Original Research Article

Prevalence of Multidrug Resistant Uropathogenic *E. coli*: Role of β-lactamase

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A B S T R A C T

Urinary tract infection (UTI) is one of the most frequent health problems in clinical settings. *E. coli* is the most prevalent organism causing both community as well as hospital acquired UTI. Uropathogenic *E. coli* shows increasing rates of resistance to various antibiotics. The frequent and improper usage of antibiotics resulted in the development of resistant strains of bacteria. To determine antimicrobial susceptibility pattern of uropathogenic *E. coli* isolates and evaluate the production of ESBL (Extended Spectrum β-Lactamases) and AmpC by the multidrug resistant strains. Uropathogenic *E. coli* strains, over the period of the study, were collected and studied. Antibiotic susceptibility testing was done. Phenotypic detection of ESBL production was done among the multidrug resistant (MDR) strains using the Modified Double Disc Synergy Test (MDDST). All the isolates which showed a synergistic effect with cefepime were further assessed for the production of AmpC enzyme by AmpC disc test. Two hundred non duplicate *E. coli* strains were recovered during the period of the study. Out of them 139 (69.5%) strains were MDR, of which 129 (64.5%) isolates were found to be ESBL producers (P < 0.001). Pure ESBL production was seen in 98 (49%) isolates, while31 (15.5%) isolates were co-producers of ESBL and AmpC. MDR uropathogenic *E. coli* becomes prevalent in clinical setting. This is attributed to both ESBLs and AmpC production. It is essential to limit antibiotic consumption and to establish protocol for routine and regular monitoring of ESBL production among clinical isolates.

Introduction

Urinary tract infection (UTI) is major health concern, it is one of the most common infection managed in clinical setting. It is associated with a high risk of morbidity and mortality, especially in the elderly, and account for significant health care costs (Dembélé *et al.,* 2015). It is caused by wide variety of micro-organisms; both Gram-negative as well as Gram-positive bacteria. *E. coli* is the most predominant pathogen isolated from both community as well as hospital acquired UTI; More than 50% of UTI

infections is caused by *E. coli* (Belanger *et al.,* 2011).

Uropathogenic *E. coli (UPEC)* encode a number of virulence factors, which enable the bacteria to colonize the urinary tract and resist highly effective host defense and antibiotics (Niranjan and Malini, 2014). Various drug resistance mechanisms help the bacteria to resist drug action making the treatment options limited. One of the most common ways to resist the actions of antimicrobial

agents is the production of enzymes that inactivate a drug. The production of β-lactamase is the classic example of this phenomena, this enzyme inactivates the β-lactam antibiotics by opening β-lactam ring present in penicillins, cephalosporins, and monobactams (Madigan *et al.,* 2015).

The widespread use of broad spectrum antibiotics favors the development of bacterial resistance. Extended-spectrum β-lactamases (ESBLs) are those enzymes that hydrolyze beta lactam antibiotics including third and fourth generation cephalosporins and aztreonam. Most ESBLs do not break down cephamycins or carbapenems and are susceptible to β-lactamase inhibitors as clavulanic acid. ESBLs are plasmid-mediated enzymes which may be transferable from one bacterial strain to other and between different species (Rupp and Fey, 2003).

The production of ESBLs limit the therapeutic options as it is usually associated with co-resistance to other classes of antimicrobial agents as fluoroquinolones, cotrimoxazole, tetracyclines, and aminoglycosides (Paterson and Bonomo, 2005).

New kinds of β-lactamase enzymes appeared. Various bacteria use these enzymes to resist the newest β- lactam antibiotics (Queenan and Bush, 2007).

AmpC enzyme is an example of these new β-lactamases that able to hydrolyze wide range of β-lactam antibiotics especially broad-spectrum cephalosporins like ceftazidime, ceftriaxone, cefepime, cephamycinsand monobactams. AmpC enzyme cannot be inhibited by clavulanic acid *(Jacoby, 2009).*
The worldwide emergence of antimicrobial resistance becomes a major public health problem that significantly narrows down the treatment options and result in poor outcomes.

UPEC shows high resistance to the usual treatment of UTI so we evaluate the susceptibility pattern of these uropathogenic strains with special concern about ESBL and AmpC production specially among MDR isolates.

Materials and Methods

Setting

The study was carried out from January 2017 to June 2017 at the Microbiology Diagnostics and Infection Control Unit at the Medical Microbiology and immunology Department, Faculty of Medicine, Mansoura University.

Specimen

Mid-stream urine specimens were collected aseptically from clinically suspected patients of UTIs attending outpatient and inpatient departments during their standard patient care investigation. Urine specimens were transported to laboratory and processed immediately.

