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Prevalence of bacterial antibiotic resistance pattern among patients with uropathogenic *E.coli* in Hilla City, Iraq

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Urinary tract infections are the most widespread extra intestinal bacterial illnesses. Currently , it is the most prevalent illness seen in medical practice, affecting patients of all ages, from newborns to the elderly. 57 *E.coli* isolates of urine samples of both male and female patients suffering from urinary tract infection, were collected from three central hospitals in Hilla city in Iraq (Al Noor Children's Hospital, Maternity and Children's Hospital, and Teaching Merjan Hospital) in the period from August to the end of November 2023. The major affected groups were 21–30 years old (15; 26.3%); 37 (65%) females, and 40 (70.2%) from rural areas. 39 (68.4%) cases were married, mature patients of both sexes. These isolates were found to have the ability to produce hemolysin (25; 43.8%) and 36 (63.1%) produced siderophores;57 (100%) were mannose resistant hemagglutinin type IV and 35 (61.4%) produce bacteriocin. while 57 (100%) could produce protease and 52.6% were forming biofilm. The antibiotic susceptibility test found that beta-Lactam (amoxicillinclavulanate) as the highest resistance represent 100% of the resistance, The resistance percentage to ceftazidime was 70% and cefotaxime 67%, whereas the antibiotic aztreonam was 30%, and gentamicin 40%. In addition, the trimethoprim group is 50%. whereas resistance to Nalidixic acid is 59%, Norfloxacin was 37%. Pefloxacin resistance was 30% ,Ciprofloxacin resistance was 35%. The results of minimum inhibitory concentration MIC showed that 22% of isolates were sensitive to both ciprofloxacin and nalidixic acid, but resistant to both Pefloxacin and Norfloxacin . 49% of isolates had resistance to every type of antimicrobial drugs. 9% sensitive to all antimicrobial drugs. Only 5% were sensitive to all antimicrobial drugs except ciprofloxacin solely displayed resistance. Only 3% were resistant to all antimicrobial drugs except nalidixic acid displayed sensitive. Only 7% of isolates were sensitive to all antimicrobial drugs except nalidixic acid demonstrate resistance. Only 5% of the isolates shown resistance to all antimicrobial drugs except ciprofloxacin displayed sensitive.

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INTRODUCTION

Urinary tract infections are the most widespread extra intestinal bacterial illnesses. It is currently the most prevalent illness seen in medical practice, affecting patients of all ages, from newborns to elderly (Bien et al., 2012). A urinary tract infection (UTI) is defined as the invasion and multiplication of microorganisms in any one of the urinary system's structures. The degree of the infection might vary, ranging from asymptomatic colonization (i.e., no tissue degradation) to symptomatic invasion of any tissue within the urinary system structure (Gharbi, et al., 2019).

Numerous microorganisms can cause urinary tract infections (UTTIs), although *Escherichia coli* and other Enterobacteriaceae are the most common pathogens impacting common UTIs in the public, accounting for approximately 75% of isolates. It has been demonstrated that one of the main causes of severe UTIs may be the growing resistance to the commonly used antimicrobial drugs because of their routine usage in UTI treatment without performing a susceptibility test. (Abat et al., 2015). Urinary tract infections (UTI), which comprise cystitis and pyelonephritis, are one of the urologic pathogenic *Escherichia coli* (UPEC) infections that affect people most often (Bien et al., 2012).and(Wiles et al.,2008).). Antibiotics can successfully cure UTIs, but the issue of bacterial resistance to antibiotics is growing, making UTIs more likely to recur, especially when biofilm development and virulence factors are present. (Cook and McArthur 2013) .

Additional research revealed that UPEC strains are associated with several key virulent characteristics, like bacterocin, hemolysin, siderophore ,mannose resistance and protease production and biofilm formation. (Šmajs et al ., 2010). pro-forming proteins called hemolysin broke down red blood cells and released hemoglobin, which can be released by a variety of substances, including bacterial hemolysin, which seems to support the invasive ability of bacteria. Bacteriocin production is a main specific of *E.coli* and several associated species in the Enterobacteriaceae family. In the *Escherichia* genus, bacteriocin creation is completely associated with strains of *E. coli* (Smarda et al.,2002). Bacteriocins are proteinaceous toxins created by bacteria to inhibit the growth of similar related bacterial strains (Farkas-Himsley ,1980). UPEC strains typically produce siderophores, which are likely crucial for bacteria to acquire iron during or after colonization (Todar, 2006) . Bacterial growth requires iron, and the capacity to obtain iron from the host is a requirement for the creation and perpetuation of infections. (Dal et al.,2003).

