Research article

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Occurence of Antibiotic Resistant Bacteria Causing UTIs Among Children Under School Age in Soran City, North of Iraq

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ABSTRACT

The aim of this study to research the event of antibiotic resistant bacteria causing UTIs among children under six years old in Soran, North of Iraq. Totally, 200 urine samples were taken from children suffering from UTIs, in Ashti hospital and Childbirth hospitals between 2017 and 2018 years. It is determined that 70 bacterial uropathogens have been isolated while 130 samples showed negative culture, and the incidence of UTIs was significantly higher in little girls (57.1%) than in little boys. The majority of uropathogenic isolates have resisted ampicillin, ceftazidime, ceftriaxone and cefotaxime. Other antibiotics differently showed moderate susceptibilities. The DNA profile showed that only the isolate K 61 (*Klebsiella pneumoniae*) was bearing *qnrB.*

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Introduction

Urinary tract consists anatomically of lower urinary tracts include the bladder, urethra and upper urinary tract include the kidneys and ureters [1]. During urination the urinary system expels metabolic wastes from the blood and discharges it as urine to the outside [2]. UTI (Urinary tract infection) is characterized as the notable of microscopic organisms in urine along with symptoms of infection or a bacterial infection of the urinary bladder (cystitis), the kidneys (pyelonephritis), or both. UTIs are widely recognized in childhood [3, 4, 5]. By adolescent age, about 11% of girls and 7% of boys have had at least one episode of UTI, with recurrent infections reported in many cases [6-9].

UTIs are infections resulting from the existence and growth of microorganisms anywhere in urinary tract and may be one common bacterial of human infection [10-11]. Microorganisms that causes UTIs originate from the stool [12]. UTI may be present when

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microscopic organisms and white blood cells are available in the urine [13]. *Healthcare-associated Infections* is widely recognized inconvenience from hospitalized patients [14-15]. The spread of antimicrobial resistance among urinary pathogens has been expanding everywhere throughout the world [16-18]. Many strains of bacteria were developed that cause urinary tract infection usually resistant to various types of antimicrobial agents such as amoxicillin and ampicillin [19-21]. UTIs are generally known for all aggravations of urinary tracts, which contains kidney injury (pyelonephritis), bladder (cystitis) and the urethra (urethritis) [22-23].

The resistance of quinolone was correlated to mutations that prompt amino acid changes in the QRDRs inside the subunits which are incorporated in the synthesis of DNA [24].

The objective of this study was to find antibiotic resistance patterns of microorganisms grown most frequently in Soran, North Iraq, and to determine empirical treatment options according to the results obtained. The results of our study will support the technicians working in the governmental laboratories with new data about the drug of choice for new multi drug resistant strains repeatedly arisen.

Materials and Methods

This study was carried out in Soran city- North of Iraq. Most people live in villages and have rural lifestyle. They depend on domestic animals and ground water. Rural people there are generally poor and ignorant of the rules of health. In Soran most of families attend Ashti hospital and Childbirth hospitals. 200 patients of UTI intended to Ashti hospital and Childbirth hospitals laboratories. The period of sample collection was from July 2017 to March 2018. Samples had been taken before antibiotic use. At the firstly have written all information on a questionnaire list of each patient.

Ethics committee approval was obtained from the Ministry of Health General Directorate of Health-Erbil Committee for the study (19/06/2018/98). This study was conducted in accordance with the Declaration of Helsinki.

All reagents used in the current study were prepared as follows by Harley and Presccott [25]. The uropathogenic isolates were tested for antimicrobial susceptibility by the Kirby-Bauer disk diffusion method on Mueller Hinton agar plates by Merlino et al. [26]. The antibiotics

that was widely used in the current study for the treatment of UTIs are Cefotaxime, Meropenem, Ciprofloxacin, Ampicillin, Tobromycin, Trimethoprim-sulphamethoxazole, Gentamycin, Augmentin, Levofloxacin, Ceftazidime, Impenem, Azithromycin, Vancomycin and Ceftriaxone. The antibiotic discs which are used in identification of the isolates are Novobiocin, Bacitracin, Bacitracin and Rifampin.

MacFarland standard solution is utilized to standardize the density for the susceptibility test. So to preparations of turbidity standard by Cheesbrough [27]. The girl patients were asked their parents to clean their external genitalia. If the patient was under 2 years they used Pediatric urine collector according to the method of Morello et al. [28]. First isolation and purification of bacterial species were on suitable culture media (Nutrient agar, MacConkey agar and blood agar). The recommended procedure uses a sterilized calibrated metal loop to transfer 1µl of uncentrifuged urine specimen and was streaked on Nutrient agar, Blood agar and MacConkey agar, at one and same time as per the biochemical and bacteriological tests utilizing standard techniques by Vandepitte et al. [29], additionally after marking and streaking the plates were put in the incubater at 35-37°C for 18-24 hours. Identified isolated bacteria determined with the techniques described in Bergey's manual by Holt et al. [30].

Differentiation of isolated bacteria by CHROMagar^{TM} orientation, is known as chromogens in which depended on soluble colourless. When the target organism's enzyme cleaves the colourless chromogenic conjugate, the chromophore is released [26].

Identified isolates were presserved for long time according to Mcfaddin [31]. The susceptibility was tested by Kirby-Bauer disc diffusion technique [32]. Disc diffusion test was carried out according to [33].

Molecular method

In this study, specific primers (Metabion, Germany) were designed for amplification of *qnrA*, *qnrB*, *qnrS*, and bla_{kpc} genes. For isolation of nucleic acids from Bacteria, DNA extraction kit (Roche, USA) was used for purification of genomic DNA of the isolates. Details of primer sequences are shown in (Table 1). The method described by Sambrook and Russell [34] was used for gel electrophoresis.

