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Journal of Medical and Life Science
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Prevalence of multi-drug resistant *Staphylococcus aureus* and *Escherichia Coli* isolated from urinary tract

Assala Arkan Jawad*[1], Ammar Jawad Kadhim[2], Mohammed Hayder Hashim[3]

[1]Department of Biology, Al-Farabi University College of Baghdad, Iraq.

[2]Ibin Sina University for medical and pharmaceutical sciences, Baghdad, Iraq.

[3] Dijla University College, Baghdad, Iraq.

*assala.jawad1202@sc.uobaghdad.edu.iq, ammarjkadhim@ibnsina.edu.iq, Mohammed.h.hashim@gmail.com

DOI:10.21608/jmals.2024.383094

Abstract

The current research aimed to investigate the prevalence of antibiotic resistance among Staphylococcus aureus and *Escherichia coli*. About 72 samples were suspected to have urinary tract infections and were further identified by using a biochemical and VITIK 2 compact system. The results showed that 21 samples were diagnosed as *Staphylococcus aureus* isolates and 25 samples were diagnosed as *Escherichia coli* isolates. The VITIC 2 compact system was applied to determine the bacterial isolates' sensitivity to antibiotics. The results showed that *E. coli* isolates were 100% resistant to Cephalexin, Amoxicillin, Penicillin, and Amoxicillin-clavulanic acid, while *S. aureus* showed 100% resistance against Cefepime and Cefixime. In another hand, the lower resistance of *E. coli* showed against Clindamycin and Erythromycin was 0.0%, besides the same resistance ratio of *S. aureus* against Imipenem, Nalidixic acid, Trimethoprim / Sulfamethoxazole and Cefotaxime.

Keywords: Infection of the bladder**,** Escherichia coli, Staphylococcus aureus, susceptibility to antibiotics.

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1. Introduction

Among the most prevalent bacterial infections is a urinary tract infection (UTI). 150 million individuals around the world are affected. Simple cystitis to serious infections like pyelonephritis and other consequences are all included in the spectrum of UTI disorders. Due to the female urethra's structural inferiority in preventing bacterial invasion, urinary tract infections (UTIs) are often more common in women than in men (1).

There are two categories of UTIs: simple and complicated. Simple UTIs typically have an impact on individuals in good health who do not have neurological, structural, or physiological abnormalities of the urinary system. Upper UTIs and Lower (urethritis, cystitis, and pyelonephritis, respectively) have been related to infections of this type (2,3). While, individuals who have obstructions and foreign bodies in their urinary system (which impair its ability to function), urine retention brought on by an illness or immune problem, and catheter-associated UTIs (CAUTIs), are at an increased risk of developing complicated UTIs (2).

UTI is a common infectious illness that frequently recurs in all age groups is urinary tract infection (UTI). Bacterial biofilms have a critical role in producing infection in the urinary tract, which results in recurrences and relapses (4).

UTIs account for more than 40% of all infections acquired in hospitals and 50% of the bacterial

Received: July 10, 2024. Accepted: August 29, 2024. Published: September 30, 2024

infections causing mortality and prolonged persist (5)

Several uropathogens, such as *Escherichia coli* (around 85%), *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus saphrophyticus*, *Staphylococcus aureus*, group B *Streptococcus*, *Enterobacter* and *Enterococcus species*, and *Candidaspp*., are responsible for UTIs (6).

More than 95% of urinary tract infections are recognized to be caused by a single species of bacteria. *Escherichia coli* is the most common bacteria responsible for acute illnesses (7). It's gram-negative bacilli and a member of the *Enterobacteriaceae* family, some of its strains can lead to gastrointestinal problems, food poisoning, and urinary tract infections **(8)**. The increased rate of antibiotic resistance may be a significant factor in the development of complicated and recurring UTIs (9).

According to certain Iraqi investigations, the most common pathogenic microorganisms that cause UTI were *Staphylococcus spp* (10), coccoid bacteria belonging to the class Firmicutes, and Gram-positive, non-motile, and coagulase-positive (11,12). *S. aureus* is one of the most important opportunistic bacterial infections of humans, It frequently invades human populations and is a major worldwide cause of disease and mortality (13). Diseases caused by *S. aureus* range widely, from superficial skin infections to sepsis. and deadly pneumonia, resistance to antibiotics makes treating *S. aureus* infections more difficult (14). Nonspecific antibiotic resistance resulting from the formation of biofilms is a common feature of many biofilm-related *S. aureus* infections (15).