This pathogen's propensity to build biofilms is one of its main virulent factors. This biological evolution increases the pathogen's resistance to antibiotics and shields it from host immunity. Based on estimates, biofilm development is responsible for around 80% of infectious illnesses. Numerous chronic and persistent bacterial infections are caused by this pathogen's capacity to build biofilms and its resistance to antibiotics. (Elmanama et al.,2020). Biofilm formation is an important virulence factor that increases the pathogenicity infectious of *E. coli*(Issa et al.,2018).

Enzymes known as proteases accelerate hydrolytic processes, or hydrolysis peptide bonds, which result in the breakdown of protein molecules into peptides and amino acids. (Sumantha et al.,2006). The objectives of this study were to distinguish the utmost communal reason for UTI ,virulence factors , antimicrobial susceptibility and minimum inhibitory concentration of *E. coli* isolated from Hilla City patients exhibiting clinical UTI symptoms.

MATERIALS AND METHODS

Collection of urine samples and identification of bacteria

In this study, 57 *E.coli* isolates of urine samples of both male and female hospital patients suffering from urinary tract infection, from three central hospitals in Hilla city in Iraq (Al Noor Children's Hospital, Maternity and Children's Hospital, and Teaching Merjan Hospital) in the period from August to the end of November 2023. The identification of *E.coli* isolates was carried outby standard methods include identification with morphological characters and biochemical tests .The MacConkey agar and blood agar(Himedia- india) were used to identify *E. coli* and other Gram-negative bacteria initially; eosin methylene blue medium was also used for identification according to Bergey's manual of determinative bacteriology [(Salehzadeh and Zamani ;2018). The biochemical tests Voges-Proskauer, Simmons citrate, indole production, methyl red, lactose fermentation, and sucrose fermentation, were conducted to identify the bacterial isolates and the diagnosis confirmed by using Vitek system.

Detection of virulent factors and enzyme production
Bacteriocin production: This test was achieved by using the Cup Assay Method as described by Al-Qassab and Al-Khafaji (1992) . Zones of growth inhibition surrounding stabs indicate the presence of bacteriocin. (Chung, 2003).

Hemolysin production: Hemolysin formation was tested on human blood agar dishes according to De Voy et al. (1980). A greenish zone surrounding the colonies indicated α-hemolysis, or partial hemolysis, whereas no color change indicated γ-hemolysis, or non-hemolysis. (Collee et al.,1996).

Siderophore production assay: The production of siderophore was tested for on M9 media according to Collee et al (Collee et al.,1996). The results were seen if the growth of microorganism was present or not .

Mannose resistant hemagglutination (MRHA): It was carried out to detect fimbriae other than type-1 fimbriae according to Karr et al., (1990).

Protease formation test: It was tested according to the method of Benson, (1998).

Biofilm Formation (Tube Method): After inoculating tryptone soy broth(Biolife) with 1% glucose that had been previously prepared, two to four isolates growing on nutrient agar were incubated for twenty-four hours at 37 °C. Following incubation, the bacterial growth was eliminated, the tubes were cleaned with distilled water, and they were then allowed to dry. To each tube, 5 milliliters of 0.5% concentration crystal violet was added. They were rinsed with distilled water after ten to fifteen minutes and then turned upside down to dry. Positive results were noted since rings could be seen around the inner walls and bottom of the tubes. The test is deemed ineffective if no rings were seen.(Christensen et al.,1982)

Antibiotic susceptibility test

Susceptibility to antimicrobial agents was assessed by the disc diffusion technique on Mueller–Hinton agar(Oxoid-UK) .The sensitivity test against eight antibiotics was done according to Kirby-Bauer method and interpretation of results was as recommended by CLSI-2023. Tested antimicrobials are Norfloxacin(10µg), ,Trimthoprim (30µg), Pifloxacin(10µg) , Cefotaxime (30µg), amoxicillin-clavulanate 30µg, ceftazidime 30µg, aztreonam 30µg, gentamicin 10µg, Nalidixic acid (30 µg) and ciprofloxacin (5 µg), as defined by references (Humphries et al., 2018) (Borty et al., 2016) by disk diffusion method utilization as in Table 1. The inhibition zones were interpreted as resistant and susceptible based on criteria provided by the Clinical and Laboratory Standards Institute (CLSI).