Identification of the isolates

Urine samples were inoculated onto CLED agar plates and incubated at 37°C for 24-48 hours and the colonies identified based on colony morphology, Gram staining and the biochemical reactions.

Antimicrobial susceptibility testing

E. coli isolates were tested for their antimicrobial susceptibilities by the disc diffusion method according to the CLSI guidelines (CLSI, 2011). Ampicillin (10μg), ceftriaxone (30μg), cefotaxime (30 μg), cefepime (30μg), amikacin (30μg), ciprofloxacin (5μg), norfloxacin (10μg), nitrofurantoin (100μg), piperacillin/ tazobactam (100/10μg), cefuroxime (30μg),

cotrimoxazole (25μg), and imipenem (10μg) antibiotics were used. Quality control was done using *E. coli* ATCC 25922.

Testing for the ESBL Production (Kaur *et al.,* 2013)

All the strains which showed a diameter less than 27mm for cefotaxime and less than 25mm for ceftriaxone, were selected for testing ESBL production by the Modified Double Disc Synergy Test (MDDST) by using a disc of amoxicillin-clavulanate (20/10 μg) along with four cephalosporins; third generation cephalosporins -cefotaxime, ceftriaxone, cefpodoxime and fourth generation cephalosporins -cefepime. A lawn culture of the organisms was made on a Mueller-Hinton agar plate. A disc which contained amoxicillin-clavulanate was placed in the center of the plate. The discs of third generation cephalosporins and cefepime were placed 15mm and 20mm apart respectively, center to center to that of the amoxicillin-clavulanate disc. Any distortion or increase in the zone towards the disc of amoxicillin-clavulanate was considered as positive for the ESBL production. Multidrug resistant *E. coli* was defined as resistance to at least three classes of antibiotics.

AmpC Detection (Black *et al.,* 2005)

All the isolates which showed a synergistic effect with cefepime only in MDDST were further tested for the production of AmpC enzyme by AmpC disc test. AmpC disk test is based on the use of Tris-EDTA which allows the cell to release β- lactamases. The Mueller-Hinton agar plate was inoculated with a lawn culture of cefoxitin - susceptible *E. coli* ATCC 25922. A cefoxitin disc (30 μg) was placed on the agar. Tris-EDTA disc (6mm) inoculated with several colonies of test organism was placed almost touching the cefoxitin disk. The plate was then incubated

overnight at 37°C. An indentation or flattening of zone of inhibition indicated inactivation of cefoxitin which indicated the enzyme inactivation of cefoxitin (positive result). Absence of a distortion indicated no significant inactivation of cefoxitin (negative result).

Statistical analysis

Statistical analyses will be performed using IBM-SPSS version 22.0 for Windows 10 (SPSS Inc., Chicago, IL, USA). The susceptibility pattern of ESBL producers and non-producers was evaluated. 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 and Discussion

Out of the 412 processed midstream urine samples, 300 (72.8%) samples were culture positive. Gram-negative bacilli were the most frequent isolated organisms; *E. coli* recovered from 200 samples (66.7%) (Table 1). Patients infected by UPEC had different ages ranged from 8 years up to 67 years. The patients were 80 males and 120 females. Seventy-three patients had UTI for the first time, while 127 patients had recurrent UTI.

Antibiotic susceptibility testing was done for the 200 consecutive non-duplicate *E. coli* isolates. High level of resistance was seen with ampicillin (96%), ciprofloxacin (80%), nitrofurantoin (80%), cotrimoxazole (78.5%), ceftriaxone (78%), cefotaxime (74%) and cefuroxime (71%). Sensitivity to imipenem was 62.5%. Up to 42% of the isolates were resistant to cefipime (Table 2).

There was a wide spectrum resistance to different antibiotics. MDR isolates represented 69.5% (139/200), while

susceptible strains detected in 61 (30.5%) isolates (Figure 1). The difference between susceptible and MDR isolates was highly significant (p < 0.001).

Among these MDR strains, ESBLs positive strains were 129 (64.5%) isolates while, the number of AmpC positive strains were 31 (15.5%) isolates. All AmpC producer were co-producers of ESBL, the remaining 98 (49%) isolates were pure ESBLs (Figure 1).

ESBLs positive isolates were found to be more resistant than ESBLs negative isolates to other classes of antimicrobials (Table 3). None of the ESBL positive strains were sensitive to all the antimicrobial agents tested.

The difference between the resistance pattern of ESBL-producing and non-ESBL-producing strains was highly significant (P < 0.0001).