Table 1 *qnrA, qnrB, qnrS,* and bla_{kpc} primer sequences

Isolates No.	Resistance Genes	Primer Sequence	Target size(bp)
E. coli E 7, E 51, E 57, E 59, E 62	*qnrA*	(F)5'-TCAGCAAGAGGATTTCTCA-3'	(R)5'-516
	qnrB	GGCAGCACTATTACTCCCA-3' GATCGTGAAAGCCAGAAAGG-3'	(F)5'- (R)5'-469
Klebsiella pneumoniae K 61	*qnrS*	ACGATGCCTGGTAGTTGTCC-3' ACGACATTCGTCAACTGCAA-3' TAAATTGGCACCCTGTAGGC-3'	(F)5'- (R)5'-714 (F)5'-
	bla_{kpc}	ATGTCACTGTATCGCCGTCT-3' TTTTCAGAGCCTTACTGCCC-3'	(R)5'- 893

Result and Discussion

Incidence of urinary tract infection

Our results showed that, out of 200 urine specimen gathered from patients complaining of signs and symptoms of UTIs attending Ashti hospital and Childbirth hospitals in Soran city, 70 (35%) were positive for bacterial infections while 130 (65%) samples showed culture negative. The study of Alsammani et al. [35] showed that 203 (66.7%) of children had urinary tract infection.

Incidence of the isolated uropathogens associated with UTIs

Uropathogens isolates from 200 samples, 70 strains of bacteria were belonging to 9 species (Table 2).

Table 2 Uropathogens obtained from urine culture

No.	**Isolated uropathogens**	**Number**	**Percentage**
1	*Escherichia coli*	27	38.6%
2	*Staphylococcus aureus*	13	18.6%
3	*Coagulase-negative Staphylococci*	8	11.4%
4	*Klebsiella pnuemoniae*	7	10%
5	*Enterococcus faecalis*	6	8.6%
6	*Streptococcus spp.*	4	5.7%
7	*Pseudomonas aeruginosa*	2	2.9%
8	*Staphylococcus saprophyticus*	2	2.9%
9	*Proteus vulgaris*	1	1.4%

Most common causative organism was *E. coli* (38.6%). This result was consistent the study by Sharma et al. [36] from Nepal and from Aligarh, India by Akram et al. [37] Studies by Islam et al. and Mantadakis et al. [38-39] showed *E. coli* as most common organism but with

varying proportionsut. According to Schlager (2001), in children, *Staphylococcus aureus* is uncommon without in-dwelling catheters or many other factors of infection after instrumentation of the urinary tract [40].

Incidence of UTIs in relation to sex and age groups

70 (35%) patients out of 200 showed to be urine culture positive (Fig. 1).

UTI is predominantly a disease of female. According to Ouno et al. (2013) and Ramazan et al. (2004), the length of male urethra provides a distance barrier that eliminates bacteria from the urinary bladder [41-42].

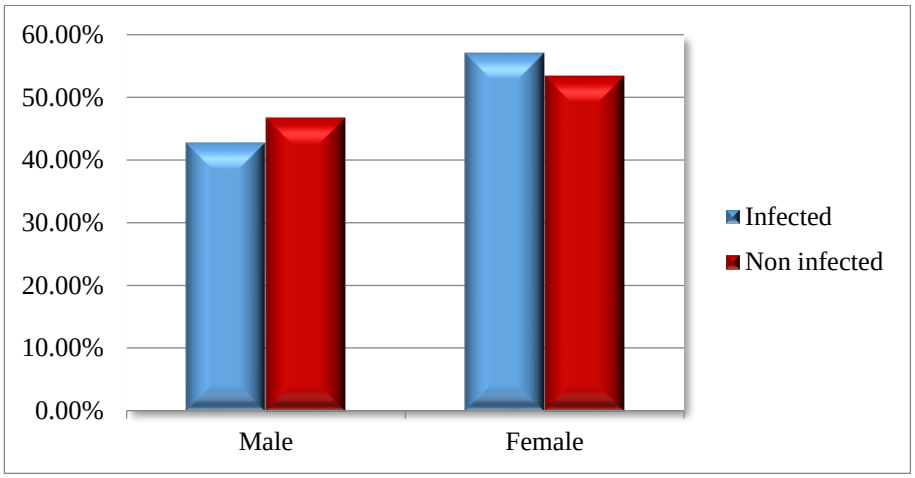

Fig. 1 Frequency of UTIs according to the gender

The majority of children 26 (37.1%) were among those less than two years old, and more than half of them were male 15 (57.7%) (Fig. 2). The same situation reported by Alsammani et al. [35]. The high incidence of UTIs in females under one year old might to be as a result of a distinct type of factors, such as the short and wide female urethra (3-4 cm length) and its proximity to the anus according to Kolawole et al.[43].

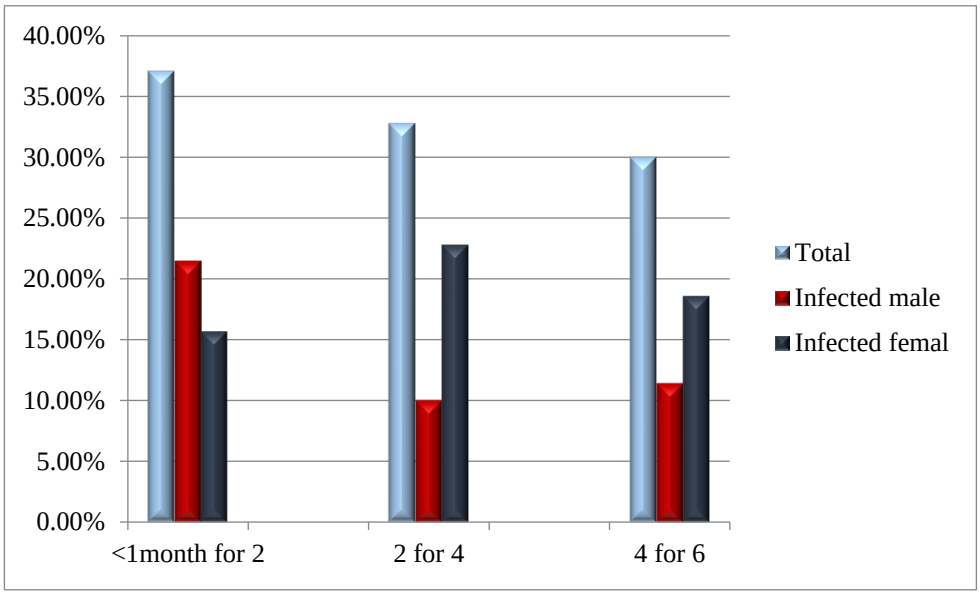

Fig. 2 Distribution of UTIs in relation to age groups

Incidence rate of UTIs according to the residence of the patients

In rural area, the incidence rate of UTIs was significantly high in comparison to urban The incidence rate of UTIs at urban was 34.3% and 65.7% in rural. The most probable explanation, is might to be due to poor diagnostic facilities available in the health centers in rural areas and also the lower socioeconomic, hygienic standards and the absence of a proper sewage system.

