
F -primer	1
R -primer	1
Free Water Nuclease	7.5

Continue Table 2. Materials of Polymerase Chain Reaction used in study	
DNA	3
Total	2 5
per single- rxn	22µl - Master mix per tube and 3 µl of template.

Table 3. Programs of PCR thermo-cycling in this study

Steps	Temp.℃	Time	No. of Cycles
Initial -denaturation	(95)	(5) m	(1)
Denaturation	(95)	(30)s	(30)
Annealing	(55)	(30)s	
Extension	(72)	(30)s	
Final extension	(72)	(7)m	(1)
Hold	(10)	(10)m	

3. Result

3.1. Isolation and identification of samples

During the period from April to August 2024, 100 urine samples were collected from patients infected in urinary tract for both sexes. All isolates were directly cultured in mannitol salt agar and blood agar then incubation at 37°C for 18-24 h. Fifty-seven isolates (57%) showed positive growth, of which 45 isolates (45%) were tentatively diagnosed as S. aureus,12 isolates (12%) were unknown and 43 isolates (43%) showed no growth.

3.2. Culture characteristics and Microscopic examination

Gram stain test of Staphylococcus was carried out. The isolates examined under light microscope, and these cells appeared as Gram positive bacteria (grape-like clusters). S. aureus isolates identified according to morphological characteristics, and were in the form of medium to large colonies, 1-3 mm in diameter, with regular and smooth edges and a gelatinous consistency. The colonies were characterized by bright yellow color when grown on mannitol salt agar(MSA), due to their ability ferment of mannitol sugar and appear white colonies in blood agar.

3.3. Identification of methicillin-resistant Staphylococcus aureus (MERSA)

Twenty isolates out of 45(44%) isolates were phenotypic diagnosed as (MERSA) based on several phenotypic characteristics, the most important of which was growth on Hi-Chrome MeReSa agar (Himedia, India) Fig.1, which is a selective medium for the diagnosis of MRSA based on color.

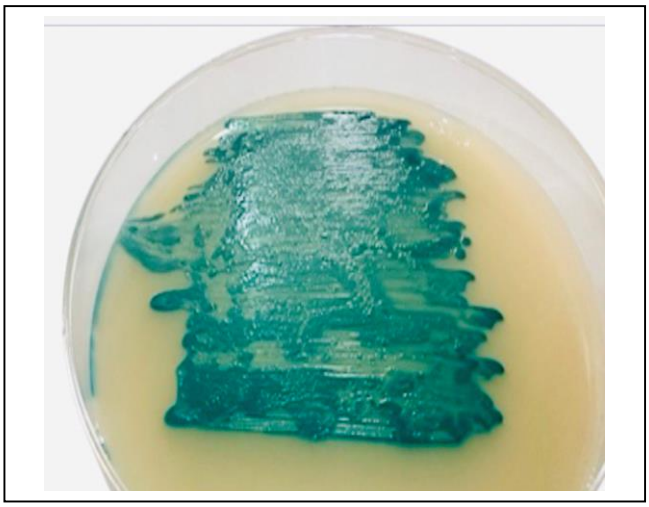

Fig. 1. Colonies *S. aureus* bluish-green color on HiCrome MeReSa Agar

All isolates were cultured and incubated for 18-24 hours. If the result was not clear, they were incubated for 48 hours. The result is positive if the colonies appear blue-green, as shown in Fig. 2. Isolates of MRSA identified by (PCR) techniques according to presence of *mec A* gene. The results showed that all isolates which gave positive growth on Hi-Chrome MeReSa agar media, contained *mec A* gen as percent 100%. with molecular weight of 147 bp, as show in the Fig. 2.