Despite the high pathogenicity of uropathogenic *Escherichia coli* strains, the host activates various immune systems, such as innate and adaptive immunity., using antibiotics such -lactams, trimethoprim, nitrofurantoin, and quinolones as part of routine UTI treatment is common in many countries to eradicate them from the urinary tract (16). *E. coli*, a mostly facultative anaerobic Gram-negative bacterium, colonizes the digestive tract of infants and aids in the maintenance of normal intestinal homeostasis (17).

Many bacteria, particularly antibiotic-resistant pathogens, and biofilm producers are capable of penetrating urinary tract tissues, colonizing, and establishing potentially fatal infections (18). Antibiotic use as a cure for urinary tract infections poses a major threat to public health. There are many counterfeit and spurious pharmaceuticals of questionable quality in circulation, especially in developing countries where there is a high level of poverty, illiteracy, and poor hygienic standards. Because the medications are so inexpensive and widely accessible without a prescription, abuse is a possibility (19).

Currently, Cephalosporins of the second or third generation from the -lactam antibiotic class, including ciprofloxacin, ampicillin, trimethoprim (TMP), sulfamethoxazole (SMX), cotrinoxazole (TMP/SMX), and ampicillin, are the most frequently advised and utilized during UTI therapy against UPEC isolates (15,20).

B-lactam inhibitors are the safest and probably most often given medicines for UTIs. B-lactam inhibitors are broad-spectrum antibiotics that have a beta-lactam ring in their structure. Numerous studies show that beta-lactamase medicines are less effective against *E. coli* in UTIs (21).

Materials and Methods

Samples collection

From November 2022 to March 2023, urine samples from women and men of various ages who had UTI symptoms were collected from Iraqi Hospitals in Baghdad. Afterward, they were cultured onto Nutrient, MacConkey, mannitol salt agar, and blood agar and incubated there overnight at 37 °C. About 72 samples were identified as having UTIs. Identification was accomplished by following the protocols of established biochemical tests.

Identification of bacterial isolates and Antibiotic susceptibility test (AST) using VITEK2 compact system

Using the VITEK2 compact system, bacterial isolates were identified following the company's instructions.

Many bacterial colonies were inoculated into glass tubes to set the turbidity at 0.5 (McFarland standard). The sample was loaded into the VITEK 2 compact system equipment, which used negative pressure to transfer the bacterial suspension to a cassette. The cassette was then incubated for 12 hours to finish the AST and biochemical reaction. The data were interpreted using the custom software for the VITEK 2 compact system to identify the different species and strains of bacteria as well as to determine the susceptibility results.

Results

Identification of bacteria

All samples supposed to have UTI were cultured on nutrient agar, MacConkey agar, mannitol salt agar, and blood agar to identify their physiological, morphological, and biochemical characteristics. The results showed that 21 samples were diagnosed as *Staphylococcus aureus* isolates and 25 samples were diagnosed as *Escherichia coli* isolates. These characters are shown in Table (1) and Figures (1, 2) below:

Table (1): The characters of *E. coli* and *S. aureus*

Characters	***Escherichia coli***	***Staphylococcus aureus***
Gram stain	G-ve	G+ve
Morphology	Bacilli	Cocci
Motility	Motile	Non motile
TSI	A\A +, -	A\A +, -
MacConkey	Lactose fermenter (pink colonies)	No growth
Mannitol salt agar	No growth	Yellow colonies (mannitol fermenter)
Blood agar	Hemolytic	Hemolytic