Identification of isolated bacteria

By comparing results of microscopic and cultural characteristics, biochemical characteristics and differential susceptibility to special antibiotics like: Rifampin and Bacitracin 10 µ to differentiate *Staphylococcus* from *Micrococcus*, Novobiocin 5µ to differentiate *Staphylococcus saprophyticus* from other staphylococci, Bacitracin susceptibility 0.04µ to differentiate *Streptococcus pyogenes* from other negative gram positive cocci. The microscopic examination showed that 37 (52.8%) of isolates were gram negative (*Escherichia coli, Klebsiella pnuemoniae, Pseudomonas aeruginosa and Proteus vulgaris)*. Also showed that there were 33 (48.2%) of isolated were gram positive (*Staphylococcus aureus,* coagulase-negative Staphylococci (CoNS), *Enterococcus faecalis, Staphylococcus saprophyticus* and *Streptococcus spp*).

Four species of isolates showed positive growth on MacConkey agar are belong to gram negative bacteria were isolated accordance to the growth on MacConkey agar. These were differentiated preliminarily into lactose fermenters and lactose non fermenters which include; *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris*.

Most of *Pseudomonas aeruginosa* isolates were easily recognized on different media due to their ability to produce green pigment confirmed with positive oxidase.

Gram positive bacteria

33 (48.2%) of isolates were gram positive show the characteristics used in the diagnoses and distribution cocci of gram positive, which were confirmed by Holt et al. [44]. and Harley and Prescott [25].

Differentiation of isolated bacteria by CHROMagar^{TM} orientation

This technology is supported the result of identification and making final diagnosis, in which bacterial pathogenes on this medium [26]. Bacterial pathogenes on this medium as the following; *Eschercia coli* is dark pink to reddish, *Enterococcus* is turquoise blue, *Klebsiella sp. is* metallic blue, *Proteus sp.* is brown halo, *Pseudomonas* is cream and translucent, *Staphylococcus aureus* is golden and small, *S.saprophyticus* is pink, opaque and small, *Streptococcus spp.* light blue-green.

Antimicrobial susceptibility

The results of antibiotic susceptibility are appeared in (Fig. 3a-n).

The results of antibiotic susceptibility are appeared in (Fig. 3a), most of our bacterial isolates were highly resistant to ampicillin, we found that 96.3% of *E. coli*, 85.7% of *Klebsiella pneumonia* and all others bacteria in our bacterial isolates were resistant to ampicillin.

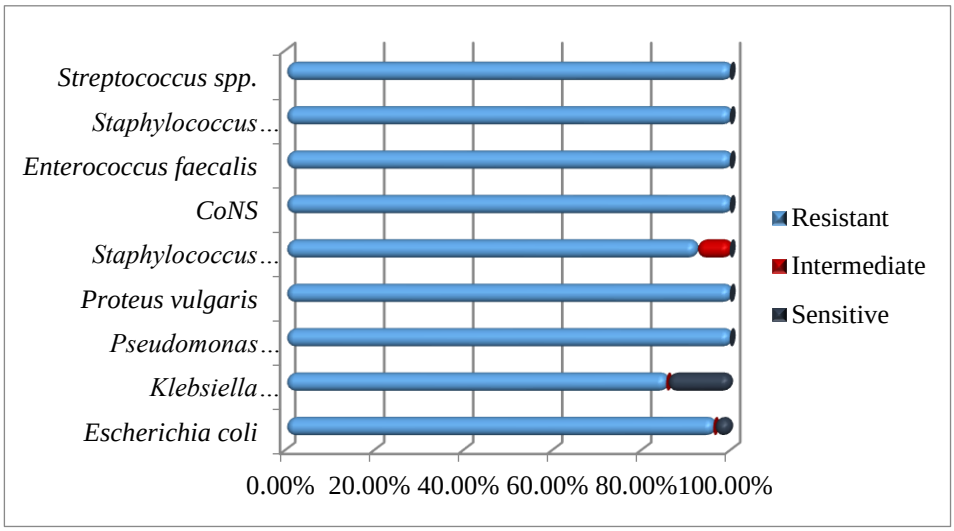

Fig. 3a Antimicrobial susceptibility to Ampicillin

Our result appeared that most of the isolated bacterial uropathogens are resistant to the ceftazidime and ceftriaxone (Fig. 3b).