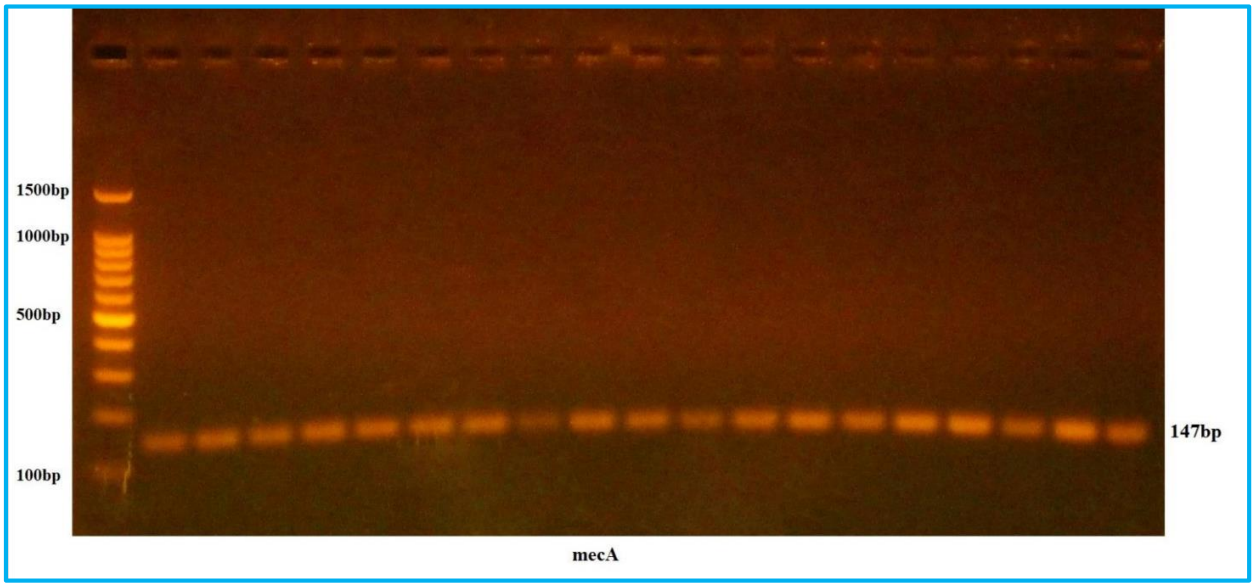

Fig. 2. Electrophoresis for PCR products to determine presence *mec A* gene at size molecular 147bp for methicillin-resistant *S. aureus*

3.4. Distribution of MRSA isolates by sex

The results in Fig. 3 highlights the incidence of MRSA among males and females. The highest incidence was recorded in females, reaching 15 (75%), while in males, it reached 5 (25%).

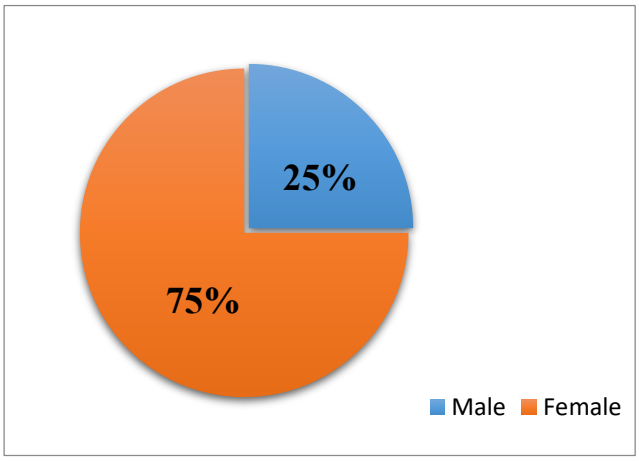

Fig. 3. Distribution MERSA isolates according to the Sex

3.5. Antibiotic susceptibility test

Susceptibility testing for 20 MRSA samples to 14 antibiotics, performed by Kirby - Bauer method according to the CLSI [20]. Table 4 and Fig. 4 indicates all MRSA isolates which resistant to cefepime, cefoxitin, Erythromycin, Azithromycin, and penicillin antibiotics at 100%, then ciprofloxacin and Chloramphenicol more than 90%. The lowest resistance rate was to amikacin, clindamycin, and imipenem by 56%, 60%, and 65% respectively. The isolates were classified according to the number of antibiotics were resistant to determine the resistance pattern that is important to explain their behavior in infection. The results showed all isolates diagnosed as MRSA showed multiple resistance for different types of antibiotics in a rate of 100%. Also 20 isolates, 14 (70%) resistant to more than 10 antibiotics and 6 (30%) were resistant to less than 10 antibiotics.

