



Predominance of antibiotics resistance of *Escherichia coli* isolated from Urinary tract infection patients in diabetic and non- diabetic woman

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Abstract : *Escherichia coli* is the commonest bacterial uropathogen of UTIs, the commonest infections in diabetic and non-diabetic patients. Better understanding of their main resistance mechanisms to commonly used antibacterial agents will help to reduce the burden of this infection. The prevalence of drug resistant uropathogenic *E.coli* isolates from diabetic and non-diabetic patients attending health facilities. These patients were further categorized into the 'diabetic group' based on the following definitions: the diabetic group consisted of patients with prior diagnosis of DM (complicated or uncomplicated), with a blood HbA1C level above 6,5% . Patients with T2DM were selected in line with the standard definition of the American Diabetes Association. This study aimed to define the antimicrobial Resistance of *Escherichia coli* isolated from diabetic and non-diabetic from UTI patients in al.najaf , iraq. By assessing the susceptibility patterns of these bacterial strains, we can gain valuable insights into the burden of antibiotic resistance in this population. Such information can help guide the development of appropriate treatment strategies and inform public health policy decisions. In this study, 300 clinical individuals were collected urine samples to patients divided into groups (A and B), each containing 150 samples. all isolates were tested for antimicrobial sensitivity using the Kirby-Bauer disk diffusion method in accordance with the definition provided by the Committee of Clinical Laboratory International Standards (CLSI, 2020) on diagnostic sensitivity test plates. The McFarland standard was used to prepare bacterial inoculum suspensions for antimicrobial susceptibility testing. Mueller Hinton agar plates were streaked using a sterilized cotton swab. sterile Antibiotics were used. its were highly Resistance to most of the selected antibiotics. *E.coli* showed a high resistance to ampicillin, Both group A and group B demonstrated a high level of resistance to ampicillin 61(96.82%) vs 47(92.15%) respectively,Cefotaxime 59(93.65%) vs 45(88.23%),Ceftazidime 58(92.06%) vs 43(84.31%), Trimethoprim-sulfamethoxazol, 58(92.06%) vs 44(86.27%). also results showed that broad-spectrum antibiotics such as imipenem, meropenem and nitrofurantoin would be the first line and the most effective antibiotics for the empirical treatment of urinary tract pathogens due to their low resistance rates. its were 6(9.52%) vs 3(5.88 %),4(6.34%) vs 2(3.92 %) and 23(36.5%) vs 12(23.52%) respectively.

Keywords: Type 2 diabetes mellitus, T2DM, UTI.,*E.coli* ,Antibiotic Resistance

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Introduction

Diabetes and urinary tract infections (UTIs) are two most prominent diseases all-around, Diabetes is associated with an earlier onset and increased severity of UTIs, resulting in costly and debilitating complications. Several investigations showed the higher distribution of UTIs, urethritis, pyelonephritis, cystitis and bladder dysfunction in diabetic in compare with non-diabetic patients (1). Type 2 diabetes (T2DM) is a chronic complex disease triggered by the interaction of genetic and environmental factors, affecting over 425 million people worldwide (2,3). It has become one of the foremost chronic noncommunicable diseases distressing the health of people worldwide (4). In 2016, about 2 million patients were suffering from diabetes mellitus in Iraq (5). this a heterogeneous class of disease, and >90% of individuals with DM have T2DM, occurs as a result of deficient insulin action and inadequate insulin secretion rather than insufficient use of insulin. Individuals with T2DM are at high risk of moderate or severe infections, including gangrenous cholecystitis, foot infections, soft-tissue infections and urinary tract infections (UTI) (6). Documented data revealed that near to 50% of people have been affected by UTIs all-around the world (7). People living with diabetes are more susceptible to infections than people without diabetes, One of the commonest infections in these immunocompromised diabetic patients is UTI, This results from decreased cellular responses and poor pathogen clearance that are very common in these patients (8).

(UTIs), which can occur in community and hospital, are among the most prevalent bacterial illnesses. Sothat, UTIs affect 150 million people worldwide each year and are one of the most pervasive infections seen in

modern medical treatment, affecting people of all ages, from newborns to the elderly (9,10). The major etiologic agent in the uncomplicated UTIs in females is uropathogenic *E. coli* (UPEC), which accounts for 75-95% of cases (11,12).