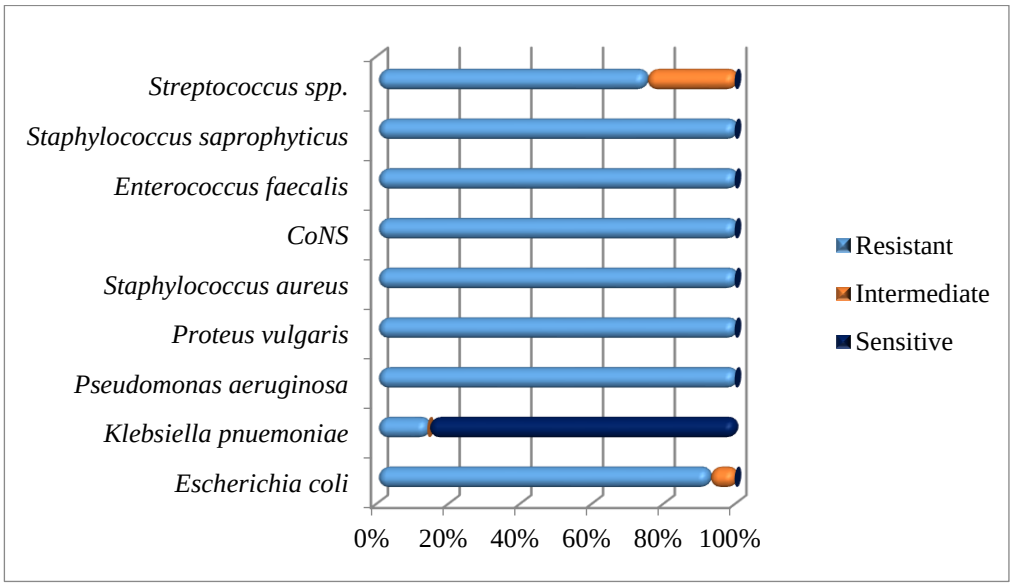

Fig. 3b Antimicrobial susceptibility to Ceftazidime

Most of isolates bacterial uropathogens were resistant to the ceftriaxone (Fig. 3c).

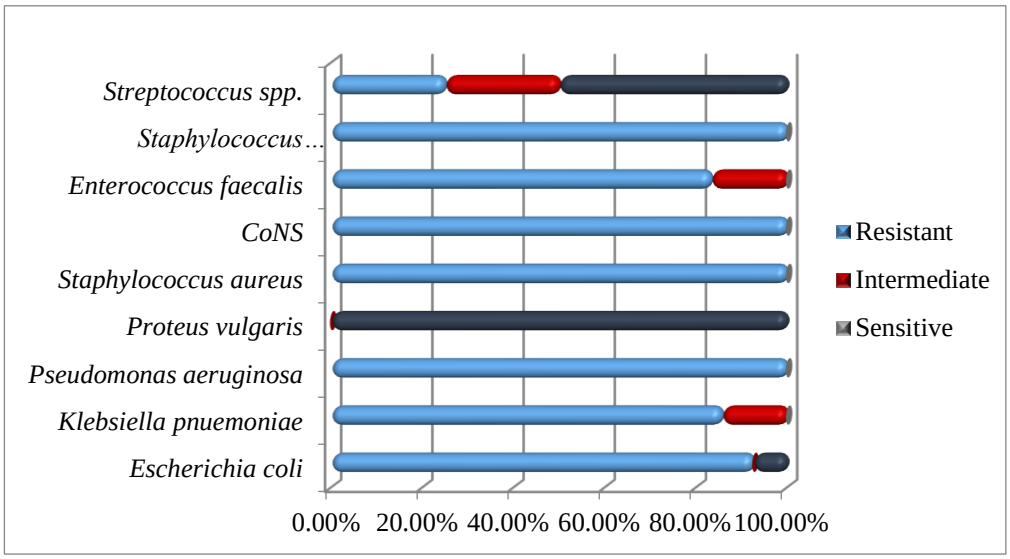

Fig. 3c Antimicrobial susceptibility to Ceftriaxone

As shown in the (Fig. 3d), 55.5% of *Escherichia coli*, 42.8% of *Klebsiella pneumoniae*, 84.6% of *Staphylococcus aureus*, 87.5% of CoNS and 50% of *Enterococcus faecalis* were resistant to azithromycin.

According to Zuckerman et al. (2009), Azithromycin inhibits protein synthesis due to reversibly ties to the bacterial ribosome [45].

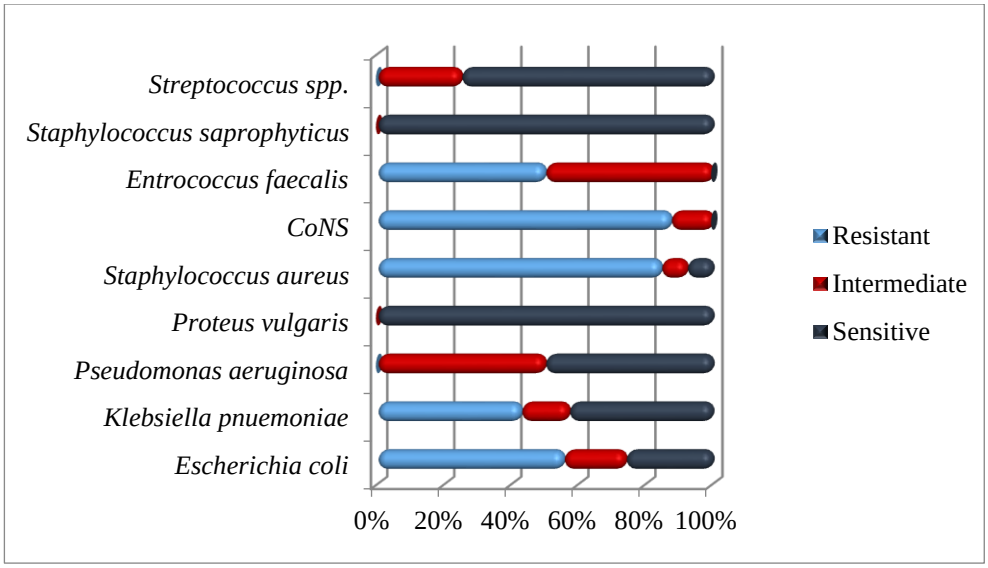

Fig. 3d Antimicrobial susceptibility to Azithromycin

The percentage of resistance to cefotaxime are shown in (Fig. 3e), Bacteria can resist these cefotaxime and ceftriaxone by production of β-lactamase especially CTX-M beta-lactamases class A [46].