MIC determination

The agar dilution method was used to measure the minimum inhibitory concentration (MIC) in Mueller Hinton agar (Oxoid). Media plates were serially diluted two times, ranging from 0.25 to 512 mg/L, in accordance with the Clinical and Laboratory Standards Institute's recommendations (CLSI, 2007) (Abass et al.,2014).

RESULTS

Escherichia coli was identified from the urine of UTI patients in 57 clinical samples, with the patients' ages ranging from one year to 50 years , the major affected group was (21-30) 15(26.3%), 37(65%) females and 20(35%) males ,17(29.8%) from urban resident and 40(70.2%) from rural areas. The common of UTI infections involving *Escherichia coli*, 39(68.4%) cases were married mature patients for both sex, 18(31.6) cases of *E.coli* in patients which not married as in Table-2 . The medical personnel at the hospital diagnosed each patient with a UTI based on a combination of laboratory testing and clinical symptoms. The hospital patients were all admitted to the hospital and had one or more UTI symptoms.

Identification of isolates

The processes of identification and confirmation were carried out using morphological, biochemical, and phenotypic features. It was discovered that every isolate was a Gram-negative rod. Biochemical testing included oxidase-negative, lactose, glucose, and sucrose fermentation; the IMVIC test revealed indole positivity; the Voges-Proskauer and citrate utilization tests yielded negative results .They have capability of reaction on eosin methylene blue (EMB) medium and gave green metallic sheen, on MacConkey gave pink colonies and on blood agar giving alpha or gamma haemolysis as in table-3 . The Vitek-2 test system verified the identifications.

Virulence factors and Enzyme production

When *E. coli* was examined for its ability to produce hemolysin, it was discovered that 25 (43.8%) of the 57 isolates could do on blood agar as a zone of hemolysis surrounding bacterial colonies, and 36 (63.1%) of the isolates could make siderophores on M9 media as in Table 3. After the detection of mannose resistance hemoglutinin type IV, it was discovered that all isolates possessed this virulence factor (57, 100%). The 57 *E.coli* isolates showed that 35(61.4%) isolates had the ability to produce bacteriocin.

Additionally, after 24 hours of incubation, all 57 (100%) of the isolates of *E. coli* were reported to be able to produce protease on M9 media (supplemented with 20% glucose and 1% gelatin). The colonies were surrounded by a transparent, clear region after 3 milliliters of 5% trichloroacetic acid was added. Using the tube method to examine the isolates' capacity to form biofilms, it was discovered that 30 isolates (52.6%) produce biofilm, and 27 isolates (47.4%) were not able to do so.

Antibiotic susceptibility test

Using the disc diffusion method, the susceptibility of fifty seven Uropathogenic *E. coli* (UPEC) isolates to ten distinct antibiotic types was assessed. The pathogenic isolates that were examined for these medicines had different patterns of sensitivity and degrees of resistance. The antimicrobial test yielded the following findings: the beta-Lactam (amoxicillin clavulanate) as the highest resistance represent 100%. The resistance of the cephems group, where it was ceftazidime 70% and cefotaxime 67%, whereas the resistance to monobactams group (aztreonam) was 30%, and aminoglycosides group (gentamicin) 40%. Furthermore, the resistance to trimethoprim group was 50%. whereas resistance to nalidixic acid was 59%. Resistance to norfloxacin was 37%. Pefloxacin resistance was 30% . And 35% of isolates were resistant to ciprofloxacin.(Table 4).

Minimum inhibitory concentration MIC determination

Agar dilution method was used to assess the minimum inhibitory concentrations (MICs) of four antimicrobial drugs (ciprofloxacin, norfloxacin, pefloxacin, and nalidixic acid) for isolates separately. The isolates' general resistances to various antimicrobial drugs were as follows: 22% of isolates were sensitive to both ciprofloxacin and nalidixic acid, but resistant to both pefloxacin and. 49% of isolates had resistance to every type of antimicrobial drugs. Only 9% of isolate were sensitive to all antimicrobial drugs . Only 5% were sensitive to all tested antimicrobial drugs except ciprofloxacin solely displayed resistance. Only 3% were resistant to all tested antimicrobial drugs except nalidixic acid displayed sensitive. Only 7% sensitive to all antimicrobial drugs except nalidixic acid demonstrate resistant. Only 5% of the isolates shown resistance to all antimicrobial drugs except ciprofloxacin displayed sensitive. (Table 5).

Table 1-Antimicrobial agents and their concentrations (Oxoid, United Kingdom).

