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Isolation and Diagnosis of Resistant Bacteria from Pregnant Women with Urinary Tract Infections in Karbala

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ABSTRACT

Background & Objective: Increasing urinary tract infections (UTI) in pregnant women was a reason for which this study aimed to find out the spread of bacteria in them and also study the resistance of bacteria to antibiotics and the type of resistance.

Materials & Methods: During November 2022, 50 urine samples were collected from pregnant women with symptoms of UTI. Direct microscopy tests were conducted on the samples. The samples were cultured on the media of MacConkey agar (MAC) and blood agar. Biochemical tests were performed and diagnosed using the VITEC-2 system. Antibiotic susceptibility screening test was done for all isolates.

Results: Of the 50 bacterial isolates diagnosed and isolated from pregnant women with UTI, 84% were gram-negative and 16% were gram-positive. The most prevalent bacteria were *E. coli*, with a rate of 60%, followed by *Proteus mirabilis*, with a rate of 12%. All *E. coli* isolates were resistant (100%) to the AMOX antibiotic, and the isolates showed high resistance (87%) to CFR, CN, CZ, CXM, CAE, CPD, CRO NA and SXT antibiotics. 13.3% of *E.coli* isolates were extended detection and response (XDR), 50% and 25% of *Staphylococcus hominis* and *aureus* isolates were XRD, respectively.

Conclusion: *E. coli* is the most common and most resistant bacteria of type XRD, and gram-positive bacteria, staph bacteria, showed resistance to type XRD. In addition, gram-negative bacteria showed high resistance to many antibiotics, including AMOX, CFR, CN, CZ, CXM, and CAE. Gram-positive bacteria showed complete resistance against BENPEN, OXA, CLIN, TEC, VAN, TET, FUS and VAN.

Keywords: Resistance Bacteria, Urinary Tract Infections, Pregnant Women, Extensively Drug-Resistanct (XDR), Multiple Drug Resistance (MDR)

Introduction

In hospitals as well as the general population, urinary tract infections are among the most prevalent bacterial infections, coming in second only to respiratory infections **(1)**. Around 150 million people worldwide are thought to have urinary tract infections (UTIs) **(2)**. Women are more prone to UTI than men are, and this is partly because of a shorter urethra, the lack of prostatic secretion, pregnancy, and the ease with which fecal microbes can enter the urinary tract **(3)**. Around 50% of women will get at least one urinary tract infection (UTI), including while pregnant. Pregnant women can develop UTIs from a variety of pathogenic microbes, including bacteria, fungus, protozoa, and viruses. *E. coli* and other Enterobacteriaceae make up around 75% of the isolates and are the most common bacterial infections **(4)**.

The use of antibiotics as a treatment for UTIs is among the most widespread around the globe, and its significance for world health cannot be overstated. Notwithstanding how important they are, the emergence of resistance threatens the long-term efficacy of antibiotics. The primary cause of antibiotic resistance has been the excessive and unneeded use of antibiotics **(5)**. Antimicrobial resistance (AMR), which is thought to be responsible for more than 700,000 deaths annually worldwide, is a problem that is becoming more and more of a worry on a global scale **(6)**. A general term used to describe resistance to all antimicrobial substances is "antimicrobial resistance." Due to antimicrobial resistance, traditional treatments are less effective and take longer to complete, which increases the risk of diseases spreading **(7)**. The public's misuse of antibiotics is one of the major causes of antibiotic resistance **(8)**, and some research has revealed that some pregnant women are unaware of how to treat common infections, which contributes to AMR **(9)**.

Methods

In November 2022, certain private laboratories in the province of Karbala collected samples. 50 pregnant individuals with urinary tract infections provided urine samples. Following general urine examination (G.U.E.) and urine cultivation for bacterial isolation, patients were deemed to have a positive UTI.

Patients with symptomatic UTIs had approximately 10 ml of clean-catch mid-stream or transurethral catheterization urine specimens taken in sterile containers. Each urine sample was divided into two portions in the medical laboratory: the first portion was centrifuged and stored at -80 ºC, and the second portion was directly inoculated on standard culture media (MacConkey and Blood agar) and incubated aerobically at 37 ºC for 24–48 hours using conventional methods. To prepare urine fragments for direct microscopic analysis, the remaining pee was centrifuged (1500 rpm for 5 minutes).

