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PREVALENCE OF MULTIDRUG RESISTANT *E. coli* AND ROLE OF B-LACTAMASE

Abou Dobara, M. I.[1]; Heba E. Al-Degla[2] and A. H. Elnagdy[1]
[1] Botany Department, Faculty of science, Damietta University, Egypt
[2] Microbiology Department, Faculty of Medicine, Mansura University, Egypt

ABSTRACT

Urinary tract infection (UTI) is one of the most common bacterial infections and Gram negative bacteria are among the most prevalent bacteria detected from UTI patients. E. coli is the most common organism causing both community as well as hospital acquired UTI. The high incidence of UTIs in the general population, the potential for complications, and the associated costs of treatments emphasize the importance of appropriate antibiotic therapy. The resistance rates of uropathogenic *E. coli* to various antibiotics have been reported as beta-lactams, trimoxazole, quinolones, gentamicin, amikacin, cefuroxime, nalidixic acid

Keywords: Urinary tract infection, *E. coli.,* β-lactamase, Extended-spectrum β-lactamase.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections and Gram negative bacteria are among the most prevalent bacteria detected from UTI patients. Urinary tract infections have an estimated figure of 150 million per annum worldwide. In fact, UTIs are the leading cause of Gram-negative bacteremia in patients of all ages and are associated with a high risk of morbidity and mortality, especially in the elderly, and account for significant health care costs (1).

Gram-negative bacteria causing UTI include *Escherichia coli (E. coli), Klebsiella species, Enterobacter species, Proteus species* and Gram-positive bacteria include *Enterococcus species,* and *Staphylococcus saprophyticus. E. coli* is the most common organism causing both community as well as hospital acquired UTI (2). More than 50% of UTI infections in patients is accounted for *E.coli* (3).

The pathogenic *E. coli* strains are classified into: Enteric or diarrheagenic *E. coli* strain which gives rise to gastroenteritis, but rarely causes disease outside of the intestinal tract (4), Extraintestinal *E. coli* strain: which exist in the gut without consequence, but can also disseminate and colonize in other host niches including the blood, the central nervous system and the urinary tract, resulting in disease. An important subtype of the second strain is Uropathogenic *E. coli* (5).

UPEC strains encode a number of virulence factors, which enable the bacteria to colonize the urinary tract and resist highly effective host defense. Virulence factors of *E. coli* that are essential for development of UTIs can be divided into two groups (6): (i) virulence factors associated with the surface of bacterial cell and (ii) virulence factors which are secreted and exported to the site of action. Surface virulence factors of UPEC include a number of different types of adhesive organelles (adhesins or fimbriae), which promote bacterial attachment to host tissues within the urinary tract and determine its pathogenicity. Secreted virulence factors include toxins which are produced by colonizing *E. coli* may cause an inflammatory response which leads for UTIs symptoms (7).

One of the most common ways for bacteria to resist the actions of antimicrobial agents is the production of enzymes that inactivate a drug. The classic example of this phenomenon is β-lactamase production which are enzymes that open the beta-lactam ring, inactivating the β-lactam antibiotic (8). Extended-spectrum β-lactamase(ESBL) are enzymes that inactivate most of β-lactam antibiotics, including penicillins, cephalosporins and the monobactam aztreonam. They are found exclusively in Gram-negative organisms, primarily *Klebsiella pneumoniae, Klebsiella oxytoca and E. coli*. Most ESBLs do not break down cephamycins or carbapenems and are susceptible to β-lactamase inhibitors as clavulanic acid (9). The aim of this study to investigate the prevalence and antimicrobial resistance pattern of multidrug resistant E. coli causing UTI in patients from Mansoura. Also, to detect production of ESBL (Extended Spectrum β-Lactamases) and AmpC by the multidrug resistant *E. coli*.

MATERIALS AND METHODS

This study will include all patients with UTI attending Mansoura University Hospitals whether outpatients or inpatients. Midstream urine samples will be collected by the clean-catch technique. Microscopic examination of a fresh film of the sediment will be done to detect the presence of pus cells, R.B.Cs, epithelial cells, crystals and others. Urine samples will be inoculated onto CLED agar. The standard disk diffusion microbial sensitivity test based on Kirby–Bauer method on Mueller Hinton agar will be done for all the isolates to assess the antibiotic resistance as per Clinical and Laboratory Standard Institute 2010 guidelines. Phenotypic detection of ESBL production in multidrug resistant (MDR) *E. coli* by the Modified Double Disc Synergy Test (MDDST). All the isolates which will show a synergistic effect with cefepime only in MDDST will be further tested for the AmpC enzyme production by AmpC disc test.