Antibiotic resistance in *E. coli* is a serious problem, especially for diabetic patients (13). The overuse and misuse of antibiotics have led to the development of antibiotic-resistant strains of *E. coli*, making it difficult to treat infections, due to the country's ongoing conflict and limited healthcare resources, access to appropriate antibiotics is often limited (14). This has led to the widespread use of broad-spectrum antibiotics, contributing to the emergence and spread of resistant bacteria. The increase of antibiotic resistance and appearance of multi-drug resistant (MDR) pathogens in the course of UTI is related to high rates of inadequate antibiotic empirical therapies prescribed without the antibiotic susceptibility testing and finally result in an ineffective UTI treatment (15).

Bacteria that are resistant to multiple antimicrobial agents are called multi-drug resistant (MDR), those with extensive drug resistant (XDR) or totally drug resistant (TDR) are sometimes called "superbugs" (16).

Materials and Methods

In this study, 300 clinical individuals were collected urine samples to patients divided into two group were obtained mainly from Al-Najaf governorate, AL-Sadr Medical City/ Diabetic Center, Al-Hakim General Hospital, and Al-Najaf Teaching Hospital, as well as in some private clinics and laboratories, for the period from 1st August /2022 to end July / 2023. The ages of patients ranged between (20-75) years.

Sample collection and processing

1-The first group (A) women patients have type 2 diabetes mellitus and

urinary tract Infection (UTI) with *E.coli*: 150 midstream urine samples were collected from women patients, who have T2DM and symptoms of urinary tract infection (UTI). suffering from symptoms of UTI (defined as a combination of the following symptoms:the presence of clinical signs or symptoms of UTI in the host, including dysuria and frequency or urgency of urination). These 150 patients were further categorized into the 'diabetic group based on the following definitions: the diabetic group consisted of patients with prior diagnosis of DM (complicated or uncomplicated), with a blood HbA1C level above 6.5% . Patients with T2DM were selected in line with the standard definition of the American Diabetes Association.

2 -As for the second group (B) : women patients have urinary tract Infection non-diabetic:150 midstream urine samples were collected from women patients from women patients.

Each patient was instructed to fill a sterile urine container with (5 mL) of midstream urine. Within 1 hour of sample collection, samples were processed in the laboratory following proper guidelines to prevent contamination. data and other information including clinical symptoms, previous antibiotics, and length of antibiotic usage were collected using a standardized questionnaire. Each patient enrolled in this study.

Any patient with a history of antibiotic intake and who were on immune suppressive drugs in the preceding 2 weeks, showing impaired blood glucose level with HbA1C <6.5% were excluded from the study.

Identification of Isolated Microorganisms

Overall, 300 urine samples (5 mL) were collected from the selected patients both group. Isolation and identification of isolates were done

following their morphology in Gram's staining, cultural characteristics and biochemical properties, as per the Manual of Clinical Microbiology. Identified as *E.coli* by cultural, biochemical characteristics and Vitek-2 system. The positive urine samples were subcultured on specific media.

Antimicrobial susceptibility testing:

Investigation for the susceptibility pattern of bacteria is considered a useful method for determining the future challenges of effective therapy Disc diffusion method was used in this study. sixty three from group A and fifty one from group B isolates of *E.coli* were tested for their susceptibility against 13 type of antibiotics: Amoxicillin, Cefotaxime, Ceftazidime, Amoxicillin-Clavulanicacid, Trimethoprim – sulfamethoxazol, Aztreonam, Ceftriaxone, Ciprofloxacin, Amikacin, Gentamicin, Nitrofurantoin , Imipenem, Meropenem. The McFarland standard was used to prepare bacterial inoculum suspensions for antimicrobial susceptibility testing.. Plates of Muller-Hinton agar were used to find the sensitivity pattern and incubated at 37°C for 24 hours. The zone of the inhibition of the bacterial growth was measured after incubation and compared with the clinical and laboratory standards institute (CLSI, 2020).