Before using a completely automated VITEK-2 compact system to identify the bacterial species, manual biochemical tests for bacterial isolates were performed to determine the species. Bacterial identification was done using cards that were gram-positive and gram-negative. If the test organisms were isolated as pure isolates from MacConkey or Blood agar, 3 ml of 0.45% sterile saline was added to the polystyrene tube, and the organism was then homogeneously suspended in the saline. Gram-negative and gram-positive bacteria had densities of 0.5 to 0.63 in the bacterial solution. A microorganism suspension is inoculated on the identification cards. The identification card is inserted into the adjacent slot while the transfer tube is inserted into the appropriate suspension tube in a special rack (Cassette) holding a test tube containing a suspension of microorganisms.

Results

Of the 50 bacterial isolates obtained from pregnant women with UTIs, 42 were gram-negative (84%) and eight were gram-positive (16%). The results of the laboratory diagnosis using the VITEC-2 system, which are shown in Figure 1, showed the appearance of five bacterial species and six species belonging to these species. The results also show that the number of bacterial isolates isolated from pregnant women suffering from urinary tract infection was 50 bacterial isolates, distributed according to bacterial species, where *E. coli* was diagnosed in 60% of the diagnosed isolates, followed by *Proteus mirabilis* by 12%, while the percentage of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus hominis* bacteria was 8% each, and *Klebsiella pneumonia* bacteria were the least visible bacterial species with a rate of 4%.

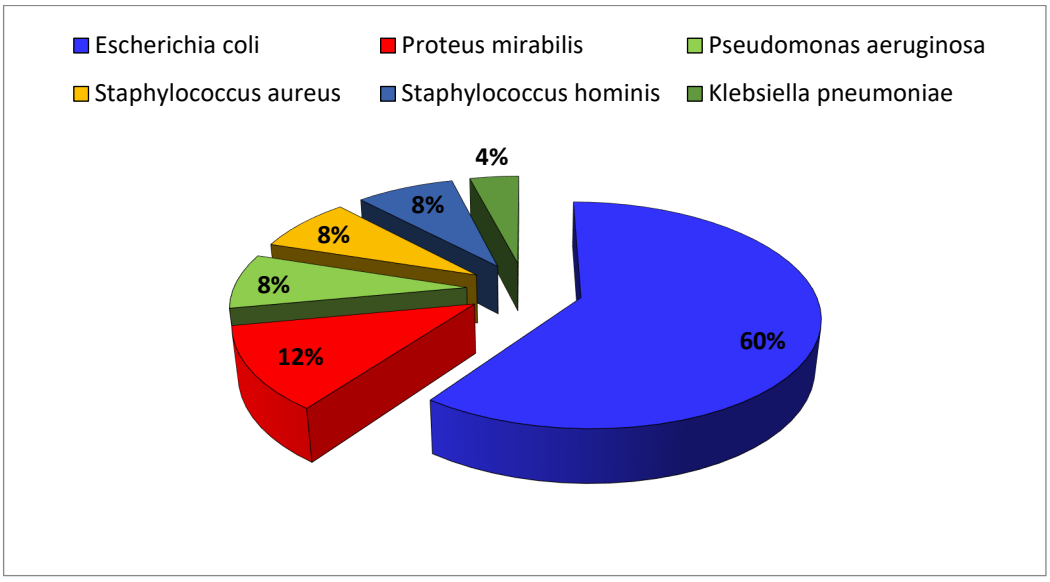


Figure 1. Isolation rate of UTI bacterial species in pregnant women.

Table 1 shows antibiotics and their abbreviations in the international system of abbreviations that were used in this study to determine bacterial resistance to them.

Table 1. Abbreviations for antibiotics used in the susceptibility test of the Vitek 2 system.