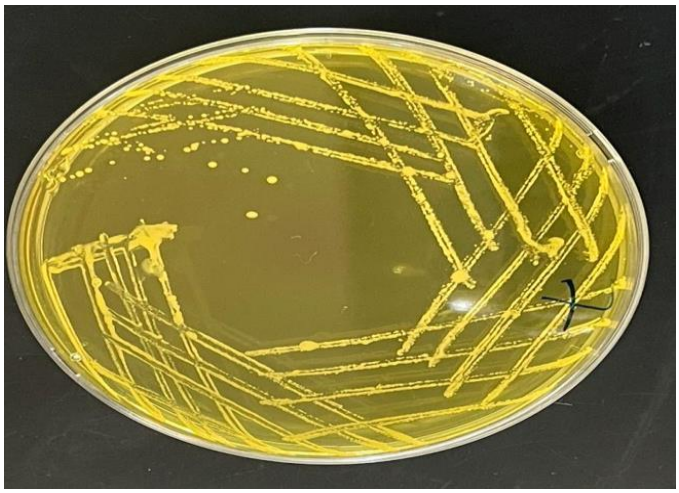

Figure (1): *S.aureus* on mannitol salt agar

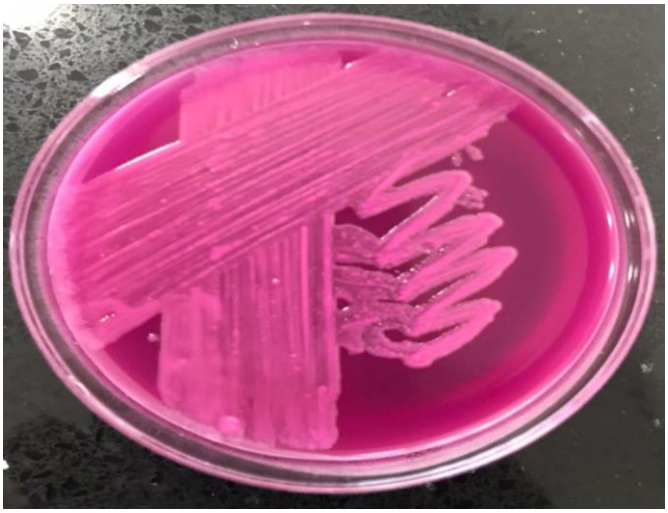

Figure (2): *E.coli* on macConkey agar

Identification by VITIK2 compact system

All bacterial isolates that were supposed to be *Escherichiacoli* or *Staphylococcus aureus* are further identified using the VITEK2 compact system to confirm the results. The results showed that twenty-five (25) isolates from the samples had *E.coli* and twenty-one (21) isolates from samples had *S.aureus* as shown in tables (2,3).

Antibiotic susceptibility test using VITIK 2 compact system

After the identification of bacterial isolates, the antibiotic sensitivity test was finished. by using the VITIK 2 compact system. The results are shown in Figures (3, 4) and Tables (4, 5).

From Figure 3, the higher resistance of *E. coli* isolates was against Amoxicillin, Cephalexin, Penicillin, and Amoxicillin-clavulanic acid which were 100%.

While the lowest bacterial resistance of *S.aureus* was against Clindamycin and Erythromycin which is 0.0 %.

Also, the *E. coli* resistance was different as shown in Figure (3), the resistance percentage of bacterial isolates were 73.7, 71.9, 70.3, 70, 67.9, 67.6, 66.7, 64.3, and 60 against Cefotaxime, Tetracycline, Doxycycline, Nalidixic acid, Cefixime, Azithromycin, Trimethoprim / Sulfamethoxazole, Ceftazidime, and Cefepime respectively. Additionally, the resistance ratio of *E. coli* to Ceftriaxone, Ciprofloxacin, Levofloxacin, Gentamycin, Cefoxitin, and Aztreonam were 51.3, 48, 45.5, 38.9, 38.2 and 38.1 respectively. Finally, the bacterial resistance was 22.7, 14.3, 12.5, and 9.8 to Imipenem, Chloramphenicol, Amikacin, and NIT respectively. From Figure 4, we find that *S.aureus* isolates have a higher resistance percentage against Cefepime and Cefixime which were 100%. The lowest bacterial resistance was against Imipenem, Nalidixic acid, Trimethoprim / Sulfamethoxazole, and Cefotaxime which is 0.0 %. Furthermore, the resistance of *S.aureus* to Penicillin, Erythromycin, Ceftriaxone, and Azithromycin were 86.7, 83.3, 75, and 70.6 respectively. Moreover, *S.aureus* showed a 54.7, 54.2, 50, 40.7 37.3, and 33.3 resistance percentage against Clindamycin, Gentamicin, Aztreonam, Ciprofloxacin, Levofloxacin, and Cefoxitin. Finally, the resistance percentages against Chloramphenicol, Tetracycline, Doxycycline, Vancomycin, and Nitrofurantoin were 26.9, 23.9, 20.3, 15.1, and 9.8 respectively.