Results

Identification of Sample

Depending on the gram stain, morphological features on culture media (MacConkey agar, Blood agar , Eosine methylene blue agar (EMB) and Vitek 2 system, out of 300 urine samples; preliminary results showed there is a growth in 235 (78.33%) specimens, there were 204 (68.%) specimens diagnosed as Gram-negative bacteria, while 35 (10.33%) specimens were diagnosed as Gram-positive bacteria and 65 sampel (21.67%) with no any growth as shown in Figure (1).

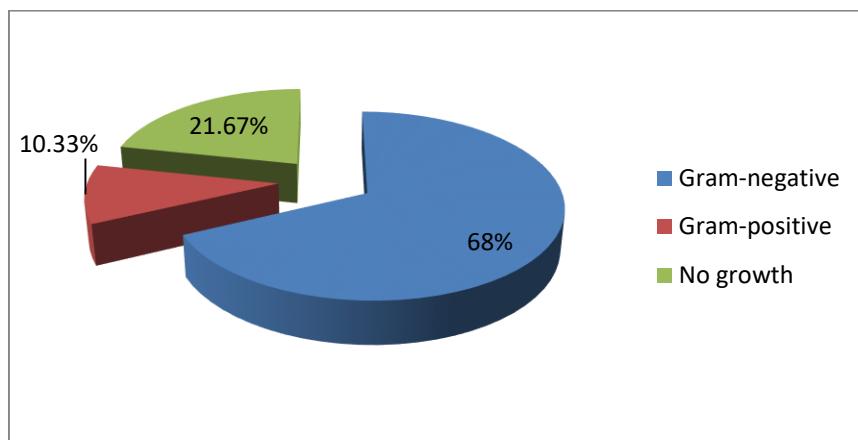


Figure (1): percentage of total specimens (N=300)..

After confirming the examination with Vitek 2 system. the results showed Prevalence of many types of bacteria,

The results were shown in this study : *E.coli* 63(51.63%) to group A vs 51(45.13%) to group B , *Klebsialla pneumonia* 21(17.21%) group A vs 17(15.04%) to group B, *Proteus mirabilis* 11(9.01%)to group A vs 10(8.84%) to group B, *Staphylococcus aureus* 8(6.55%) to group A vs

11(9.73%) to group B, *Pseudomonas aeruginosa* 7(5.73%) to group A vs 10(8.84%) to group B, *Acinetobacter baumannii* 6(4.91%) to group A vs 5(4.42%)to group B, *streptococcus epidermidis* 4(3.27%) to group A vs 8(7.07%) to group B, *Enterobacter spp* 2(1.63%) to group A vs 1(0.88%) to group B. distributed as following: (Table 1)

Table (1): percentage of total bacterial growth isolated

Bacterial growth	Group(A) B.G. N=122, %	Group(B) B.G. N=113 ,%	Total B.G. N=235
<i>E.coli</i>	63(51.63%)	51(45.13%)	109(47.18%)
<i>Klebsialla pneumonia</i>	21(17.21%)	17(15.04%)	36(15.58%)
<i>Proteus mirabilis</i>	11(9.01%)	10(8.84%)	21(9.09%)
<i>Staphylococcus aureus</i>	8(6.55%)	11(9.73%)	15(6.49%)
<i>Pseudomonas aeruginosa</i>	7(5.73%)	10(8.84%)	16(6.92%)
<i>Acinetobacter spp</i>	6(4.91%)	5(4.42%)	8(3.46%)
<i>Strept. epidermidis</i>	4(3.27%)	8(7.07%)	13(5.62%)
<i>Enterobacter spp</i>	2(1.63%)	1(0.88%)	7(3.03%)
<i>No growth</i>	28(18.66%)	37(24.67%)	65(23%)
Total	150(100%)	150(100%)	300(100%)

Antimicrobial resistance of UPEC

UTIs are associated with significant use of antibiotics that cause implications for bacterial ecology and spread of resistance to antibiotics, especially when it stems from the empirical antimicrobial treatment of

recurrent UTIs. Antimicrobial resistance in UPEC and the spreading of MDR UPEC in recent decades is a clinical problem, particularly in women with recurrent UTIs. The increasing frequency of MDR UPEC, especially in developing countries, results in

excessive use of broad-spectrum antibiotics such as fluoroquinolones, cephalosporins, and aminoglycosides that raise the cost of treatment and hospitalization (19.20). Antimicrobial

resistance between UPEC is increasing in many countries and shows the time- and area-related variability .