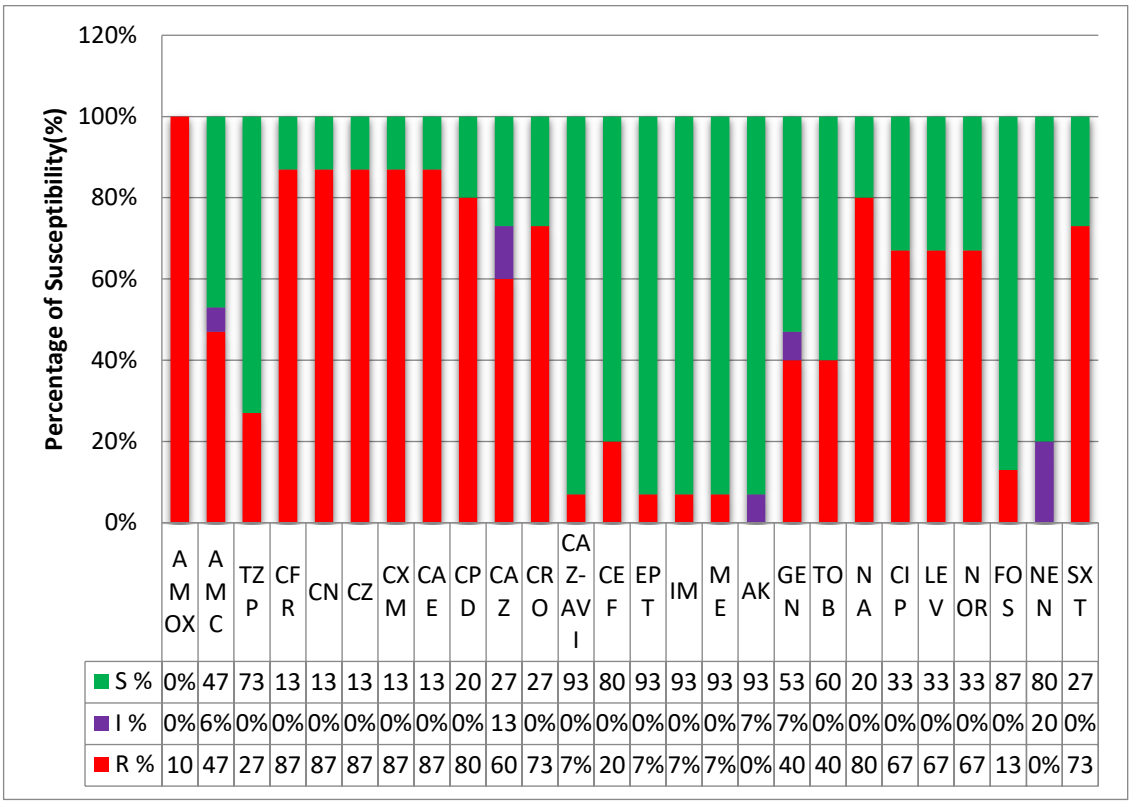
Abbreviation	Antibiotic	Abbreviation	Antibiotic
AK	Amikacin	**ERY**	Erythromycin
AMC	Amoxicillin/Clavulanic Acid	**ETP**	Ertapenem
AMOX	Amoxicillin	**FAM**	Ampicillin/Sulbactam
AZT	Aztreonam	**FOS**	Fosfomycin
BENPEN	Benzylpenicillin	**FUS**	Fusidic Acid
CAE	Cefuroxime Axetil	**GEN**	Gentamicin
CAZ	Ceftazidime	**IM**	Imipenem
CAZ-AVI	Ceftazidime/Avibactam	**LEV**	Levofloxacin
CEF	Cefepime	**ME**	Meropenem
CFM	Cefxime	**MOX**	Moxifloxacin
CFR	Cefadroxil	**NA**	Nalidixic Acid
CIP	Ciprofloxacin	**NEN**	Nitrofurantoin
CLIN	Clindamycin	**NIT**	Nitrofurantoin
CMZ	Cefmetazole	**NOR**	Norfloxacin
CN	Cefalixin	**OXA**	Oxacillin
CPD	Cefpodoxime	**RIM**	Rifampicin
CRO	Ceftriaxone	**SXT**	Trimethoprim/Sulfamethoxazole
CTX	Cefataxime	**TCC**	Ticarcillin/Clavulanic Acid
CX	Cefoxitin	**TEC**	Teicoplanin
CXM	Cefuroxime	**TET**	Tetracycline
CZ	Cefazolin	**TIG**	Tigecycline
CZX	Ceftizoxime	**TOB**	Tobramycin
ENR	Eurofloxacin	**TZP**	Piperacillin/Tazobactam
ERT	Ertapenem	**VAN**	Vancomycin

When examining the sensitivity of 30 *E. coli* bacterial isolates towards antibiotics, the results are shown in Figure 2. All isolates were resistant (100%) to the AMOX antibiotic, and the isolates showed high resistance (87%) to CFR, CN, CZ, CXM, CAE, CPD, CRO NA and SXT antibiotics, while the isolates were highly sensitive (93 %) to CAZ-AVI, EPT, IM, ME, AK and FOS antibiotics.

The six *Proteus merabilis* isolates showed total resistance (100 %) against NEF, while they were 100% sensitive to the following antibiotics: TCC, TZP, CFR, CAZ-AVI, AZT, ERT, EPT, ME, AK and FOS (Figure 3).