Table (2): Results from the VITIK 2 compact system that displayed isolates of *E. coli*

bioMérieux Customer: **Microbiology Chart Report** Printed February 1, 2022 6:54:10 AM GMT-06:00

Patient Name: bahega, bahega
Location: urine
Lab ID: 86

Patient ID: 86
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli
Source:

Collected:

Comments:	

Identification Information	Analysis Time:	4.87 hours	Status:	Final
Selected Organism	97% Probability Bionumber:	Escherichia coli 0405611570526210		
ID Analysis Messages				

Table (3): Results from the VITIK 2 compact system that displayed isolates of *S.aureus*

bioMérieux Customer:
System #:
Patient Name:

Laboratory Report

Printed by: Labadmin
Patient ID:

Isolate: 14-1 (Qualified)
Card Type: GP Bar Code: 2421825103387751 Testing Instrument: 0000148FEFA8 (AL.MAHMUDIA HOSP.)
Card Type: AST-P592 Bar Code: 3721767203443796 Testing Instrument: 0000148FEFA8 (AL.MAHMUDIA HOSP.)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 050612163361231
Organism Quantity:

Selected Organism: Staphylococcus aureus

Comments:	

Identification Information	Card: GP	Lot Number: 2421825103	Expires: Nov 25, 2022 12:00 GMT-06:00
	Status: Final	Analysis Time: 7.77 hours	Completed: Sep 25, 2022 18:07 GMT-06:00
Organism Origin	VITEK 2		
Selected Organism	86% Probability Bionumber: 050612163361231	Staphylococcus aureus Confidence: Acceptable identification	
Analysis Organisms and Tests to Separate:			
Analysis Messages: The following antibiotic(s) are not claimed: Ampicillin, Gentamicin High Level (synergy), Streptomycin High Level (synergy), A positive ICR test is indicative of inducible resistance to macrolides, lincosamides, and type B streptogramin. This isolate is presumed to be resistant to clindamycin; however, clindamycin may still be effective in some patients.			
Contraindicating Typical Biopattern(s) Staphylococcus aureus AMAN(1),AlaA(1),MBdG(94),			

Susceptibility Information	Card: AST-P592	Lot Number: 3721767203	Expires: Sep 28, 2022 12:00 GMT-06:00
	Status: Final	Analysis Time: 12.17 hours	Completed: Sep 25, 2022 22:30 GMT-06:00

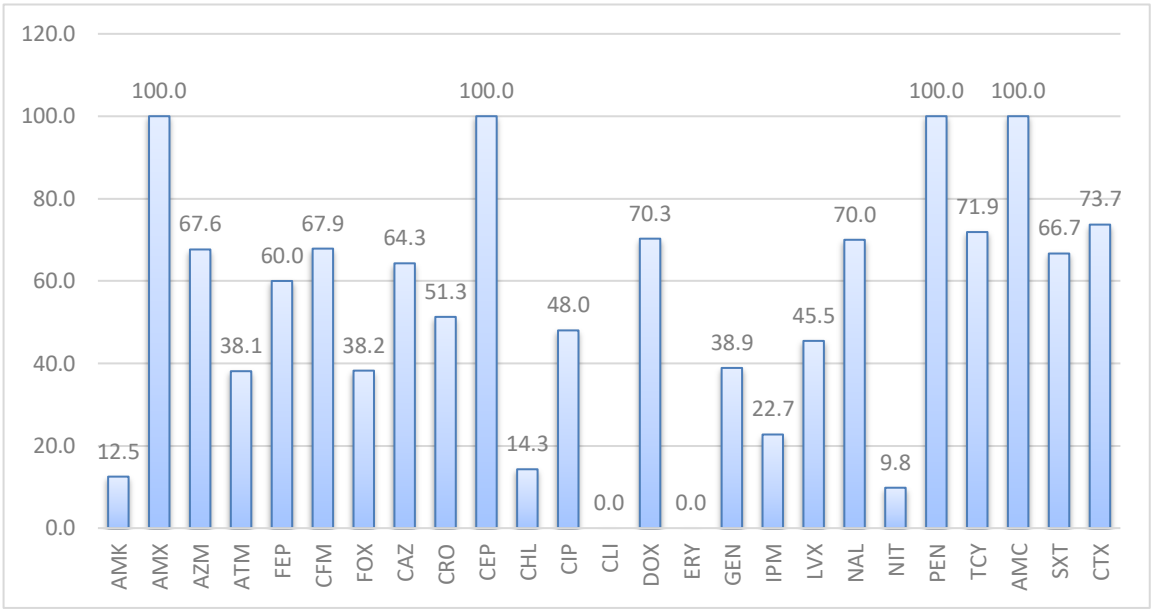

Figure (3): Antibiotic susceptibility test of *E.coli* that shows bacterial resistance to different antibiotics