Antibiotics	Concentration µg/ml
Gentamicin	10
Aztreonam	30
Norfloxacin	10
Ceftazidime	30
amoxicillin-clavulanate	30
Trimethoprim	20
Pefloxacin	10
Ciprofloxacin	5
Cefotaxime	30
Nalidixic acid	30

Table2. Gender, residency and marital status in relation to no. of cases

	Gender		Residence		Marital Status	
Cases	Male	Female	Urban	Rural	Married	Not Married
No.	20	37	17	40	39	18
Percentage	35	65	29.8	70.2	68.4	31

Table 3. Virulence factors of *E.coli*

Enzyme production	+	-
Hemolysin production	25 (43.8%)	32(56.2%)
Siderophore production	36 (63.1%)	21(36.9%)
Bacteriocin	35(61.4%)	22(38.6%)
Protease production	57(100%)	0(0%)
Mannose resistance hemagglutinin	57(100%)	0(0%)
Biofilm formation	30 (52.6%)	27(47.4%)

Table 4. Antibiotic susceptibility of tested *E. coli* isolates

Antimicrobial	Resistance%	Sensitivity %
Amoxicillin clavulanate	100	0
Ceftazidime	70	30
Cefotaxime	67	33
Aztreonam	30	70
Gentamicin	40	60
Trimethoprim	50	50
Nalidixic acid	59	41
Norfloxacin	37	63
Pefloxacin	30	70
Ciprofloxacin	35	65

Table 5. Minimum inhibitory concentration

No. (out of 57)	Resistant isolates%	Equivalent MIC Nalidixic acid	Equivalent MIC Ciprofloxacin	Equivalent MIC Norfloxacin	Equivalent MIC Pefloxacin
12	22	Sensitive	Sensitive	Resistant	Resistant
28	49	Resistant	Resistant	Resistant	Resistant
5	9	Sensitive	Sensitive	Sensitive	Sensitive
4	7	Sensitive	Sensitive	Sensitive	Resistant
3	5	Sensitive	Resistant	Resistant	Resistant
3	5	Sensitive	Sensitive	Resistant	Sensitive
2	3	Resistant	Resistant	Resistant	Sensitive

DISCUSSION

One of the most frequent bacterial diseases in humans, urinary tract infections, are primarily caused by *E. coli*. Uropathogenic clones are chosen from the fecal flora, and the existence of virulence factors is assumed to be a prerequisite for the pathogenic potential of *E. coli* strains. (Lane et al.,2007). This study showed a greater percentage of UTI in females 37(65%) than in males 20(35%) . These results agreed with Abass et al(2014), Muhaimeed and Ghareeb (2023) and Ahmed et al ., (2023), who found that 60% were females, whereas only 40% were men . This study observed a higher infection in females with urinary illness than in males. This is due to the different dissection of the urinary system in males and females and the nature of the physiological composition; where the length urethra is short when compared with males. Moreover, hormonal variation can stimulate infections. Also, males are extra protected because of the anatomy of the urine system (Odoki, et al ., (2019).).The study agrees with Jadhav et al (2011). The age group 21 to 30 years showed the highest incidence of UTIs. Among these, diabetes mellitus, urinary tract blockage, and impaired bladder emptying are probably more common in older people. These elements contribute significantly to UTI and encourage bacterial colonization. (Gales et al.,2002). Other studies by Ulleryd (2003) and Abass et al(2014) have revealed comparable results. Iraq has a high rate of infection, with genitourinary infections being particularly common. This could be because some economically disadvantaged groups don't have access to inexpensive personal or community cleanliness. These results also agreed with Ahmed et al., (2023) who found the highest proportion was from the age group of 20-39 years (56.7%) .

The results of hemolysin and siderophore production agreed with the results of Ali (2012), Al-Sayigh et al. (2013), Al-Hamawandi (2014) and Shah et al (2019) who found that numerous *E. coli* strains produce hemolysin, which oversees creating the hemolysis zone that surrounds bacterial colonies on blood agar. Mannose resistance hemagglutinin type IV results agreed with results obtained by Chin et al, (2011) and AL-Hamawandi (2014), while the results of bacteriocin agreed with Smajs, (2010) and Ali (2012). Also, the protease production results agreed with results of Choong etal, (2001) and AL-Hamawandi (2014) who found that all tested isolates were producing protease.

