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The Dissemination of Multidrug Resistance (MDR) and Extensively Drug Resistant (XDR) among Uropathogenic E. coli (UPEC) Isolates from Urinary Tract Infection Patients in Babylon Province, Iraq

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Abstract:

Antibiotic resistance is a problem of deep scientific concern both in hospital and community settings. Rapid detection in clinical laboratories is essential for the judicious recognition of antimicrobial resistant organisms. So, the growth of Uropathgenic *Escherichia coli* (UPEC) isolates with Multidrug-resistant (MDR) and Extensively Drug-resistant (XDR) profiles that thwart therapy for (UTIs) has been detected and has straight squeezed costs and extended hospital stays. This study aims to detect MDR- and XDR-UPEC isolates. Out of 42 UPEC clinical isolates were composed from UTI patients. The bacterial strains were recognized by standard laboratory protocols. Susceptibility to antibiotic was measured by the standard disk diffusion method Out of 42 Uropathogenic *E. coli*, 37 (88.09%) were found to be MDR while 5 isolates (11.90%) were XDR. The present study concluded high prevalence of uropathogenic *Escherichia coli* (UPEC) with Multidrug-resistant (MDR) isolated from urinary tract infection in Babylon province – Iraq.

Key words: Antibiotic susceptibility, Extensively Drug-resistant (XDR), Multidrug-resistant (MDR), Uropathogenic *Escherichia coli*, UTI.

Introduction:

Uropathogenic *Escherichia coli* (UPEC) strains are the most significant causative agent of UTIs in humans (1). The total prevalence of UTIs caused by the UPEC strains is about 30–70% (2, 3, 4). UTIs caused by UPEC strains often requires antibiotic therapy. Accurate prescription of beta-lactams, aminoglycosides, quinolones, sulfonamides, tetracyclines, penicillins, and cephalosporins groups of antibiotics is effective for the control and treatment of UTIs. Resistance of pathogenic organisms to countenance antibiotics has become a worldwide problem with serious consequences on the treatment of infectious diseases. The heightened use/ misuse of antibiotics in human medicine, agriculture and veterinary is primarily contributing to the phenomenon. There is an alarming increase of antibiotic resistance in bacteria that cause either community infections or hospital acquired infections. Of particular interest is the multidrug resistant pathogens, *Escherichia coli*.

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A strain of UPEC would be considered as Multi Drug Resistant (MDR) bacteria if it was resistant to at least three different classes of antibiotics and Extensively Drug resistant (XDR) bacteria if it had a sensitivity to only one class of antibiotics (5). UPEC isolates can acquire antimicrobial resistance by DNA mutation or by horizontal gene transfer (HGT). Mutations occur spontaneously, at a variable frequency, depending on the antibiotic and the microorganism. Sometimes, the bacteria need to accumulate mutations in a stepwise process to develop fully functional clinical resistance, e.g., in the resistance to fluoroquinolones, and inactivation of hydrolytic enzymes by β-lactamases; permeability alteration through active efflux pumps contribute in resistance (6). The aims of this study is to detect the MDR- and XDR-UPEC isolated from urinary tract infection in Babylon province – Iraq.

Materials and Methods:

Diagnosing Bacterial isolates:

Two hundred and eight urine specimens were collected from patients with UTI admitted to Al-Hillah General Teaching Hospital and Al-Hashimyah General Hospital, during the period from February to June 2017.The age of the patients

ranged from 5 to 69 years of male and female. The identification of UPEC was performed according to the standard microbiological and biochemical protocols (7).

Ethical Approval:

The experimental work was approved by the Ethical Committees of the hospital and in compliance with recommendations of the Ethical Committees Committee; privacy was maintained regarding patient data.

Antibiotic Susceptibility Study:

Antibiotic susceptibility patterns of the bacterial isolates were evaluated using a disk diffusion assay (8). The antibiotic discs were purchased from Conda-Spain. The names and concentrations of antibiotic discs are as follows; Trimethoprim - Sulphamethazol TMP-SMX (5/250 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), norfloxacin (10 µg), levofloxacin (5 µg), ceftazidime (30 µg), cefotaxime (30 µg), Cefepime (30 µg), ceftriaxone (30 µg), cefpodoxime (30 µg), gentamicin (10 µg), amikacin (30 µg), and amoxicillin-clavulanic acid (20/10 µg), nitrofurantoin (300 µg), Doxycyclin (10 µg), Meropenem (10 µg), Imipenem (10 µg), Piperacillin -Tazobactum (110 µg), nalidixic Acid (30 µg). standardized overnight culture of each isolate was used to seed melted Mueller-Hinton agar (MHA) at 45◦C and poured into sterilized plates (in triplicate) aseptically. These were allowed to solidify and the antibiotic disks were aseptically placed on the surface of the culture media. The MHA plates were then incubated at 37◦C for 24 h. After 24 h incubation, the inhibition zones were measured and interpreted by the recommendations of the Clinical Laboratory Standards Institute (9).