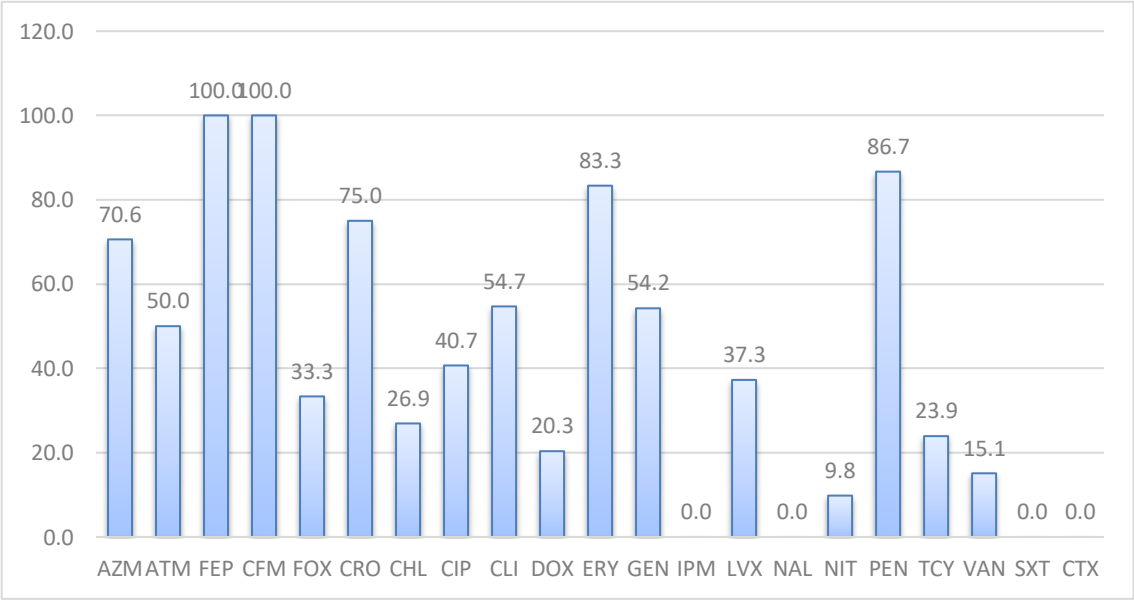

Figure (4): Antibiotic susceptibility test of *S. aureus* that shows bacterial resistance to different antibiotics

Table (4): The VITIK 2 compact system results that showed antibiotic susceptibility test of *E. coli* isolates

bioMérieux Customer: Microbiology Chart Report Printed February 1, 2022 6:54:10 AM GMT-06:00

Patient Name: bahega, bahega
Location: urine
Lab ID: 86
Patient ID: 86
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli
Source: Collected:

Comments:	

Identification Information	Analysis Time:	4.87 hours	Status:	Final
Selected Organism	97% Probability	Escherichia coli		
	Bionumber:	0405611570526210		
ID Analysis Messages				

Susceptibility Information	Analysis Time: 9.98 hours	Status: Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ticarcillin	>= 128	R	Amikacin	4	S
Ticarcillin/Clavulanic Acid	16	*R	Gentamicin	>= 16	R
Piperacillin	>= 128	R	Tobramycin	>= 16	R
Piperacillin/Tazobactam	8	*R	Ciprofloxacin	>= 4	R
Ceftazidime	4	S	Pefloxacin		
Cefepime	2	S	Minocycline	<= 1	S
Aztreonam	16	R	Colistin		
Imipenem	<= 0.25	S	Rifampicin		
Meropenem	<= 0.25	S	Trimethoprim/ Sulfamethoxazole	<= 20	S

*= AES modified **= User modified

AES Findings	
Confidence:	Consistent

Table (5): The VITIK 2 compact system results that showed antibiotic susceptibility test of *S. aureus* isolates

bioMérieux Customer:
System #:
Patient Name:
Isolate: 14-1 (Qualified)
Card Type: GP Bar Code: 2421825103387751 Testing Instrument: 0000148FEFA8 (AL.MAHMUDIA HOSP.)
Card Type: AST-P592 Bar Code: 3721767203443796 Testing Instrument: 0000148FEFA8 (AL.MAHMUDIA HOSP.)
Setup Technologist: Laboratory Administrator(Labadmin)