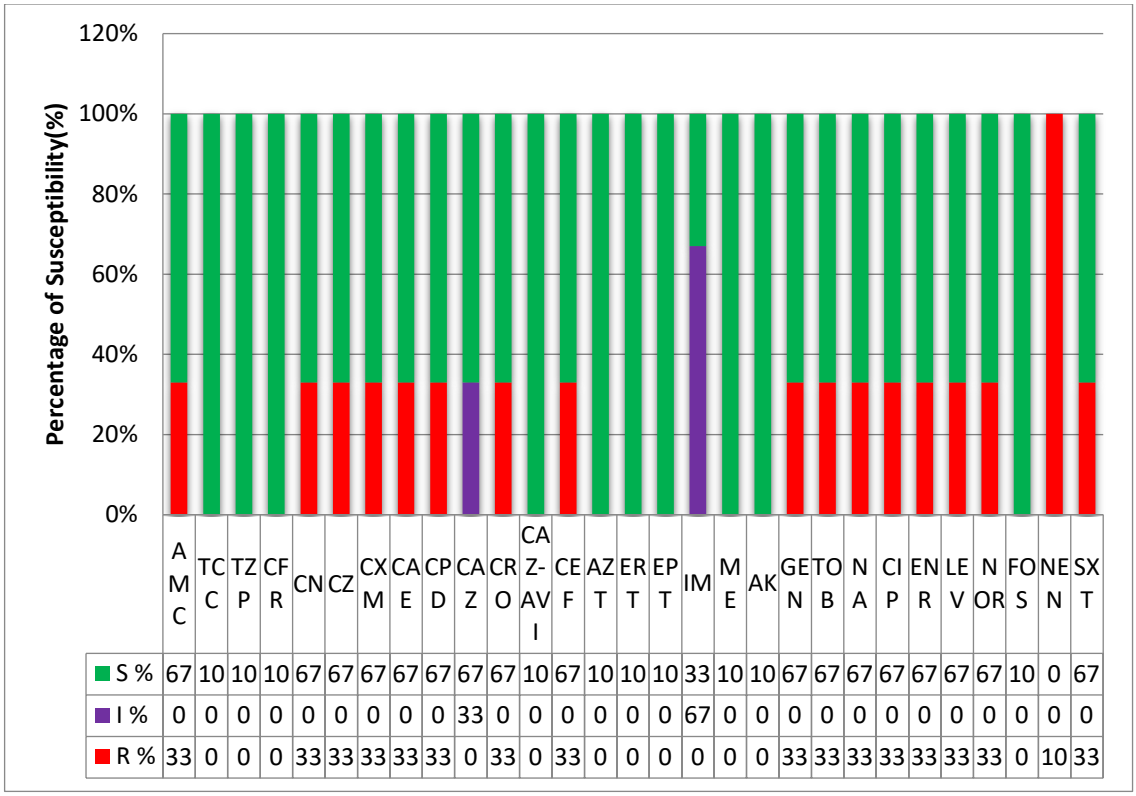
As the results show in Figure 4, the four isolates of *Pseudomonas aerouginosa* bacteria were completely resistant (100 %) to each of the following antibiotics: AMOX, FAM, CFR, CN CZ, CMZ, CX CFM, CPD, CTZ, CZX and NA.. while it was completely sensitive (100%) to the following antigens: TZP, CAZ, CAZ-AVI, CEF, IM, ME, AK, GEN, TOB, CIP, LEV and NOR.

In addition, the results in Figure 5 indicate that the two isolates of *Klebsiella pneumoniae* bacteria were completely sensitive (100 %) to all antibiotics used except for their total resistance (100 5) to the antibiotic AMOX and their moderate resistance (100 %) to the antibiotic NEN.



	AMOX	AMC	TZP	CFR	CN	CZ	CXM	CAE	CPD	CAZ	CRO	CAZ-AVI	CEF	EPT	IM	ME	AK	GEN	TOB	NA	CIP	LEV	NOR	FOS	NEN	SXT
S %	0%	47	73	13	13	13	13	13	20	27	27	93	80	93	93	93	93	53	60	20	33	33	33	87	80	27
I %	0%	6%	0%	0%	0%	0%	0%	0%	0%	13	0%	0%	0%	0%	0%	0%	7%	7%	0%	0%	0%	0%	0%	0%	20	0%
R %	10	47	27	87	87	87	87	87	80	60	73	7%	20	7%	7%	7%	0%	40	40	80	67	67	67	13	0%	73

Figure 2. **Antibiotic Susceptibility profile for 30 isolates of *Escherichia coli* by Vitek 2 system.**