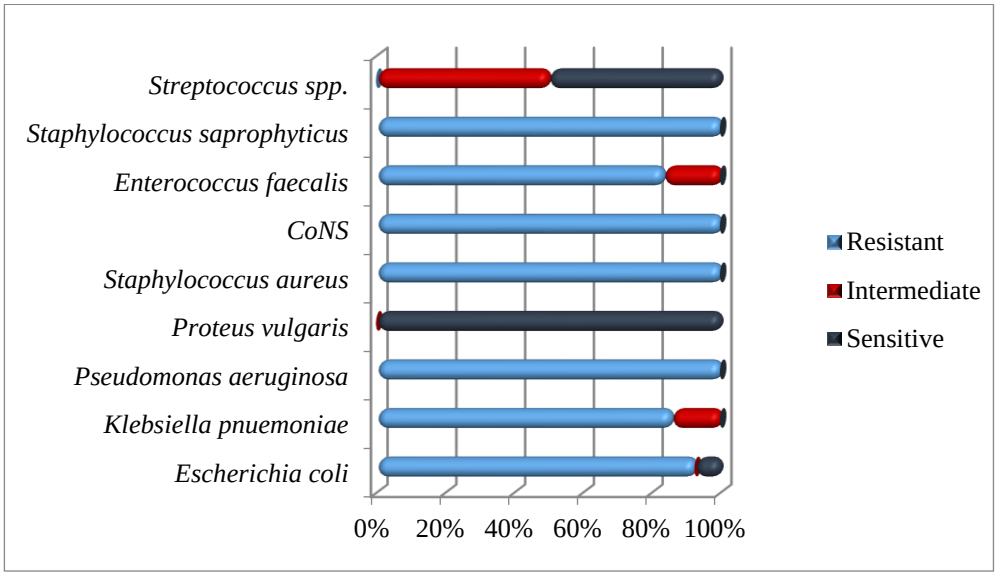

Fig. 3e Antimicrobial susceptibility to Cefotaxime

The results that the resistance percentage of isolates bacterial uropathogens for augmentin (amoxicillin-clavulanic acid) shown (Fig. 3f).

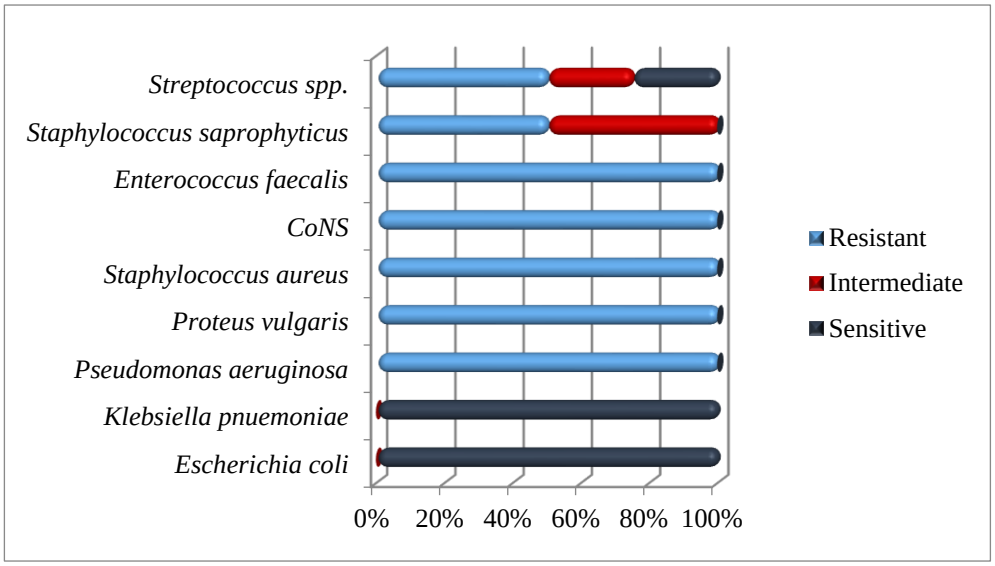

Fig. 3f Antimicrobial susceptibility to augmentin

In our study, the percentage of resistance to tobromycin are shown in and (Fig. 3g) for isolated bacterial uropathogens.

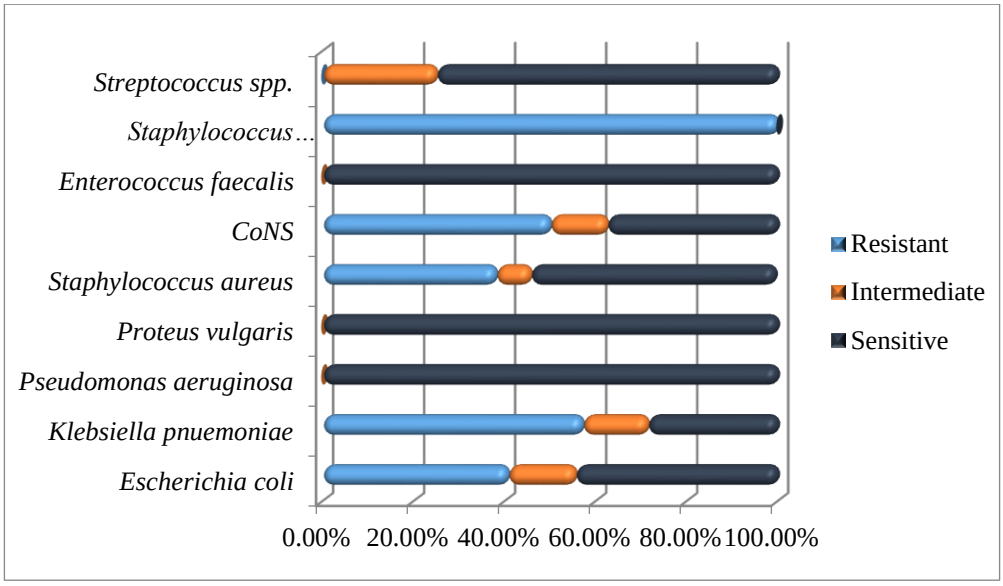

Fig. 3g Antimicrobial susceptibility to tobromycin

As shown in (Fig.3h), we found that 44.4% of *E. coli*, 28.6% of *Klebsiella pneumonia*, 15.4% % of *Staphylococcus aureus*, 12.5% of CoNS and 50% of *Staphylococcus saprophyticus* were resistant to ciprofloxacin. According to Diver and Wise (1986) Ciprofloxacin inhibit bacterial DNA synthesis and promote cleavage of DNA leading to bacterial cell death [47].