Urinary tract infection is a serious health problem affecting human both in community and clinical settings. It is encountered in different age groups and both sexes. Although the majority of UTI episodes are attended at primary care settings, they also a frequent cause of admission to some hospitals (Niranjan and Malini 2014). Among the etiologic agents of UTI, *E. coli* were the most common isolated organism (66.7%). Other studies were in accordance with our study, in which *E. coli* was the predominant uropathogen accounting for more than 50% of UTI (Islam and Yusuf, 2014; Farzana *et al.,* 2013). In study conducted in Egypt by *Elsayed et al.,* (2017), they stated that *E. coli* account for only 38.2% of UTI cases which was lower than that of our study indicating that the etiological agents of UTI varies from one locality to another even within the same country.

Antimicrobial resistance is of special concern in treating UTI. The antimicrobial susceptibility testing showed high level of resistance to different β- lactam antibiotics mainly ampicillin (96%), ceftriaxone (78%) and cefuroxime (71%). Resistance to other classes of antibiotics was detected as ciprofloxacin (80%), norfloxacin (62%), nitrofurantoin (80%) and cotrimoxazole (78%). These results were in consistent with that observed in other studies (Niranjan and Malini, 2014; Elsayed *et al.,* 2017; Guptaet al., 2013; Fody *et al.,* 2017 ; Guessennd *et al.,* 2008). Such level of resistance could be due to prescription of antibiotics in health care setting without prior microbiological investigations (Yandaï *et al.,* 2014).

Our study showed a susceptibility of 62.5% for imipenem, this frequency was dissimilar with other studies in which susceptibility varied from 95 up to 100% for imipenem (Niranjan and Malini 2014; Fody *et al.,* 2017).

Table.1 Distribution of isolated bacteria in UTI

Bacteria	No.	(%)
E. coli	200	66.7%
Other Gram negative bacilli	76	25.3%
Gram positive cocci	24	8.0%
Total	**300**	**100%**

Table.2 Resistant pattern of *E. coli* isolates

Antibiotic	No. (%)of sensitive isolates	No. (%) of resistant isolates
Ampicillin	8 (4%)	192 (96%)
Ciprofloxacin	40 (20%)	160 (80%)
Nitrofurantoin	40 (20%)	160 (80%)
Cotrimoxazole	43 (21.5%)	157 (78.5%)
Ceftriaxone	44 (22%)	156(78%)
Cefotaxime	52 (26%)	148 (74%)
Cefuroxime	58 (29%)	142(71%)
Norfloxacin	76 (38%)	124(62%)
Piperacillin/tazobactam	96 (48%)	104(52%)
Amikacin	104 (52%)	96(48%)
Cefipime	116 (58%)	84 (42%)
Imipenem	125 (62.5%)	75(37.5%)

Table.3 Antibiotic resistance of ESBL producers and non-producers to various
non β-lactam antibiotics

Antibiotics	ESBL producers (N= 129) No. (%)	Non ESBL producers (N= 71) No. (%)
Ciprofloxacin (160)	121 (75.63%)*	39 (24.37%)
Nitrofurantoin (160)	116 (72.5%)*	44 (27.5%)
Cotrimoxazole (157)	122 (77.7%)*	35 (22.3%)
Norfloxacin (124)	113 (91.1%)*	11 (8.9%)
Amikacin (96)	88 (91.7%)*	8 (8.3%)

*P value <0.001

Fig.1 Extended spectrum beta-lactamase (ESBL) detection
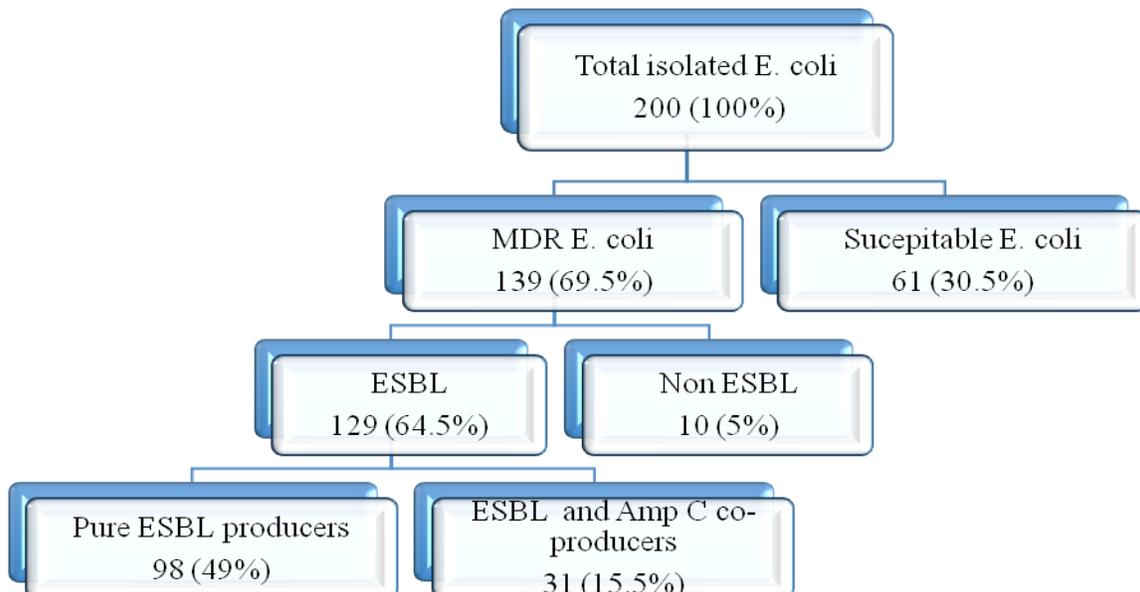
ESBL producing bacteria is one of the most encountered causes of treatment failure and prolonged stay in hospitals.