The results shown Antibiotics Resistant to (Table 1)

Table (2): Results Antibiotics Resistant from *E.coli*

No	Antibiotics	Con.	Resistant Group A	Resistant Group B
1	Ampicillin	25 μ g	61(96.82)%	47(92.15%)
2	Cefotaxime	30 μ g	59(93.65%)	45(88.23%)
3	Ceftazidime	30 μ g	58(92.06%)	43(84.31%)
4	Trimethoprim –sulfamethoxazol	1.25/23.75 μ g	58(92.06%)	44(86.27%)
5	Amoxicillin + Clavulanic acid	20/10 μ g	57(90.47%)	43(84.31%)
6	Aztreonam	30 μ g	55(87.3%)	39(76.47%)
7	Ceftriaxone	30 μ g	48(76.19%)	37(72.54%)
8	Ciprofloxacin	5 μ g	46(73.01%)	35(68.62)
9	Amikacin	30 μ g	40(63.49%)	30(58.82%)
10	Gentamicin	10 μ g	37(58.73%)	28(54.9%)
11	Nitrofurantoin	300 μ g	23(36.5%)	12(23.52%)
12	Imipenem	10 μ g	6(9.52%)	3(5.88 %)
13	Meropenem	10 μ g	4(6.34%)	2(3.92 %)

Discussion

This study showed the prevalence of *E. coli* as a Gram-negative bacteria that considered a major cause of urinary tract infection followed by *Klebsiella pneumonia*. Its is in agreement with studies conducted in Iraq, who recorded that *Escherichia coli* was the most common pathogen, followed by *Klebsiella* ssp. (17).

According to the results showed in table (2), The results for were:

There was difference between two groups A,B as regard to most resistant antibiotics in urinary tract infection with *E.coli*. In this study, *E.coli* showed a high resistance to ampicillin, Both group A and group B demonstrated a high level of resistance to ampicillin 61(96.82%) vs 47(92.15%) respectively. Similar approximately results recorded by Hussien. , he found the resistance percentage toward ampicillin among *E.coli* strains isolated from urine were (97.78%)(18). Another studie in Iraq mentioned by Fadhil of the 201 bacterial isolates studied, 174

(86.60%), that *E.coli* were the most resistant isolates to Ampicillin (19). In contrast Shah et al., who mentioned that (51%) of isolates were resistant to Ampicillin (20).The resistance of *E.coli* to Ampicillin is related to the widespread use of this antibiotic without the real need and due to the multiple use of this antibiotics by hospitals for different infections (21).

The increasing of *E.coli* resistance to Cefotaxime two groups A,B 59(93.65%) vs 45(88.23%). Similar results recorded by researchers Hussien, Sabir , who found the resistance percentage toward Cefotaxime among *E. coli* strains isolated from urine were (95.56% , and 89.7%) respectively(18.22). While this finding was slightly higher than the resistance percentage mentioned in previous studies, who recorded (79% and 74.4%) of the Cefotaxime resistance, respectively. These studies conducted in different regions ; India and Egypt (23.24).

The resistance percentage toward Ceftazidime was 58(92.06%) vs 43(84.31%). the present results are in agreement with studies in Iraq mentioned by Assafi, *et al.*, of the 71 bacterial isolates studied, 62 (87.32%), that *E.coli* were the most resistant isolates to Ceftazidime(25). On the other hand the results didn't agree with the studies other researchers who indicated that (55.3%)(56%) of *E.coli* isolates were resist to this antibiotics(26.27).

Trimethoprim-sulfamethoxazol resistance rate of the *E.coli* from UTI was 58(92.06%) vs 44(86.27%) close enough to a study done by Al-Shaboot *et al.*, which recorded resistance percentage of *E.coli* for Trimethoprim-sulfamethoxazol (92.5%)(28).While another study in Sudan mentioned the percentage of Trimethoprim - sulfamethoxazol was (88.3%) (29).