The results of biofilm formation agreed with prior studies done by Shah et al (2019) and Ahmed (2021) while disagreeing with Sabah (2018) who discovered that all tested *E. coli* isolates produced biofilm. This study found that 100% of participants showed the maximum level of resistance to beta lactams (amoxicillin-clavulanate), which was consistent with other results obtained by Al-Hilali (2010) and Abass et al ,.(2014); most *E. coli* isolates had beta-lactamase, which could be the cause of its enhanced resistance. Khalaf and Flayyih (2024) found 92% resistance to amoxicillin. The resistance to cephems group varied., where it was 70% to ceftazidime , while 67% to cefotaxime, these results agreed with the Al-Hilali (2010) results in Najaf they discovered that, respectively, resistance to cefotaxime and ceftazidime was 68.2 and 72.7%, Shakhatreh et al. (2019) and Ghaffoori and Suleiman; (2022) which supported the findings of the current study on ceftazidime, while Muhaimeed and Ghareeb (2023) found highest resistance to Cefotaxime was recorded (80%). Abbas et al. (2024) found 70% resistance to ceftazidime while Khalaf and Flayyih (2024) found 52% resistance to ceftazidim .

Resistance to the monobactams group included aztreonam, was 30%, this result agreed with the results obtained by Abbood and Alwan (2023) in Al-Hilla who found that the resistance to aztreonam was 26%, and Khalaf and Flayyih (2024) who found 40% resistance to aztreonam . Additionally, 40% of the Uropathogenic *E.coli* isolates had gentamicin resistance, according to the data. This result aligned with Shamki et al(2012) and Al-Taai.(2018) who found 57.14% of isolates were resistant to gentamycin. Al-Azawi (2023) found 26% resistant to gentamicin, Abbas et al., (2024) found 47% resistance to gentamicin. While Khalaf and Flayyih (2024) found 22% isolates were resistant to gentamicin.

In addition, while the trimethoprim group represented about 52% of the population, this result of the trimethoprim group reported 50% resistance ,this finding was compatible with another study by Estrada-Garcia et al(2005) in Mexico City , who found that the resistance to trimethoprim was 67%. Albert et al (2009) in Kuwait , found that the resistance to trimethoprim was 43.2%. also in Najaf , Al-Hilali (2010) found resistance to trimethoprim was 72.7% , and Khalaf and Flayyih (2024) who found 36% resistance to trimethoprim.

Antibiotic sensitivity was the most effective test showed resistance 59% to nalidixic acid and 35% to

ciprofloxacin, the nalidixic acid and ciprofloxacin resistance agreeing with the results obtained by Shamki et al(2012) in al Kut city , Abass et al (2014) , Al-Azawi (2023) found that 52% of the tested *E.coli* isolates were resistant to ciprofloxacin and Khalaf and Flayyih (2024) that found 20% isolates were resistant to ciprofloxacin, while Abbas et al., (2024) found 70% resistance to ciprofloxacin .In our study, 30% of our isolates were resistant to pifloxacin and 37% to norfloxacin. The results of this study agreed with the results of Vila et al (2002) and the finding of Abass et al (2014) who demonstrated It is commonly known that resistance to ciprofloxacin, norfloxacin, nalidixic acid, and pifloxacin is growing in various regions of the world. Another research indicates that 25% of urine samples have quinolone-resistant *E. coli*, which is consistent with our results of 36 (55.3%). (Soto et al.,2006).

In previous years, it appeared that the rising rate of drug impedance and multi-resistant phenotypes observed in *E. coli* turned into a major issue around the globe (Shah et al., 2020). The present study showed that urinary infection spread via *E. coli* in the people, it coincides with other Iraqi surveys studied. One study showed that the prevalence of *E. coli* in patients with urinary illness in hospitals of Baghdad is more than other species of bacteria (Almutawif and Eid ;2023). The improper and excessive use of antibiotics when treating diarrhea and UTI may be the cause of the high level of antibiotic resistance.

CONCLUSION

In this study, we tried to screen the antibiotic resistance pattern among patients with urinary *Escherichia coli* in the city of Hilla, Iraq, the most susceptible gender for *E. coli* was females. also, the most susceptible age group was 21-30 years, and those living in the rural areas were more susceptible than those living in the urban ones. while in most cases (39-68.4%) were married adult patients for both sexes. Antibiotic susceptibility test showed that all *E. coli* isolates were completely resistant to beta-Lactam (amoxicillinclavulanate),while resistant in different rates to ceftazidime (70%), cefotaxime (67%), aztreonam (30%), gentamicin (40%), trimethoprim (50%), nalidixic acid (59%), norfloxacin (37%), pefloxacin (30%) and ciprofloxacin (35%). In the light of these results, it is recommended to focus on the most affected groups in future prophylactic plans.

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