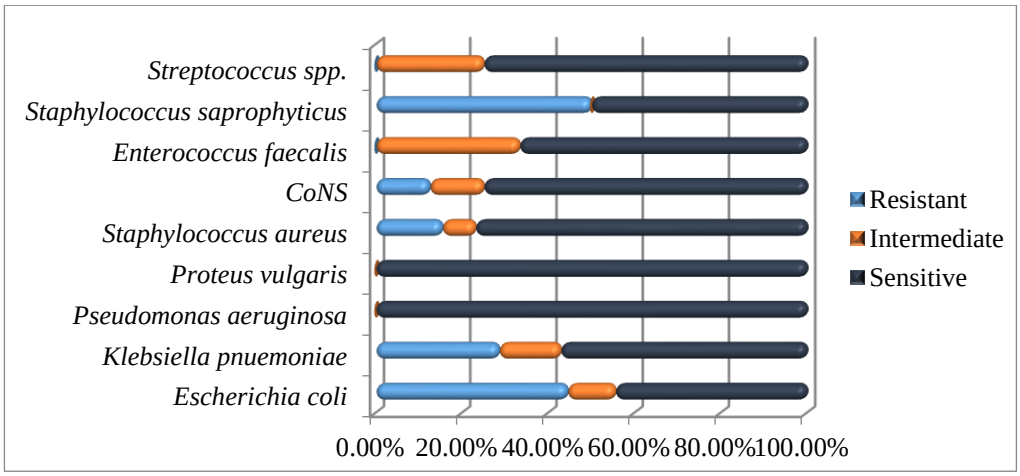

Fig. 3h Antimicrobial susceptibility to ciprofloxacin

In this study, the antibiotic susceptibility tests shown that 85.1% of *E. coli*, 100% of *Klebsiella pneumonia*, 100% of *Pseudomonas aeruginosa*, 100% of *Proteus vulgaris*, 76.9% of *Staphylococcus aureus*, 100% of CoNS, 66.66 % of *Enterococcus faecalis* and 100% of *Streptococcus spp.* were sensitive to meropenem (Fig.3i).

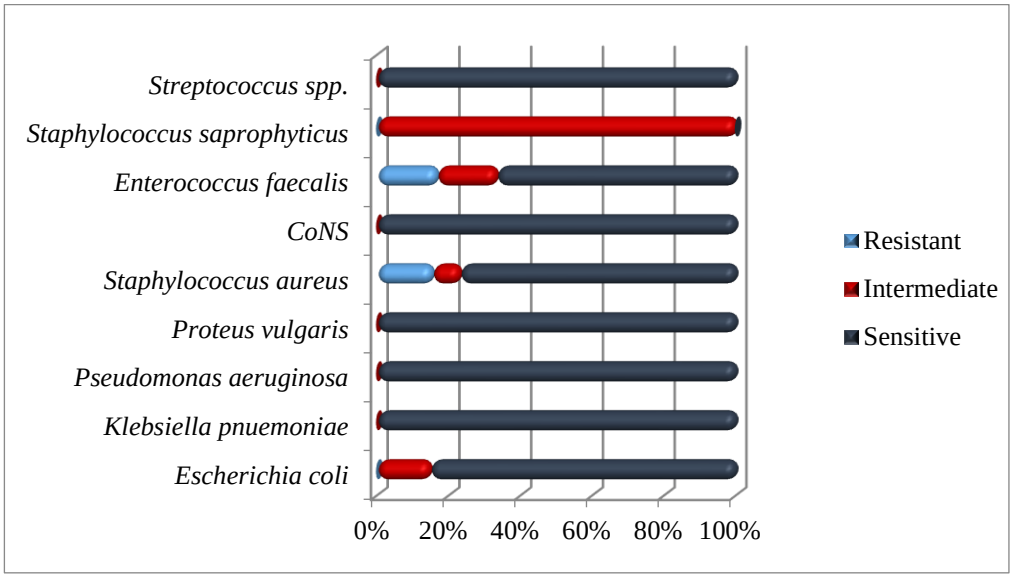

Fig. 3i Antimicrobial susceptibility to meropenem

Antimicrobial susceptibility to trimethoprim-sulphamethoxazole showed in the (Fig. 3j). High resistance to trimethoprim sulphamethoxazole seen in many other studies in different countries [10, 18].

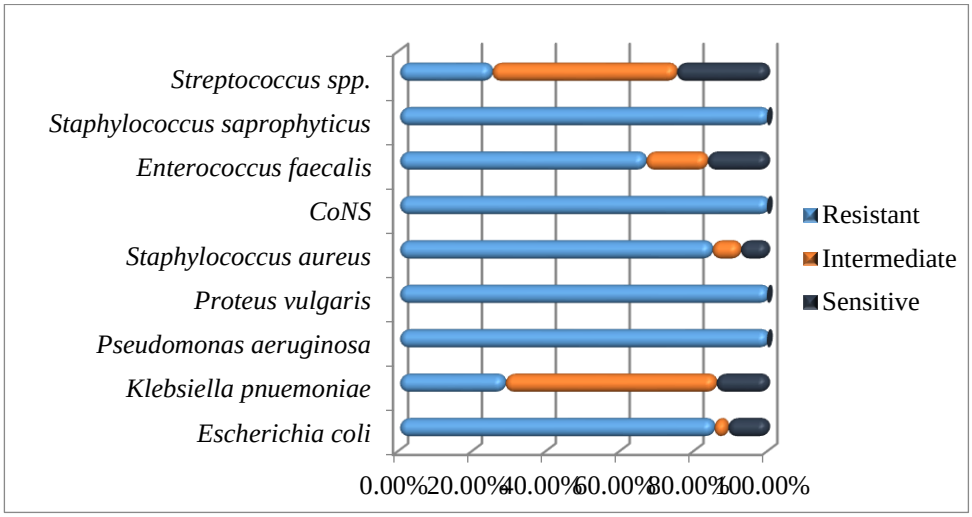

Fig. 3j Antimicrobial susceptibility to trimethoprim-sulphamethoxazole

Antimicrobial susceptibility to gentamycin showed at (Fig. 3k).