Results and Discussion:

Patients and Clinical Isolates

Out of 208 urine specimens collected from the patients with suspected UTIs, 54.80%of specimens were found to be with significant bacteriuria, 20.67% with non-significant bacteriuria and 24.51% with negative culture (Fig.1).

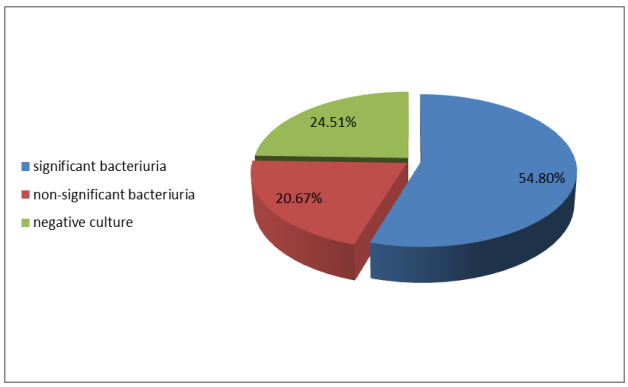

Figure 1. The percentage of significant bacteriuria, non-significant bacteriuria and negative culture.

The presence of UPEC among significant bacteriuria in this study was 42 isolates (36.84%) while 63.16% represented the percentage of significant bacteriuria with other organisms (Fig. 2).

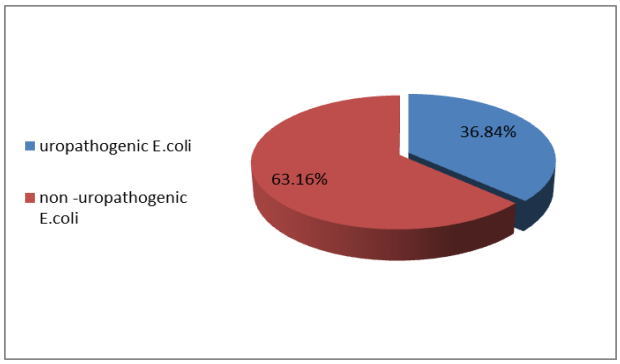

Figure 2. The percentage of uropathogenic *E. coli* among significant bacteriuria.

The frequency of UPEC in female was higher than in male and Table 1 shows the occurrence of UPEC isolates in different aged group with gender.

Table 1. The frequency of 42 uropathogenic *E. coli* isolates according to the gender in different age groups.

Age groups	No. of isolates		Total
	Female	Male	
6-15	4(9.52%)	0(0%)	4(9.52%)
16-25	8(19.04%)	1(2.38%)	9(21.42%)
26-35	11(26.19%)	6(14.28%)	17(40.47%)
36-45	5(11.90%)	1(2.38%)	6(14.28%)
46-55	2(4.76%)	1(2.38%)	3(7.14%)
More than 56	3(7.14%)	0(0%)	3(7.14%)
Total	33(78.57%)	9(21.42%)	42(100%)

Antibiotic Susceptibility:

In the present study, the 42 UPEC isolates were subjected to susceptibility test (DDT) according to the (9) guidelines using 19 different antibiotic disks. The resistance rate to the 3rd generation cephalosporins (ceftazidime, cefotaxime,

ceftriaxone and cefpodoxime) was [(92.8%), (90.47%), (90.47%) and (88.09%)] respectively (Fig.3). Also markedly, high resistance rate (95.23%) to the 4th generation cephalosporin (cefepime) was observed among isolates. The present study showed that there was elevation in the rate of resistance to cephalosporins especially the 3rd generation as well as the 4th generation cephalosporin compared with the results previously recorded by local studies, (10) in Najaf whereby it was found that the resistance rate for ceftriaxone and cefotaxime was [(79.8%), (85.6%)] respectively; with the moderate resistance rate to cefepime (59.6%). However, in Baghdad, (11) revealed that the resistance rate was > 70% to cefotaxime, ceftazidime, and ceftriaxone respectively. The increased the resistance rate among UPEC isolates mostly due to over use, disuse of medical prescription with empirical therapy that increased antibiotic pressure and increased the probability of resistance transfer such as plasmid-mediated antibiotic resistance found to be common in *E. coli*. Despite that, the majority of parenteral third-generation cephalosporins, e.g. cefotaxime, were administrated in a hospital setting. The long-term exposure to antimicrobial agents directly increases the selection pressure for resistance (12, 13, 14). Nevertheless, the high level of resistance to third-generation cephalosporin in present study is most likely due to the gaining of β-lactamases, which encodes by *bla* genes probably during therapy.