Patients and methods

The study was carried out from January 2015 to September 2015 at the Microbiology Diagnostics and Infection Control Unit at the Medical Microbiology and

immunology Department, Faculty of Medicine Mansoura University. The study included urine samples collected from 200 outpatients and inpatients with UTI. These patients included different ages ranged from 8 years up to 67 years. The patients were 80 males and 120 females. 73 patients had UTI for the first time, while 127 patients had recurrent UTI.

A UTI was clinically defined according to CDC criteria as at least one symptom suggestive of this infection and included fever > 38◦C, urgency, dysuria, frequency, suprapubic tenderness, costovertebral angle pain or tenderness and Positive urine culture of ≥103 and <105 CFU/ml with no more than 2 species of microorganisms. Plus one of the following: Positive Gram stain of unspun urine , Positive dipstick for leukocyte esterase and/or nitrite Pyuria (urine specimen with ≥10 white blood cells [WBCs]/mm 3c or ≥3 WBC/high power field of un spun urine).

Collection of urine specimens

In non catheterized patients, midstream urine samples will be collected by the clean-catch technique. Urine will be collected in a sterile, wide mouthed container with a tightly fitted lid. In catheterized patients, the catheter will be clamped off to allow collection of freshly voided urine. Urine will be aspirated by a syringe after disinfecting the area of needle puncture by 70% alcohol. Direct microscopic examination of a fresh film of the sediment will be done to detect the presence of pus cells, R.B.Cs, epithelial cells, crystals and others. Urine samples will be inoculated onto CLED agar. After 24-48 hours of incubation at 37ºC, the number of bacteria will be estimated by counting the number of colonies that appeared on the medium. The number of colonies will be multiplied by 100, and this represented the number of colony forming units (CFU) in a milliliter of urine. Identification of growth and antibiotic susceptibility test will be done. Phenotypic detection of ESBL production in multidrug resistant *E. coli* by the MDDST and Amp C Detection will be done (10).

Statistical Analysis

The Statistical software SPSS 15.0 was used for the analysis of the data. Results were calculated on the basis of number and percentages. P values were calculated using a 2 test. P <0.01 were considered to be statistically significant.

RESULTS

Out of the 412 urine samples processed for bacterial culture, 300 (72.8%) were positive for growth (Table 1) . The frequencies for the pathogens in the positive cases were as follows, most frequent Gram-negative bacterium was *E.coli* with 200 cases (66.7%), MDR *E.coli* was (69.5%) (Table 2). The resistance rate of *E.coli* detected from the culture was found to be 96% for Ampicillin , 80% for Ciprofloxacin & Ceftriaxone , 78 % for Ceftriaxone, 71% for Cefuroxime , 62% for Norfloxacin , 52% for Pipracillin/tazobactam , 48% for Amikacin , 42 % for Cefipime & 37.5 % for imipenem. Of the 200 *E. coli* isolates, 64.5% (129/200) were found to be ESBL producers by MDDST (P < 0.001) (Table 3). Pure ESBL production was seen in 40.4% (98/129) *E. coli*. While ESBL and Amp C co producers were 24.1% (31/129) (Table 4). There was positive relationship between the resistance of *E. coli* to different antibiotics and the age. The oldest age (>80) shows the maximum resistance 35.3% for all antibiotics (Table 5).