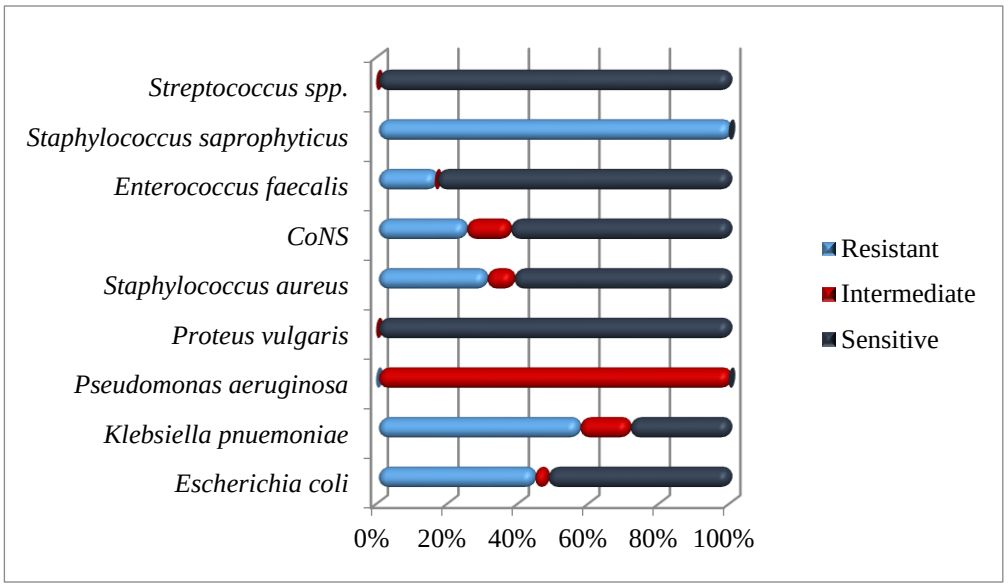

Fig. 3k Antimicrobial susceptibility to gentamycin

The results of present study showed that the sensitive percentage of isolates bacterial uropathogens for levofloxacin were shown (Fig. 3l). Levofloxacin is active against both Gram-positive and Gram-negative bacteria [48].

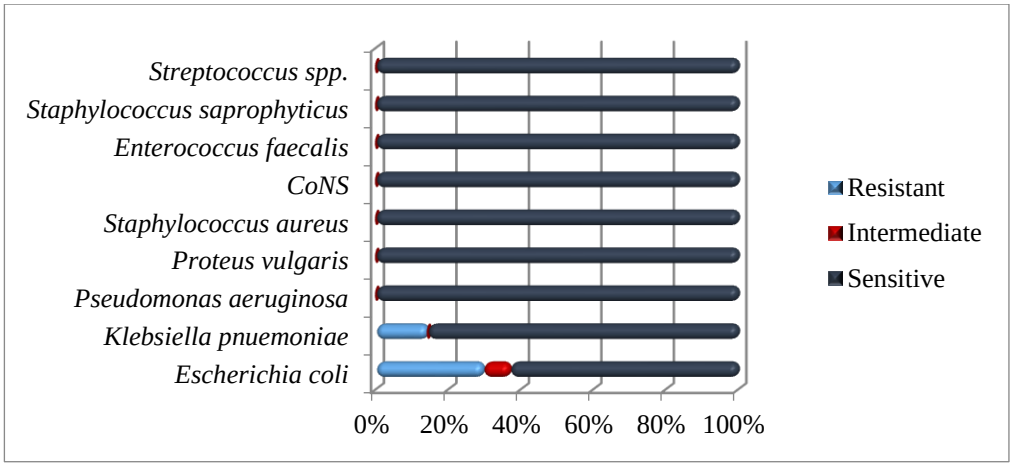

Fig. 3l Antimicrobial susceptibility to levofloxacin

In our results that the sensitive percentage of isolates bacterial uropathogens for impenem were shown at (Fig. 3m).

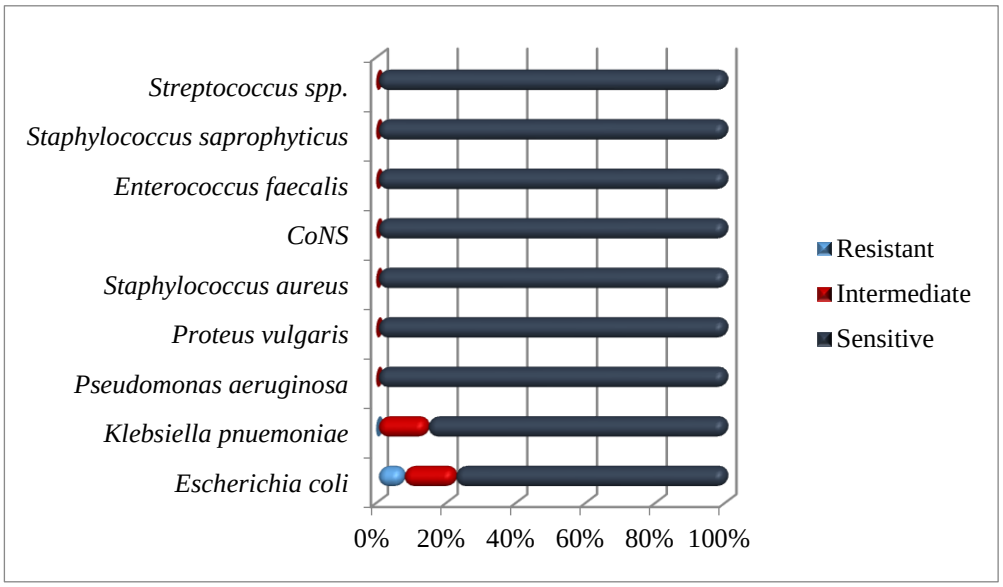

Fig. 3m Antimicrobial susceptibility to imipenem

Antimicrobial susceptibility to vancomycin showed in the (Fig. 3n). Vancomycin is active just against gram-positive organisms.