In this study showed level of resistance to Amoxicillin + Clavulanic acid 57(90.47%) vs 43(84.31%) . The present results are in agreement with some studies ,The results showed (90.1%),(88%),(85%)(27.30.31). On the other hand the results didn't agree with the study in from Korean patients,, who found a few isolates of *E.coli* resistant to Amoxicillin + Clavulanic acid (42.9%)(32)

High resistance also observed against Aztreonam 55(87.3%) vs 39(76.47%). the results was matched with results of Al-Shaboot, who recorded resistance percentage of *E.coli* for Aztreoname (88.1%)(28). But this study differs with this antibiotic in study from iraq showed resistance (97.78%) (18)

Also in the current study more than half of *E.coli* isolates were resistance to anibiotics such as Ceftriaxone 48(76.19%) vs 37(72.54%), Ciprofloxacin 46(73.01%) vs 35(68.62), Amikacin 40(63.49%) vs 30(58.82%)

and Gentamicin 37(58.73%) vs 28(54.9%). this result was reported high in comparison with study in Iraq, its results were as follows: Ciprofloxacin (52.74%), Amikacin (50.54%),Ceftriaxone (48.35%), Gentamicin (42.85%)(17).while these results were not consistent with According to Tajbakhsh *et al.*, when they are reported that *E.coli* was resistance to many antibiotics such as Ciprofloxacin (56.25%), ceftriaxone (41.25%), Amikacin (38.75%), Gentamicin (18.75%) (33), Katongole, *et al.*, who they found that uropathogens *E.coli* isolates were resistance, Gentamicin, Ciprofloxacin and Ceftriaxone (87%),(62%) and (55%) respectively (34) . In another study , who they found that uropathogens *E.coli* isolates of pediatrics in North of Iran were resist to Ceftriaxone (28%) , Amikacin (34%) Gentamicin (37%) , ciprofloxacin (76%)(35) . while Dash, *et al.*, noted that (70%) of *E.coli* isolates were resistance to gentamycin and ciprofloxacin (60%) resistance to (40%) was identified to amikacin (36).

Some isolates of *E.coli* for the samples under study were resistant to nitrofurantoin 23(36.5%) vs 12(23.52%). The results of the investigation agreed with studies for researchers, who found a few isolates of *E.coli* resistant to Nitrofurantoin (32.05%),(25.5%) (25.33). while hand the result didn't agree with the study by (Aal-Aaboda and Al-Notazy,) who indicated that (79%) of *E.coli* isolates were resist to this antibiotics(37).

The current study showed low resistance to antibiotics Imipenem and Meropnem where very few isolates of *E.coli* have resistance to this antibiotic 6(9.52%) vs 3(5.88 %) and 4(6.34%) vs 2(3.92 %) respectively. By comparing the results with other researchers, it can be said that there is similarity with the

results of Another studie in Iraq,where very few isolates of *E.coli* have resistance to this antibiotic (8%) , (4%) respectively(30). while hand the result didn't agreed with the study by Sabir, *et al.*, imipenem (43.3%)(22).

Results showed that broad-spectrum antibiotics such as imipenem, meropenem and nitrofurantoin would be the first line and the most effective antibiotics for the empirical treatment of urinary tract pathogens due to their low resistance rates. in comparison with other antibiotics high resistance like Ampicillin, Cefotaxime, Ceftazidime, Trimethoprim- sulfamethoxazole, Amoxicillin + Clavulanic acid and Aztreonam.

The prevalence of antimicrobial resistance among the human clinical isolates of *E.coli* has increased dramatically in recent years, which has created a serious problem in the treatment of these diseases. This high frequency of antibiotics resistance may be due to, the exaggerated use of antibiotics has led to the selection of new strains of bacteria that resist to antibiotics, a situation which is found in the case of UPEC strains, self-prescription policy, comparatively cheaper antibiotics intake, lack of dependency on laboratory guidance and inadequate doses of antibiotics intake, and easily available over the counter with out prescription of registered medical.

The increasing rates of antibiotics resistance isolates in Iraq may be due to limited infection surveillance program, limited labrotary facilities ,lack of communication between physicians and microbiology ists, lack of standardizod criteria to determine antibiotic resistant isolates and poor sanitation.

Conclusions

Urinary tract infection due to *E.coli* may be difficult to treat empirically due

to high resistance to commonly used antibiotics. Continuous surveillance of multidrug resistant organisms and patterns of drug resistance are needed to prevent treatment failure and reduce selective pressure.

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