Figure 3 shows that the resistance rate to β-lactam/β-lactamase inhibitor combinations including Piperacillin/Tazobactam and Amoxicillin-Clavulanic acid was 61.90% and 100% respectively. These results were relatively in agreement with (15), who revealed that 95%of *E. coli* isolates were resistant to Amoxicillin-Clavulanic Acid; this high level of resistance could be attributed to the irrational use of drug in this locality. The increasing level of drug abuse, and the patients indulge in antibiotic self-medication, commonly to treat all kinds of infections, has been recorded as one significant way of promoting antibiotic resistance (16).

Clavulanic Acid present in the Amoxicillin-Clavulanic Acid complex is meant to afford protection to the β-lactam chemical ring nucleus present in the Amoxicillin, and this protection should be expected to enhance the activity of Amoxicillin. This observed resistance is related to permeability and absorption factors influencing antibiotic transfer across the microbial cells. Thus, the Amoxicillin- Clavulanic Acid complex has a large molecule possibly with great difficulty in permeability and overall transport across the microbial cell wall (17). It may also be due to the relatively limited quantity available to exert an antimicrobial effect (17, 18). The resistance to β-lactamase inhibitors is formed mainly by numerous mechanisms: hyperproduction of β-lactamase, production of β-lactamase resistant to inhibitors, and chromosomal cephalosporinases (19). Some bacteria produce multiple β-lactamase, which may reduce the efficiency of β-lactam/β- lactamase inhibitor combinations (20).

In the present study, Imipenem and Meropenem, (penems class), were found to be effective against the majority of UPEC isolates and the resistance rate was low (11.90% and 11.90%) respectively. These results were relatively comparable with the results of (10) who found that, the UPEC strains were resistance to imipenem and meropenem (9.6% and (25%) respectively. On the other hand, (11) showed that *E.coli* demonstrate low resistance rate to imipenem (6%). Also the result of the present study was in agreement with the frequencies, recorded in India by (21), who revealed that in case of imipenem the resistance rate was (11.86%) in (2012) and (11.36%) in (2014). The low resistance rate to carbapenems may be explained by lesser use of these injectable drugs till date.

For aminoglycosides, the resistance rates to gentamicin (78.57%) and doxycycline (83.33%) were high, while low resistance rate (21.42%) for amikacin was detected (Fig. 3). In the present study, there was observable increasing rate of resistance to gentamicin and amikacin when compared with the results of (10) who obtained resistance rate to gentamicin (61.5%) and to amikacin (2.9%). The percentages of aminoglycosides resistance described in this study harmonized with those reported by (22) and (23), who found that the vast majority of *E. coli* isolates were susceptible to amikacin. Also (14) reported that isolates were resistant to amikacin and gentamicin (7% and 63%) respectively. Also the resistance rate to doxycycline (83.33%) in the present study was higher than the results of (24) and (25) who showed that the resistance rate of *E. coli* to doxycycline was (66.6% and 78.4%) respectively.

As in the quinolones demonstrated resistance rate (64.28%) Nalidixic Acid and Ciprofloxacin, (57.14%) Ofloxacin and Norfloxacin and (50%) for levofloxacin. These results were in agreement with the results recorded by (11) who showed resistance range to ciprofloxacin, levofloxacin, and > 70% to nalidixic acid. In Pakistan (26) showed that the rate of resistance against ciprofloxacin, levofloxacin and norfloxacin were remained 60%, 58% and 57%, respectively. The findings of the present study were found to be

more than the results recorded in Nigeria by (27) who reported the rate of resistance against ciprofloxacin and ofloxacin were 27%.

Along with other antibiotics, quinolone resistance is of particular interest because it is frequently recommended for the treatment of complicated cystitis in patients, quinolones are also used as a first choice for the treatment of UTIs, mainly because of certain advantages of this antibiotic over co-amoxiclav, particularly in terms of its pharmacokinetic properties (28, 29). Quinolone resistance normally arises by mutations in the chromosomal genes (30), plus decreased membrane penetrability in conjunction with the over-expression of efflux pumps, with additional low-level resistance mediated by plasmid-mediated quinolone resistance genes,which are ever more being reported (14).