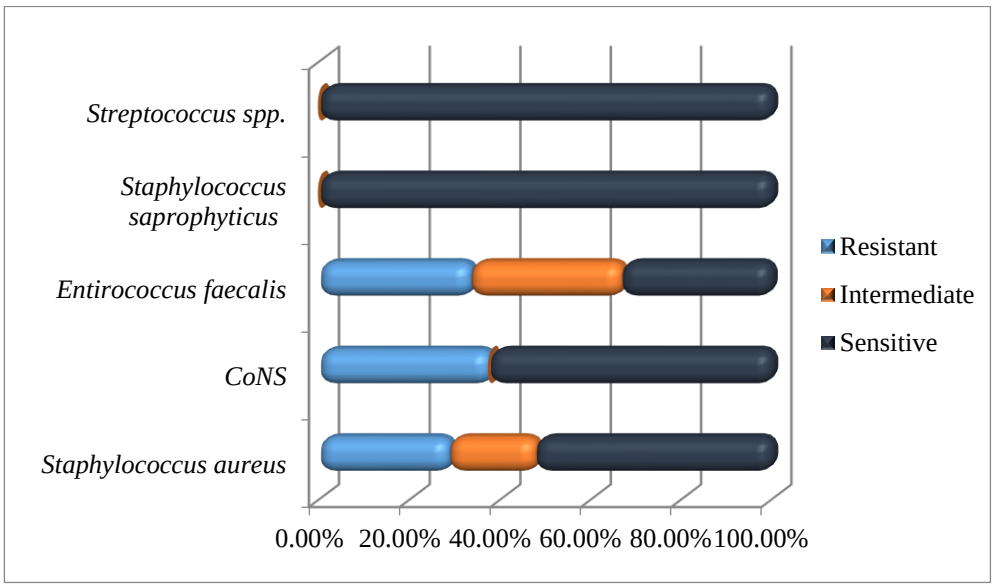

Fig. 3n Antimicrobial susceptibility to vancomycin

In our study and after we mentioned all previous susceptibility tests results to 14 different antibiotics which commonly used in hospitals, shown us that a higher prevalence rate of resistance to commonly prescribed antibiotic agent, all the bacterial uropathogens showed

the highest degree of resistance to ceftazidime, ceftriaxone, cefotaxime, trimethoprim-sulphamethoxazole, ampicillin and augmentin. This result agrees with results obtained by Sahilin et al. [49] in India, by AbdulRazzaq (2013) [18] in Mosul, and by Mezal and coworkers (2011) [50] in Basrah. while this drugs exhibited low resistant rate in another study outside Iraq such as Jha and Bapat [51] reported that all the organisms causing UTI were sensitive to ciprofloxacin. Hussein [52] reported that all the organisms causing UTI were sensitive to gentamycin and tobramycin was found to be least effective. In our study, isolates appeared high sensitivity to meropenem, and imipenem, also *levofloxacin* apeared high (more than 90%) sensitivity in all groups except of *Escherichia coli* (62.9%) were sensitive. Yasmeen et al. in Dhaka reported in their study, meropenem, and imipenem appeared high sensitivity, also *levofloxacin* appeared (47.9%) sensitivity of *Escherichia coli*. [53]. Kyoung reported that meropenem, and imipenem appeared very high (over 90%) sensitivity in all groups [54]. Abera and Kibret, in rural community of Ethiopia reported about the resistant and sensitivity of approximate resistance result to azithromycin of UTI causative agents [55].

Detection of quinolone and carbapenem genes

We have amplified four genes of carbapenem and quinolone risistance genes responsible in *Escherichia coli* and *Klebsiella pnuemoniae*, which antibiotics used more than others to treat of infection of bacteria. We have chosen six isolates to detecte the presence of resistance to carbapenem and quinolone genes by (RAPD-PCR), because both of them can share the same sequences antibiotic resistance and they may get them by gene transferring [56].

The results showed that out of five isolates of *Escherichia coli* and one of *K. pneumoniae*, no one of these isolates contained genes in their genomic DNA except of *Klebsiella pnuemoniae* (K 61) contained *qnrB* in its genomic DNA. *qnrA* was more prevalent until 2001, but since then, *qnrB* has predominated [57].

Also others had negative results for detected genes as mention because we have detected specific sizes (bp). Pathogenic bacteria carry another sequences to resist the same antibiotics, for example [58] in China reported in their study, they found that *Escherichia coli* were all resistant to meropenem and imipenem.

Conclusions and Suggestions

The main investigation directed to decide the pervasiveness of UTI. The impact of gender and age on its predominance, and their vulnerability profile to basic utilized anti-infection in the group of Soran city. The study also allows comparison of the situation in Soran city, North of Iraq with different areas outside and inside the state as well as in the country.

According to this study, we concluded, there were no control on getting the drugs and alot of it given to outpatients without physicians prescription from outside of hospital, when infection is occur in the any parts of body**'s** patient, they taking antibiotics without culturing and determination of antibiotic susceptibility for side of infection, this led to kill commensal bacteria and the emergence of different types of antibiotic resistant bacteria.

Abbreviations/Kısaltmalar

UTI: Urinary tract infection, Bp: Base pair, CFU: Colony forming unit, CLSI: Clinical and laboratory standards institute, E.coli: *Eschericia coli*, EMB: Eosin methylene blue agar, PBS: Phosphate buffered salin, PCR: Polymerase chain reaction, QRDRs: Quinolone-resistant determining region, RAPD: Random amplification of polymorphic DNA, VUR: Vesico-ureteral reflux.

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Data Availability statement
The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards
Conflict of interest
The author declare no conflict of interest.
Ethical standards
The study is proper with ethical standards.
Authors' contributions
During the study, Hasan Akan wrote the article and Rawezh Hakeem Mustafa conducted laboratory research.

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