	AMC	TCC	TZP	CFR	CN	CZ	CXM	CAE	CPD	CAZ	CRO	CAZ-AVI	CEF	AZT	ERT	EPT	IM	ME	AK	GEN	TOB	NA	CIP	ENR	LEV	NOR	FOS	NEN	SXT
S %	67	10	10	10	67	67	67	67	67	67	67	10	67	10	10	10	33	10	10	67	67	67	67	67	67	67	10	0	67
I %	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	67	0	0	0	0	0	0	0	0	0	0	0	0
R %	33	0	0	0	33	33	33	33	33	0	33	0	33	0	0	0	0	0	0	33	33	33	33	33	33	33	0	10	33

Figure 3. **Antibiotic Susceptibility profile for 6 isolates of *Proteus merabilis* by Vitek 2 system.**

Figure 4. **Antibiotic Susceptibility profile for 4 isolates of *Pseudomonas aerouginosa* by Vitek 2 system.**

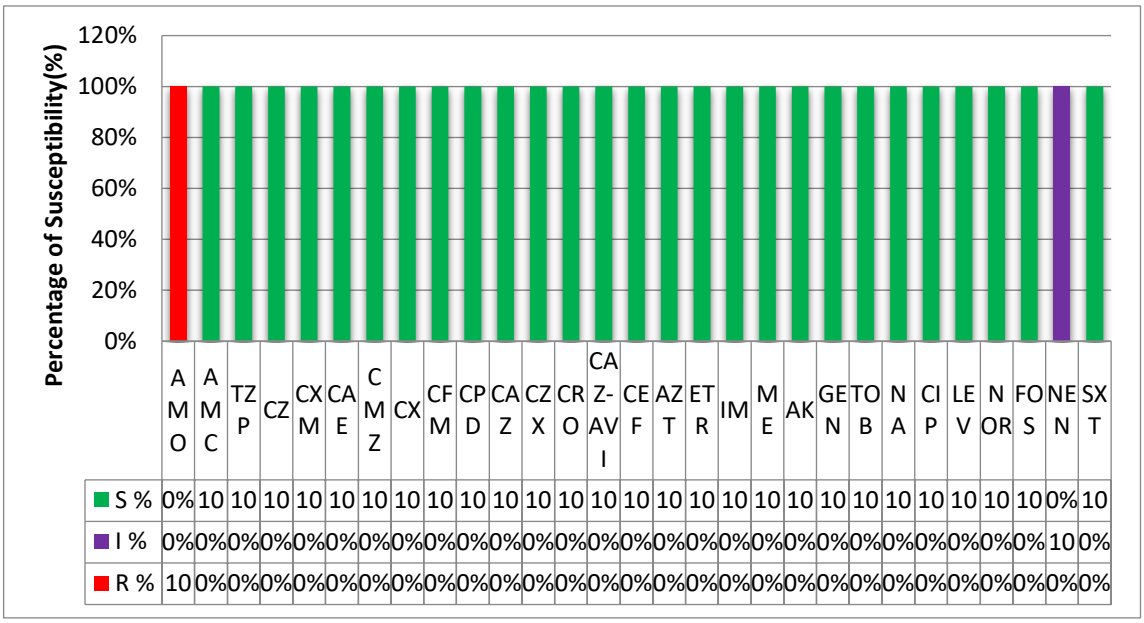
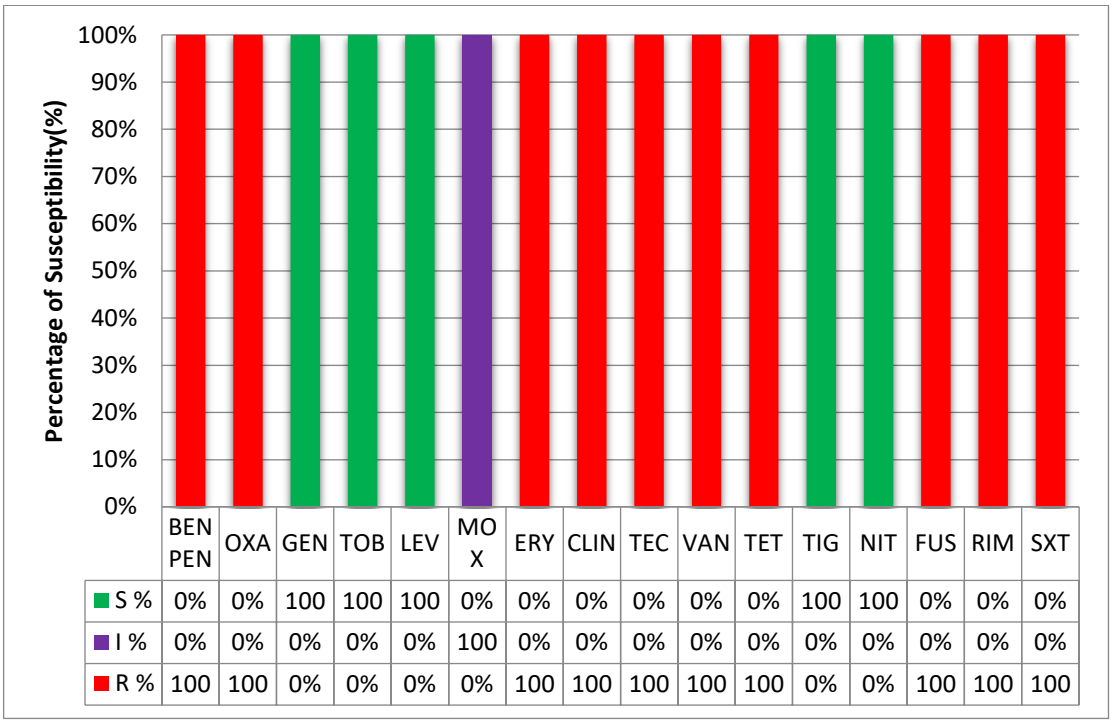