In the present study (69.04%) of the UPEC isolates were resistant to Trimethoprim-sulfamethoxazole, this occurrence of resistance recorded in the present study to Trimethoprim-sulfamethoxazole is in agreement with (11) who pointed that the resistance of *E.coli* to Trimethoprim- sulfamethoxazole was (68%). Also (31) in Mexico conducted that the resistance rate of *E. coli* to Trimethoprim- sulfamethoxazole was (66%).The resistance to TMP-SMX was due to widely used as a first choice of treatment for UTI infections . In some countries, the use of TMP-SMX has become limited, for example, German national guidelines do not recommend this agent as a first choice for the treatment of uncomplicated cystitis (32). Since TMP-SMX resistance is associated with the development of concomitant resistance to other antibiotics thereof, limited use of TMP-SMX may help to sustain its effectiveness over the long run.

The results revealed (42.85%) of the UPEC isolates were resistant to Ntrofurantoin. These results agree with the frequencies, recorded in Mexico by (31) who showed that *E. coli* had resistance to Nitrofurantoin as (44.8%). Since a greater percentage of the UTI isolates in this study were sensitive to Nitrofurantoin, it would be an excellent choice for UTI therapy while awaiting the result of culture and sensitivity tests. Also the limited use of nitrofurantoin in hospitals in the past few years, which may have led to decreased resistance level to nitrofurantoin. However, the patient's status may warrant the choice of Ciprofloxacin or Ofloxacin. This variation further supports the fact that the distribution of *E. coli* UTI-causing pathogen, including its antimicrobial susceptibility pattern, varies from place to place and changes from time to time (28). The emerging problem of antibiotic resistance in bacterial pathogens is extremely complex. The emergence of drug resistance to trimethoprim, sulfamethoxazole, the penicillins, cephalosporins, and fluoroquinolones by UPEC has limited the choices for selecting the appropriate antibiotic for the treatment of UTIs (33).

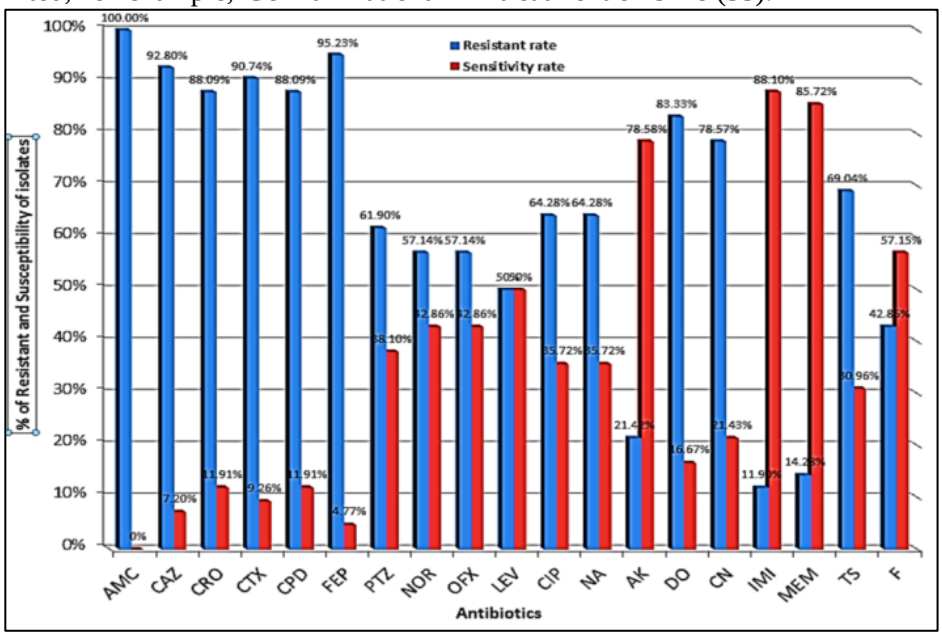

Figure 3: Antibiotic susceptibility of 42 uropathgenic *E. coli* isolates by disk diffusion test (DDT).