Table 4. Antibiotic Susceptibility Test for MRSA Isolates

Antibiotic discs	Sensitive		Intermediate		Resistant	
	NO.	%	NO.	%	NO.	%
Amikacin	7	35	-	-	13	56
Azithromycin	-	-	-	-	20	100

ContinueTable 4. Antibiotic Susceptibility Test for MRSA Isolates						
Cefepime	-	-	-	-	20	100
Cefoxitin	-	-	-	-	20	100
Chloramphenicol	1	5	1	5	18	90
Ciprofloxacin	-	-	1	5	19	95
Clindamycin	8	40	-	-	12	60
Erythromycin	-	-	-	-	20	100
Gentamicin	5	25	1	5	14	70
Imipenem	6	30	1	5	13	65
Levofloxacin	2	10	4	20	14	70
Penicillin	-	-	-	-	20	100
Rifampin	2	10	3	15	15	75
Tetracycline	4	20	4	20	12	60

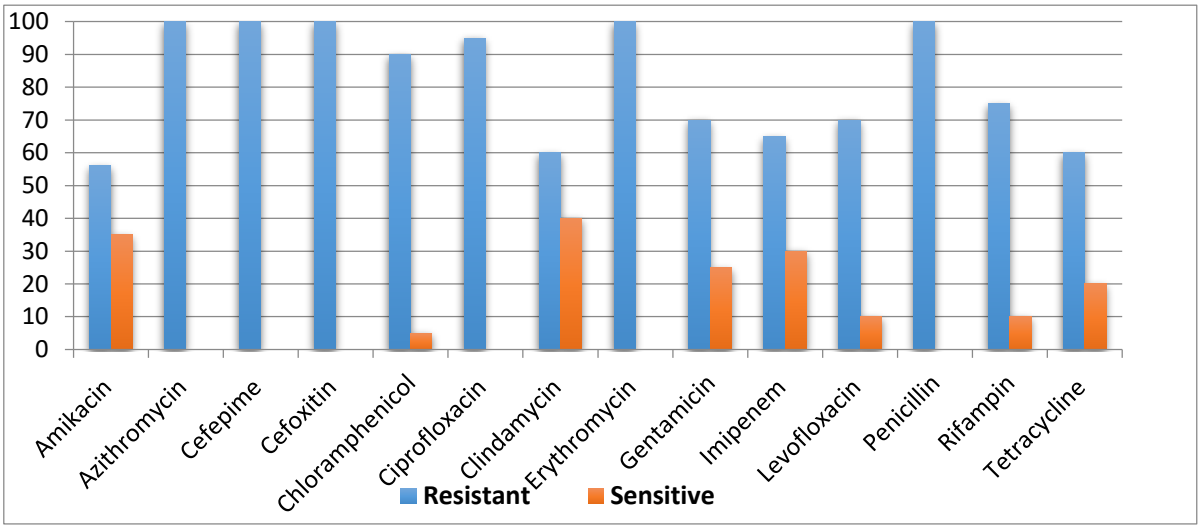

Fig. 4. Antibiotic susceptibility test for MRSA isolates

4. Discussion

This study comprehensively addressed the prevalence, virulence and antibiotic resistance of MRSA in Diyala governorate, eastern Iraq. All samples collected from urine samples of patients with urinary tract infections for various hospitals in Diyala governorate. The study used some of microbiological techniques to identify MRSA isolates, including primary culture samples in mannitol salt agar MSA, which as selective, differential medium to *S. aureus*. Then all isolates were identified based on the microscopic and morphological characteristics of colonies and cells. Many biochemical tests were performed to confirm the diagnosis of *S. aureus* is one of the most common pathogens associated with hospital, community acquired infectious worldwide. Its incidence varies from one region to another, depending on geographical characteristics, as well as the social and demographic factors of the populations [19]. Its considered an opportunistic pathogen due its part of the normal flora in the body, in addition to possessing some virulence factors that enable it to cause infection and then infection. The result of the laboratory culture showed that 45(45%) out of 100 samples showed bacterial colony growth in mannitol salt agar and blood agar. This percentage is due to the size of the sample studied, in addition to the social factor of the people, the incorrect use of antibiotics, and age, gender of the infected people. As for the samples that didn't show growth in culture medium, this may due to the presence of other pathogens such as viruses or parasites, which were not detected in this study. This may also be due to the efficiency of the antibiotics used in the treatment and their ability to kill and eliminate the bacteria [20]. MRSA isolates were diagnosed phenotypically according to color guide after culturing them in HiCrome MrReSa selective medium, where the colonies appeared green after incubated for 24 – 48 hours. The diagnosis was also confirmed genetically based on the presence of *mec A* gene, as all isolates grown in HiCrome MrReSa medium showed the presence of this gene [21]. Number of samples diagnosed as MRSA was 20 samples, and the results of our study were consistent with those obtained by researcher [22]. Who found the MRSA isolates rate to be 20%. Also, results of our study reported a much higher MRSA isolation rate compared to the study conducted by researcher, his isolation rate in his study was only 3%. In Kurdistan region /northern Iraq, in 2015 a high prevalence MRSA (53%)was reported [23]. In 2019, the prevalence of MRSA among hospital workers reached 50% after molecular confirmation by detecting *mec A* gene [24].

Several studies conducted in middle east reported prevalence of MRSA of about 78% between 1999- 2020. These studies showed that MRSA strain showed high resistance to penicillin and ciprofloxacin [25]. This high rate indicate major challenges in the treatment of MRSA infections in the middle east, as treatment options are limited due to the resistance of these strains to most common antibiotics.