Laboratory Report

Printed by: Labadmin
Patient ID:

Bionumber: 050612163361231
Organism Quantity:

Selected Organism: Staphylococcus aureus

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	POS	+	Erythromycin	>= 8	R
Benzylpenicillin	>= 0.5	R	Clindamycin	<= 0.25	*R
Ampicillin			Linezolid	2	S
Oxacillin	>= 4	R	Teicoplanin	<= 0.5	S
Imipenem			Vancomycin	1	S
Gentamicin High Level (synergy)			Tetracycline	<= 1	S
Streptomycin High Level (synergy)			Tigecycline	<= 0.12	S
Gentamicin	<= 0.5	S	Fosfomycin		
Ciprofloxacin	<= 0.5	S	Fusidic Acid	1	S
Moxifloxacin	<= 0.25	S	Rifampicin	1	*R
Inducible Clindamycin Resistance	POS	+	Trimethoprim/ Sulfamethoxazole	20	S

*= AES modified **= User modified

AES Findings:	Last Modified: Oct 24, 2021 11:13 CDT	Parameter Set: Global CLSI-based +Phenotypic 2019
Confidence Level:	Consistent	
Phenotypes flagged for review:	BETA-LACTAMS	MODIFICATION OF PBP (mecA)
	MACROLIDES/LINCOSAMIDES/ STREPTOGRAMINS	MLSB INDUCIBLE

Discussion

The results of higher resistance of *E. coli* isolates against Amoxicillin, Cephalexin, Penicillin, and Amoxicillin-clavulanic acid were nearly similar to the results of Hashim and AlKhafaji (22) they found that *E. coli* has high resistance to these antibiotics, while lowest bacterial resistance of *S.aureus* was against Clindamycin and Erythromycin was closely the same of the results reported by of Hashim and AlKhafaji (23), Zhang (24). In another hand, the findings for Clindamycin and Erythromycin resistance were 41 (39.8%) and 62 (60.1%), respectively (25).

In another study, *S. aureus* showed resistance of about (33%) against Trimethoprim/Sulfamethoxazole (TS) prescribed antibiotics for UTI (7). While in Sain, Ziaullah Mirza (26) showed that Teicoplanin, vancomycin, and linezolid all showed 100% sensitivity in *S. aureus*, while amikacin and clindamycin showed 80% sensitivity, and fluoroquinolones showed moderate sensitivity (30–40%). It was demonstrated that *S. aureus* isolates showed high resistance to Cefotaxim, Amikacin, Augmentin, and Nitrofurantoin (12.0%, 6.3%, 5.1%, 1.4%) respectively (2).

E. coli resistance showed differences among different antibiotics as shown previously, while *S.aureus* isolates showed higher resistance percentages against Cefepime and Cefixime which were 100%, so these results agreed with the results produced by Alorabi (27). Also from another study, a significant percentage of *S. aureus* strains (79.3%) showed resistance to cefoxitin (28). On another hand, a few isolates of MSSA showed cefepime (0.3%) and ceftriaxone (2.3%), while the lowest bacterial resistance was against Imipenem, Nalidixic acid, Trimethoprim / Sulfamethoxazole and Cefotaxime which is 0.0 %. *S. aureus* was shown to be highly sensitive to imipenem (98%), nitrofurantoin (97.6%), and vancomycin (95.1%). However, high resistance was observed to tetracycline (33.2%), trimethoprim/sulfamethoxazole (56.9%), and penicillin G (91.9%) (29).

Also, as shown in the results of antibiotic resistance in this study, a report from the research revealed that *S. aureus* isolates have resistance to a wide range of antibiotics, such as ceftazidime (96.7%), gentamicin (64.8%), cefoxitin (94.5%), cefepime (60.4%), cefotaxime (64.8%), ciprofloxacin (46.4%), and levofloxacin (37.4%), clarithromycin (56%), azithromycin, erythromycin, and clindamycin. (38.5%) (27). Where have been observed MRSA strains exhibited significant resistance to doxycycline (68.0%) (30,31). The results of this study also agree with (32,33).

Ethical approval

The project received approval from the local ethical commission at Ibn Sina University, Baghdad, Iraq.

Declaration of Conflicting Interests:

the authors declare that they have no possible conflicts of interest

Funding:

no funding

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