Table (1): Zone diameters interpretive standards for E. coli according to the CLSI (11)

Antibiotic	Zone diameter inmm		
	Highly Sensitive	Intermediate Sensitive	Resistant
CIP	>21	16-20	<15
AK	≥17	15-16	≤14
TZP	≥21	18-20	≤17
IPM	≥16	14- 15	≤ 13
NOR	≥17	13-16	≤12
F	≥17	15-16	≤14
CXM	≥18	15- 17	≤ 14
AM	≥ 29	21-28	≤20
FEP	≥18	15-17	≤ 14
CRO	≥21	14-20	≤ 13

Table (2): Resistance in E.coli isolates according to age

Age Group (years)	Antibiotics									
	CIP 160	NOR 104	AK 68	CXM 132	CRO 156	FEP 64	F 160	AM 192	IPM 75	TZP 92
1-20	17	4	2	15	13	5	6	10	8	8
21-40	22	12	9	19	21	8	17	21	10	13
41-60	30	22	15	23	34	12	33	22	11	19
61-80	33	30	20	34	41	18	47	51	16	24
>80	58	36	22	41	47	21	57	89	30	28

The table shows the positive relationship between the resistance of *E. coli* to different antibiotics and the age. The oldest age (>80) shows the maximum resistance 35.3% for all antibiotics.

Table (3): Resistant pattern of E.coli isolates

Antibiotic	Isolates resistant no.	(percentage %)
1- Ampicillin	192	96%
2- Ciprofloxacin	160	80%
3- Nitrofurantoin	160	80%
4- Ceftriaxone	15	78%
5-Cefuroxime	142	71%
6-Norfloxacin	124	62%
7-Pipracillin/tazobactam	104	52%
8- Amikacin	96	48%
9- Cefipime	84	42%
10-imipenem	75	37.5%

Table (4): Extended spectrum beta-lactamase (ESBL) detection

E.coli	Total ESBL Percentage %	ESBL and Amp C Co-producers	Pure ESBL Produsers
(Total No. =200)	129 (64.5%)	31 (24.1%)	98 (40.4%)

Table (5): Antibiotic resistance of ESBL producers and non producers to non β-lactam various antibiotics

Antibiotics	ESBL producers (No. = 129) Resistant No.(%)	Non ESBL producers (No. = 71) Resistant No. (%)	P value
Ciprofloxacin (160)	121 (75.63%)	12 (24.37%)	< 0.001
Norfloxacin (124)	113 (91.1%)	11 (8.9%)	< 0.001
Nitrofurantoin (160)	116 (72.5%)	44 (27.5%)	< 0.001
Amikacin (96)	88 (91.7%)	8 (8.3%)	< 0.001

DISCUSSION

MDR *E coli* has become a major public health concern, causing failure in treatment with consequent huge health burden. UTI due to MDR *E.coli* increases the cost of treatment, morbidity and mortality especially in developing countries (12). A wide range of antimicrobial agents effectively inhibit the growth of *E. coli*. The β-lactams, fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole are often used to treat community and hospital infections due to *E. coli* (13). One of the most common ways for bacteria to resist the actions of antimicrobial agents is the production of enzymes that inactivate a drug. The classic example of this phenomenon is β-lactamase production. β-lactamase are enzymes that open the beta-lactam ring, inactivating the β-lactam antibiotic (14). ESBLs are enzymes that inactivate most of β-lactam antibiotics, including penicillins, cephalosporins and the monobactam aztreonam. They are found exclusively in Gram-negative organisms, primarily *Klebsiella pneumoniae, Klebsiella oxytoca* and *E. coli*. Most ESBLs do not break down cephamycins or carbapenems and are susceptible to β-lactamase inhibitors as clavulanic acid (9).

Our results confirmed the results of Lane and Takhar, (2011) who found that *E.Coli* was the most prevalent uropathogen (49%) followed by *Pseudomonas spp*. (25%), *Proteus spp*. (10%), *Staphylococcus aureus* (5%), *Klebsiella spp*. (5%), *Serratia* (4%) and *Alcaligenes* (2%). Most of the urinary tract infections are caused by gram-negative bacteria like *E. coli*, *Klebsiella sp., Proteus vulgaris, Pseudomonas aeruginosa, Acinetobacter* and *Serratia*. 90% of UTI cases are caused by gram-negative bacteria while only 10% of the cases are caused by gram-positive bacteria (2).

In agreement with our study, Swami et al., (2012) found that the incidence of resistance to antibiotics fluoroquinolone or trimethoprim-sulfamethoxazole plus fluoroquinolone increased significantly with age. Among 1600 patients, incidence was 1110 in patients 80 years and older; 430 in those 65 to 79 years of age and 60 in 18 to 64 years of age (15).