Figure 5. **Antibiotic Susceptibility profile for 2 isolates of *Klebsilla pneumoniae* by Vitek 2 system.**

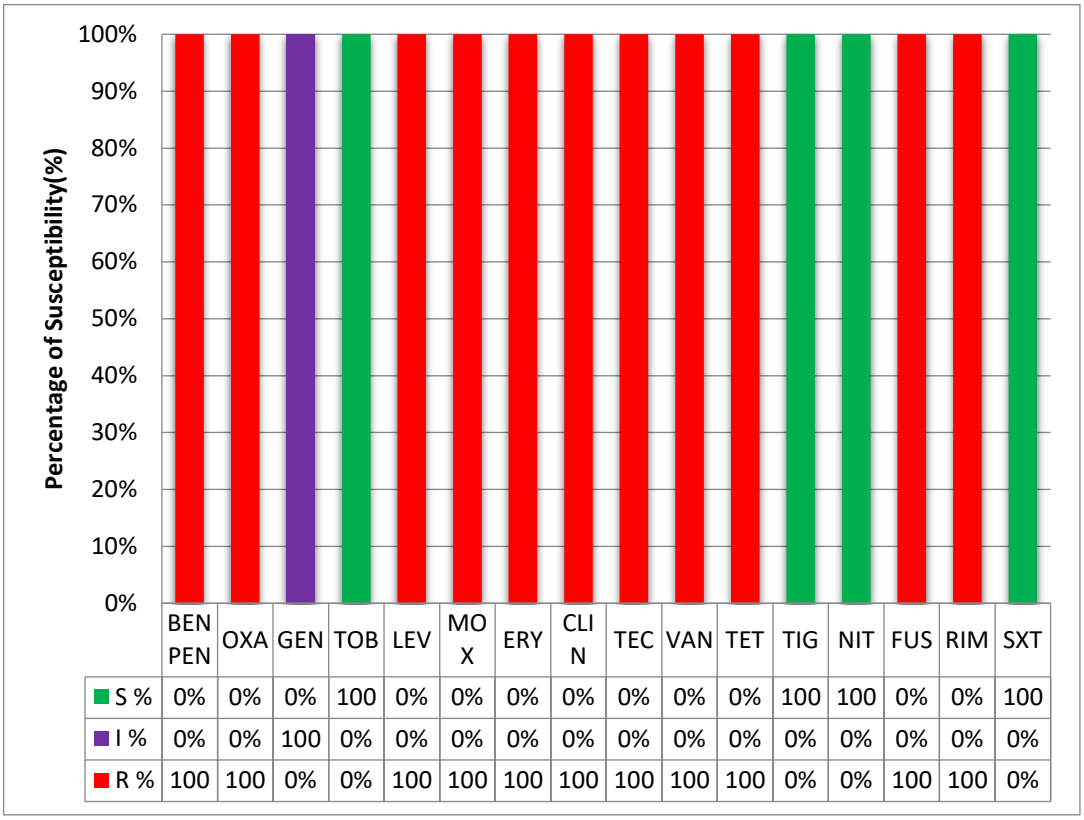
To study the sensitivity of positive bacteria isolated from pregnant women with urinary tract infections, the sensitivity of *Staphylococcus aureus* and *Staphylococcus hominis* bacteria was tested against 16 antibiotics, and the results are shown in Figures 6 and 7. The results of Figure 6 indicated that *Staphylococcus aureus* was resistant (100%) to BENPEN, OXA, ERY, CLIN, TEC, VAN, TET, FUS, RIM and SXT antibiotics, while it was fully sensitive (100%) to GEN, TOB, LEV, TIG and NIT antibiotics, it was only moderately sensitive to MOX.