Therefore, is an increasing need to develop effective strategies to control the infection and limit its spread. a study conducted in Tehran revealed that the isolation rate of MRSA from different clinical cause was 66% [26]. In Africa studies have indicated increase in the prevalence of MRSA according to the study of researchers [27], which was conducted in the microbiology laboratory of the university hospital of monastir, Tunisia, and showed that MRSA isolates were 83% from pus, 9% from blood, (6%) from endoscopic puncture, and one isolate from a venous catheter. In another Tunisian hospital, 83 clinical samples from 64 patients with diabetic foot infections were analyzed, and the prevalence of MRSA was 22%. In Algeria, a study of 211 patients with urinary tract infections showed that 126 patients were infected with MRSA, the infection was more prevalent in females than males, and most isolates had multiple antibiotic resistance [28]. The variation in the prevalence of MRSA among studies around the world is due to differences in geographical environment, health conditions, and indiscriminate and incorrect use of antibiotics [29]. In recent years, MRSA has become a serious health problem as it has become resistant to most conventional and modern antibiotics. Therefore, it is necessary to investigate resistance patterns to identify future challenges for effective treatment. In this study, the susceptibility of MRSA isolates to 14 antibiotics was tested to investigate the drug resistance pattern and to identify the most effective treatments for this type of bacteria. Table 4 showed the distribution of antibiotic resistance of MRSA isolates from different clinical sources, where it showed cefepime, cefoxitin, Erythromycin, Azithromycin, and penicillin antibiotics at 100%, then ciprofloxacin and Chloramphenicol more than 90%, this has been confirmed by many studies [30]. The reason for *S. aureus* resistance to methicillin and all beta lactam antibiotics is the *mec A* gene, located in the (SCC*mec*), this chromosome encodes an alternative penicillin binding protein (PBP2a), a chain-linked enzyme that catalyzes the peptidyl transfer reaction required to link peptide glycan chains. Because of their low affinity for all beta-lactam antibiotics, staphylococci can survive these antibiotics when exposed to high concentrations. In addition, MRSA strains often resistant to other types of antibiotics, such as macrolides, aminoglycosides, chloramphenicol, and fluoroquinolones [31,32]. For the antibiotic erythromycin, the resistance rate was (88%), and this result is largely consistent with reached by in Mosul, northern Iraq, which reached 86%, which is also close to the results of the study [33], which recorded 72%, the study [13], which showed less resistance rate of 62%. Macrolide antibiotics such as erythromycin and azithromycin are used clinically to treat infections caused by many other bacterial species. They act as inhibitors of microbial growth by disrupting protein synthesis and are effective against most Gram-positive bacteria. However, exposure of commensal staphylococci to these drugs can lead to the development of resistance. Foster noted that this resistance to erythromycin is common among clinical isolates of *S. aureus*, highlighting the need for regular monitoring and assessment of antibiotic susceptibility in clinical settings to ensure the most effective drugs are used. The rate of tetracycline resistance was 68%, which is different from what was found [34] which was (15%). Most bacteria acquire tetracycline resistance as a result of acquiring tetracycline resistance genes *tet. S.aureus* can develop resistance to antibiotics through several mechanisms, including the horizontal transfer of resistance genes as well as through the production of enzymes that inactivate the drug, or through mutations that occur in chromosomal genes that can lead to changes in target proteins or in other pathways that affect the effectiveness of the drug.