Our study showed resistance to Ampicillin, Ciprofloxacin & Nitrofurantoin, Ceftriaxone, Cefuroxime, Norfloxacin, Pipracillin/tazobactam, Amikacin, Cefipime and imipenem. But, the results of Niranjan and Malini (2014) showed high levels of resistance to ampicillin (88.4%), amoxicillin-clavulanic acid (74.4%), norfloxacin (74.2%), cefuroxime (72.2%), ceftriaxone (71.4%) and co-trimoxazole (64.2%); but, isolates were sensitive to amikacin (82.6%), piperacillin-tazobactum (78.2%), nitrofurantoin (82.1%) and imipenem (98.9%) (8).

Our study showed 69.5% MDR and Niranjan and Malini (2014) showed 76.51% MDR (8).

Our results showed that out of (64.5%) ESBL producing *E. coli*, (40.4%) are pure ESBL producers and (24.1%) are both ESBL and Amp C co producers. The study of Gupta et al., (2013) also showed that of (52.6%) ESBLs positive strains producers, (10%) of strains was AmpC screening positive and 8% of the strains are co-producers of ESBL and AmpC (16).

CONCLUSION

This study concluded that *Escherichia coli* is the most common cause of UTI. Other bacteria as

Staphylococcus aureus, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp. and *Enterobacter* spp. may also be the cause. Also, viruses or fungi may rarely be the cause. MDR E.coli develops against antimicrobial agents that are used to inhibit the growth of *E. coli* such as: β-lactams, fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole. β-lactamase production is the most important contributing factor to resistance. ESBL are enzymes that inactivate most of β-lactam antibiotics, including penicillins, cephalosporins and the monobactam aztreonam.

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إثبات مقاومة الباكتيريا(*E coli*) لأنواع متعددة من المضادات الحيوية و دور إنزيمβ-lactamase

محمد إسماعيل أبودوبارة[١] ، وهبة السيد الدجلة [٢] و أحمد حازم النجدي[١]

[١]قسم النبات- كلية العلوم – جامعة دمياط

[٢] قسم الميكروبيولوجي – كلية الطب – جامعة المنصورة

التهاب المسالك البولية هو العدوى التي تصيب جزء من المسالك البولية بداية من الكلى، الحالب، المثانة أو مجرى البول. يؤثر التهاب المسالك البولية على كل من الذكور والإناث، ولكن تعتبر الاناث أكثر عرضة بسبب تركيب الجهاز البولي والتناسلي. تعتبر البكتيريا هي المسؤل الرئيسي عن التهاب المسالك البولية خاصة الايشريشيا كولاي ولكن بعض الفيروسات و الفطريات نادرا ما تكون السبب. باكتيريا الايشريشيا كولاي تمتلك بعض العوامل التي تمكنها من غزو العائل واصابته بالمرض. وهذه العوامل قد تكون سطحيه مثل افراز مادة لاصقة تعزز التصاق البكتيريا بالأنسجة داخل المسالك البولية، أوقد تكون مواد مفرزه كالسموم التي تسبب التهابات في الانسجة محدثة أعراض عدوى المسالك البولية. تستطيع باكتيريا الايشريشيا كولاي مقاومة العديد من المضادات الحيويه مثل: البيتا لاكتام، الأمينوجليكوزيدات وميثوبريم- سلفاميثوكسازول وذلك عن طريق بعض الانزيمات التي تقوم بتكسير حلقة البيتا لاكتام . يوجد بعض الانزيمات الموسعه التي تقوم بتعطيل معظم المضادات الحيوية الحلقية. وقد اجريت أجريت هذه الدراسة في قسم الميكروبايولوجيا والمناعة الطبية في كلية طب جامعة المنصورة على ٢٠٠ مريض مصاب بالتهاب المسالك البولية. تم تجميع عينات البول و تحديد نمو باكتيريا الايشريشيا كولاي وتم اختبار مقاومتها للمضادات الحيوية المختلفة عن طريق تعيين نمو الباكتيريا في أقراص تحتوي على العديد من هذه المضادات الحيوية. بعد اجراء هذا البحث وجد أن باكتيريا الايشريشيا كولاي هي السبب الرئيسي لحدوث التهاب المسالك البولية وأن انتشار مقاومة هذه الباكتيريا للعديد من المضادات الحيويه سببه افراز بعض الانزيمات التي تقوم بتكسير حلقه البيتا لاكتام الموجودة في المضادات الحيويه.