The results of Figure 7 indicate that *Staphylococcus hominis* was completely resistant (100%) to BENPEN, OXA, LEV, MOX, ERY, CLIN, TEC, VAN, TET, FUS and RIM antibiotics, while were fully sensitive (100%) to TOB, TIG, NIT and SXT, and moderately resistant to GEN only.



	BEN PEN	OXA	GEN	TOB	LEV	MO X	ERY	CLIN	TEC	VAN	TET	TIG	NIT	FUS	RIM	SXT
S %	0%	0%	100	100	100	0%	0%	0%	0%	0%	0%	100	100	0%	0%	0%
I %	0%	0%	0%	0%	0%	100	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
R %	100	100	0%	0%	0%	0%	100	100	100	100	100	0%	0%	100	100	100

Figure 6. **Antibiotic Susceptibility profile for 4 isolates of *Staphylococcus aureus by* Vitek 2 system.**



	BEN PEN	OXA	GEN	TOB	LEV	MO X	ERY	CLI N	TEC	VAN	TET	TIG	NIT	FUS	RIM	SXT
S %	0%	0%	0%	100	0%	0%	0%	0%	0%	0%	0%	100	100	0%	0%	100
I %	0%	0%	100	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
R %	100	100	0%	0%	100	100	100	100	100	100	100	0%	0%	100	100	0%

Figure 7. **Antibiotic Susceptibility profile for 4 isolates of *Staphylococcus hominis by* Vitek 2 system.**

Of the 50 bacterial isolates, 42 of which were gram-negative and 8 gram-positive, 14% of all isolates showed the type of resistance XRD, while 70% of them were multiresistant type MRD, while 16% of the isolates were sensitive to antibiotics. It should be noted, and according to the results shown in Table 2,

the bacterial isolates of which the resistance to XRD was 4 belong to *E. coli* (which constitutes 13.3% of its isolates) and 3 isolates are positive for Gram stain, two of which are *S. hominis* (which constitutes 50% of its isolates) and one isolate *S. aureus* (which constitutes 25% of its isolates). All *Pseudomonas aeruginosa* isolates were MDR (100%).

Table 2. Antibiotic resistance patterns of bacterial isolates

Bacterial type	XDR N(%)	MDR N(%)	Sensitive N(%)	Total N
Escherichia coli	4 (13.3%)	22 (73.4%)	4 (13.3%)	30
Proteus mirabilis	0 (0%)	4 (66.7%)	2 (33.3%)	6
Pseudomonas aeruginosa	0 (0%)	4 (100%)	0 (0%)	4
Staphylococcus aureus	1 (25 %)	3 (75 %)	0 (0%)	4
Staphylococcus hominis	2 (50%)	2 (50%)	0 (0%)	4
Klebsiella pneumoniae	0 (0%)	0 (0%)	2 (100%)	2
Total N (%)	7 (14 %)	35 (70 %)	8 (16 %)	50

Discussion

In contrast to the findings of the current study, the outcomes differed among studies and nations. Staph. Bacteria were the most common among the other species in the Cameroon study by Ndamasson et al., they found that the rate of UTI in pregnant women was 45 % **(10)**. The findings of a local study on pregnant women with urinary tract infections conducted in Baghdad, Iraq, did not match those of the current study, with the findings of that study being as follows: The most common bacteria were *Escherichia coli* (34%) and *Staphylococcus aureus* (22.2%), *Klebsiella* spp. (14.6%), non-coagulase *Staphylococcus* (11.5%), *Proteus* spp. (4.5%), *Pseudomonas* spp. (3.7%), *Acinetobacter* spp. (2.8%), *Citrobacter* (2.8%), *Enterococcus* **(11)**. We find agreement between the findings of the current study and those of Yata and his team in 2021 in Zambia. The researchers came to the conclusion that the majority of the bacterial isolates that were isolated from pregnant women with urinary tract infections were *E. coli* bacteria, with a rate of 59% **(12)**. The study carried out in Uganda by Johnson and his team in 2021 had different findings from the present study, with *Klebsiella pneumoniae* coming in at 37.41%, *Escherichia coli* at 28.78%, *Pseudomonas aeruginosa* and *Proteus mirabilis* at 5.04% apiece, and *Citrobacter freundii* at 1% and *Staphylococcus aureus at 23.57%.* **(13)**.