The variation in antibiotic resistance rates in *S. aureus* among different studies can be attributed to several factors, including excessive and indiscriminate use of drugs, leading to the development and spread of resistant strains, acquisition of resistant plasmids, health, climatic, and geographical factors, as well as the level of health care and infection control practices that vary between regions. Multidrug Resistance MDR in MRSA as major problem , which emerged due to the misuse antibiotics [35] our study revealed showed all isolates varying levels of multidrug resistance , being resistance to 8 antibiotic or more, which is consistent with previous studies , such as the study, conducted in Baghdad ,which found that all MRSA isolates had multidrug resistance as percentage (100%).Also a recent Nigerian study that included approximately 110 samples of MRSA showed 85% of theme showed multidrug resistance. These result require continuous monitoring of the resistance pattern and development of novel strategies to reduce the spread of multidrug resistance. The response of isolates to antibiotics is related to several factors, included number and source of the isolates which under study, as well as their ability to escape from effects of antibiotics, in addition to the difference in concentration of these antibiotics. In this regard, many studies indicated the source of the isolates is important factors to determinant multidrug resistance, especially in cases of hospital acquired infections. Multidrug resistant also been associated with presence of efflux pumps, which are proteins that allow bacteria to expel a wide range of chemical compounds. Therefore, these pumps contribute to the development of bacterial strains with great capabilities to survive even in toxic environments. Usually in Iraq, most cases of UTI are treated by the patients themselves without consulting a doctor, as well as treatment with broad spectrum antibiotics without microbiological testing. Widespread indiscriminate use and inaccurate prescription of antibiotics used in treatment are major factors contributing to the spread and development of bacterial resistance to commonly used antibiotics. This situation is exacerbated by the spread of counterfeit and poor-quality antibiotics in the medical markets [2,37].

5. Conclusion

This study highlights prevalence of MRSA in clinical specimens of patients with urinary tract infections, revealing a fourfold higher incidence in females compared to males. The study further elucidated that these isolates exhibited multiple resistances to various antibiotics. Moreover, the selective medium HiCrome MeReSa agar was validated as an optimal medium for MRSA isolation, as the diagnostic results obtained using this medium closely corresponded with those from the molecular method employing polymerase chain reaction (PCR).

Acknowledgments

I want to thank the environmental health unit workers in the Baqubah teaching hospital of Diyala governorates, I like for thank Dr. Sinda ZARROUK / University Tunis El Manar, to providing expert technical advice and I am most grateful to all staff at the Pasteur Institute-Tunis for providing advice and analysis of DNA samples.

Nomenclature & Symbols			
MRSA	Methicillin Resistant Antibiotic	S	Sensitive
S. aureus	Staphylococcus aureus	Ak	Amikacin
UTI	Urinary Tract Infection	P	Penicillin
R	Resistance	CF	cefoxitin
RF	Rifampin	PCR	Polymerase Chain Reaction

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