Determination of MultiDrug-Resistant (MDR) and Extensively Drug-resistant (XDR) uropathgenic *E. coli*

A strain of UPEC would be considered as Multi Drug- Resistant (MDR) bacteria if it was resistant to at least three different classes of antibiotics and Extensively Drug-resistant (XDR) bacteria if it had sensitivity to only one class of antibiotics (5). Figure 4 shows that out of 42 UPEC isolates, 37 (88.09%) were found to be MDR while

5 isolates 5 (11.90%) were XDR. These results were relatively comparable with the previous study conducted by (10) who reported that, 937% of *E. coli* isolates were MDR, also (23) revealed that all *E. coli* isolates obtained from Merjan Teaching Hospital in Hilla, Iraq, were considered as MDR. However, In Guinea (25) conducted that 74.4% of *E. coli* was MDR strains, whereas only 7%of *E. coli* was XDR, despite that (24) in Pakistan found that the percentage of MDR and XDR *E. coli* were 81% and 8.7%respectively.

The levels of MDR and XDR among UPEC isolates were found to be varying from country to another. Also these XDR and MDR UPEC as previously reported (34, 35).

The increase of MDR isolates and appearance of XDR in the present study is dueto uncontrolled antibiotic use in medicine over the last several years. The careless usage, without antibiotic sensitivity testing, is the most important factor promoting the emergence of MDR, which causes the selection and dissemination of antibiotic resistant pathogens in clinical medicine.

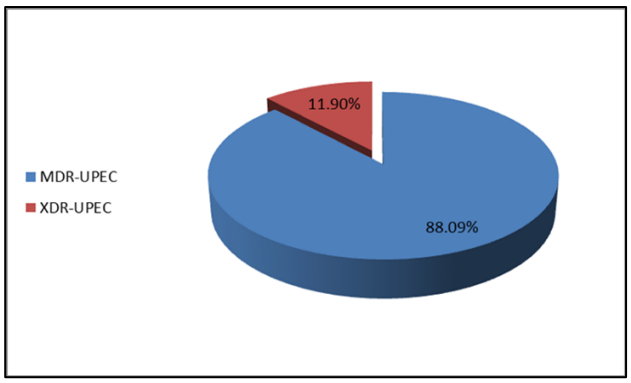

Figure 4. The Percentage of multidrug resistance among 42 Uropathogenic *E.coli* isolates.

Conclusion:

The present study reveals that the *E. coli* isolates recovered from Urinary Tract Infections in Babylon Provence have high resistance to different classes of antibiotic.

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Conflicts of Interest: None.

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انتشار صفتي المقاومة المتعددة (MDR) والمقاومة الواسعة (XDR) بين عزلات الايشيريكيا القولونية الممرضة البولية(UPEC) من مرضى التهاب المجاري البولية في محافظة بابل، العراق

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[3] فرع العلوم الطبية الاساسية، كلية التمريض، جامعة بابل، العراق.

الخلاصة:

المقاومة للمضادات تعتبر مشكلة علمية عميقة متعلقة بكلا اصابات المستشفى والمجتمع ،والتشخيص السريع في المختبرات السريرية هو ضروري و حكيم لتمييز الكائنات ذات المقاومة ضد المايكروبية ، لذلك النمو المتزايد لسلالات بكتريا القولون الممرضة للجهاز البولي ذات صفتي المقاومة المتعددة للأدوية MDR والمقاومة الشديدة للأدوية XDR مما يعقد علاج إصابات المجاري البولية وكما تم ملاحظة فيؤثر مباشرة بكلفة ومدة البقاء في المستشفى. الدراسة الحالية تهدف لدراسة صفتي المقاومة المتعددة والشديدة للأدوية بين العزلات السريرية لعزلات هذه البكتريا. حيث جمعت (42) عزلة سريرية لبكتريا القولون الممرضة للجهاز البولي من مرضى إصابات المجاري البولية، وشخصت العزلات السريرية بالطرق المختبرية القياسية، كما حسبت الحساسية المضادة لعزلات هذة البكتريا تجاة مجموعة المضادات الحيوية المقرة ضدها بطريقة نشر القرص القياسية بحسب CLSI (2016). من (42) عزلة لبكتريا القولون الممرضة للجهاز البولي، (37) 88,09% كانت ذات مقاومة متعددة للأدوية، بينما 11,90% ذات مقاومة شديدة للأدوية. استنتجت الدراسة الحالية ان نسبة إنتشار عالية لعزلات بكتريا القولون الممرضة للجهاز البولي ذات المقاومة للمتعددة للأدوية قياساً بتلك الشديدة المقاومة للأدوية، والمعزولة من مرضى إصابات المجاري البولية بمدينة بابل- العراق.

الكلمات المفتاحية: الحساسية للمضادات، فائقة – المقاومة للمضادات، متعددة – المقاومة للمضادات، الايشريشيا القولونية الممرضة البولية، التهاب المجاري البولية.