As is evident from the results of Figures 2, 3, 4 and 5 related to the resistance of gram-negative bacterial species towards the studied antibiotics, some species showed complete resistance to some antibiotics, including AMOX, and some species showed complete sensitivity to some antibiotics. These results come in light of the resistance mutations that occur in the bacterial species, which give them the characteristic of complete, high or medium resistance. The results of the current study were somewhat consistent with the findings of some researchers in the countries around the world and in Iraq, including the study conducted in Iraq, high resistance to ampicillin (85.6%), co-trimoxazole (72.2%), and tetracycline (71.3%) was found as a result. A moderate resistance to Ceftazidime, Ciprofloxacin, Amoxicillin-clavulanic acid, and Ceftriaxone was also discovered **(11)**. We found that most gram negative isolates from UTI patients showed great susceptibility to aminoglycosides and nitrofurantoin in a study conducted in Addis Abeba **(14)**. Antibiotic resistance among pathogenic bacteria is a serious health issue in developing nations and has an impact on the entire world because there aren't enough surveys for antimicrobial resistance and there aren't any laws in place to restrict prescribing. There is a greater risk to the mother and fetus due to the growing multidrug-resistant bacteria (MDR), which reduces the likelihood of giving a safe antibiotic and makes infection elimination and treatment success challenging **(15, 16)**. Due to the lack of effective medicines and the higher incidence of pyelonephritis in pregnant women, UTIs are complex diseases. Due to the high prevalence of amoxicillin- and cephalosporin-resistant isolates in our local strains, it is not recommended to use these antibiotics during pregnancy. Instead, safer alternatives were used, such as amoxicillin/clavulanate and nitrofurantoin for cystitis and fourth-generation cephalosporins for pyelonephritis **(17, 18)**.

According to the findings of a different study, nalidixic acid (88.3%), ampicillin (77.8%), and norfloxacin (58.5%) were the medications with the highest antimicrobial resistance, while chloramphenicol (20%) had the lowest resistance **(12)**. The incidence of drug resistance to various substances was assessed, and results showed rather significant levels of resistance to widely prescribed medications, including ciprofloxacin and chloramphenicol. These have likely been on the market for a long time, giving

bacteria time to develop resistance mechanisms to the antibiotics, which may account for the high level of resistance that has been documented **(19-21)**. Moreover, the availability

nless no other options are available **(22)**. Both pregnant and non-pregnant women were found in one study. In pregnant and non-pregnant women, *Staphylococ* of inexpensive over-the-counter antibiotics in underdeveloped nations like Zambia may be a contributing factor in this level of resistance **(19)**. In addition, the initial use of antibiotics prior to the appearance of antimicrobial susceptibility data in the laboratory may contribute to the high levels of resistance. Therefore, the importance of creating and enforcing antibiotic laws as well as good antibiotic stewardship in poor nations cannot be overstated.

The regular exposure to antibiotics in the area may be the cause of the resistance to commonly given antibiotics that was seen in our investigation. Cephalosporins (CEP) are generally safer to administer during pregnancy than quinolones (CIP, NOR, etc.), which are contraindicated u *cus* sp. displayed resistance to amoxicillin (AMO; 55.56%) and chloramphenicol (CHL; 100%), respectively **(10)**.

Pregnant and non-pregnant women with UT infection had uro-pathogenic isolates that had high levels of multiple antibiotic resistance to medications that were often administered. Pregnant women had considerably greater rates of multidrug resistance to quinolones (NOR, CIP) than non-pregnant women (P=0.018). Pregnant women had considerably more multidrug-resistant *Escherichia coli* isolates than non-pregnant women (P = 0.018) **(10)**.

Conclusion

From the results of the current study, we can conclude that gram-negative bacteria are more common than gram-positive bacteria in pregnant women with urinary tract infections. Also, *E. coli* is the most common and most resistant bacteria of type XRD, and gram-positive bacteria, staph bacteria, also showed resistance to type XRD. In addition, gram-negative bacteria showed high resistance to many antibiotics, including AMOX, CFR, CN, CZ, CXM, and CAE. Gram-positive bacteria showed complete resistance against BENPEN, OXA, CLIN, TEC, VAN, TET, FUS and VAN antibiotics.

Acknowledgments

None.

Conflict of Interest

None.

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