The widespread use of antibiotics to treat bacterial infections has rapidly increased the emerging multidrug resistance strains specially ESBL producing ones *(Bush, 2001).*

Our study was designed to determine the prevalence of ESBL and AmpC production among MDR strains of uropathogenic *E. coli* isolates.

In the present study, there were 139 (69.5%) MDR isolates, which was relatively high prevalence. A comparable MDR rates of 83% and 76.51% were reported in other studies done in Pakistan and India respectively (Ullah *et al.,* 2009 ; Niranjan and Malini, 2014).

In our study we investigated the prevalence of ESBL production by uropathogenic *E. coli.* More than half (64.5%; 129/200) of isolated *E. coli* strains were ESBL producer. Lower prevalence was detected in other studies carried out in other African countries such as in Benin (22%) (Ahoyo *et al.,* 2007), Nigeria (20%) (Onwuezobe and Orok, 2015), Niger (26.3%) (Fody *et al.,* 2017), Tanzania (15.1%), (Mshana *et al.,* 2016), Chad (20.09%) (Yandaï *et al.,* 2014) and the Libyan community (13.4%) (Ahmed *et al.,* 2014).

Our results showed that out of (64.5%) ESBL producing *E. coli*, 49% (98/200) were pure ESBL producers and 15.5% (31/200) were both ESBL and AmpC co-producers.

This result was in agreement with another study conducted by Gupta *et al.,* (2013) who showed that of 52.6% of the isolates were ESBLs producer strains and 10% of strains were AmpC producer. Another investigation from Egypt revealed that 57.7% of isolates were Amp C enzyme producer (Fam *et al.,* 2013).

Different studies detected much higher level of AmpC production among *E. coli* isolates as in studies carried by Shanti and Balagurunathan (2014) (76%), Rudresh and Nagarathnamma (2011) (52.2%), Getzlaffet al., (2011) (41%) and Shubha (2003) (37.5%), while other studies showed lower level of AmpC production as revealed by Mansoori *et al.,* (2009), Niakan *et al.,* (2008) and Singhal *et al.,* (2005), who reported 5.7%, 5.9%, and 7% of the isolate were AmpC producer respectively.

Gupta *et al.,* (2013) found that not all AmpC positive strains were co-producer of ESBL and AmpC, but only 8% of the strains were co-producers of ESBL and AmpC, but in our study all AmpC positive isolates wereco-producers of ESBL and AmpC.

ESBL producers were more resistant than susceptible strains to classes of antibiotics rather than beta lactams. Resistance to quinolones was recorded to be 91.1% to norfloxacin and 75.6% to ciprofloxacin. This was in consistent with Farzana *et al.,* (2013), Gupta *et al.,* (2013) and Chander and Shrestha (2013), who reported high level of resistance to quinolones more than 90%. Elsayed *et al.,* (2017) reported similar resistance to ciprofloxacin (72.2%) but less resistance to norfloxacin (59%).

The reason for that is the widespread empirical treatment with quinolones for treatment of UTI in our locality. Another notable resistance was to nitrofurantoin. In ESBL producing strains, resistance was 72.5%. This relatively high rate of resistance was reported in another studies carried by Elsayed *et al.,* (2017) and Gupta *et al.,* (2013), however Chander and Shrestha (2013) stated a lower resistance rate (11.7%).

Amikacin was another non beta lactam antibiotic which was used frequently in treatment of UTI. More than 90% of the ESBL isolates were resistant to amikacin, this result was very high as compared with other study in which resistance rate was 6.6 % (Chander and Shrestha, 2013).

Our study concluded that *E. coli* was the most common organism isolated from UTI. There were increasing incidence of MDR *E. coli* which showed resistance against different groups of antimicrobial agents such as β-lactams, aminoglycosides and fluoroquinolones. We detected high prevalence of ESBL producing uropathogenic *E. coli*, so a rational use of antibiotics, surveillance together with strict hospital infection control policies should be applied to reduce the emergence of ESBL producing *E. coli* in urinary isolates.

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