Original Article

Antimicrobial Susceptibility Pattern and Extended Spectrum ß Lactamase Production among Uropathogenic *Escherichia coli* in a Teaching Hospital in Bangladesh.

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Abstract

Background & Aims: A major portion of urinary tract infections are caused by *Escherichia coli.* It has been found that these organisms are being increasingly resistant to broad spectrum ß- lactam antibiotics mediated by extended Spectrum ß-lactamase (ESBL) enzymes. The present study was undertaken to determine the antimicrobial susceptibility pattern and the incidence of ESBL production among *Escherichia coli* strains isolated from urine.

Materials & Methods: Urine specimens from patients were subjected to culture as per Clinical Laboratory Standard Institute (CLSI) guidelines. All specimens were inoculated on to Blood agar and MacConkey agar plates and growth showing significant bacteriuria (>10^5 colony/ml of urine) were further identified by the standard biochemical procedures. Detection of ESBL production by isolated *E.coli* strains was done by Double Disc Synergy Test which is a phenotypic confirmatory test for ESBL production. Antibiotic susceptibility testing of isolates was also done as per CLSI guidelines. The study was conducted from1st January, 2014 to 30th June, 2015.

Result: A total of 377 *E.coli* strains were isolated of which 165 (44%) isolates were ESBL producers. Susceptibility pattern of isolates to Ceftriaxone, Ceftazidime, Cefixime and Ciprofloxacin was not satisfactory. More than 80% sensitivity was found only to Imipenem (96%), Nitrofurantoin (91%) and Amikacin (89%).

Conclusion: The presence of ESBL carries tremendous clinical significance. As the ESBLs are frequently plasmid encoded & same plasmid can carry genes encoding resistance to other antibiotic group, thus extremely limiting the antibiotic treatment option.

Introduction

Drug resistant bacteria are emerging world wide as a threat to treatment of common infections in community and hospital setting. Urinary tract infection is common in both community and hospitalized patients and in most of the cases is caused by *E.coli.* Also *E.coli* are well known to produce multidrug resistance. Extended spectrum ß-lactamase (ESBL) production is perhaps the most important cause of resistance to many antibiotics especially to Penicillin & Cephalosporins[1]. ESBL is the enzyme that cause increased hydrolysis of the ß-lactam drugs like Penicillins and Cephalosporins, including oxyimino-ß-lactam compounds (Cefuroxime, third- and fourth-generation Cephalosporins and Aztreonam). These enzymes have been identified in large number in various *E.coli* strains. The first ESBL-producing strains were identified in 1983 in Germany, and since then a large number of outbreaks of infection due to ESBL producing organisms have been described worldwide.[2, 3, 4, 5, 6] Most ESBLs belong to the Ambler class A of ß-lactamases and are inhibited by ß-lactamase inhibitors (Clavulanate, Sulbactam and Tazobactam).[7] These ß-lactamases are encoded by genes that can be exchanged between bacteria.[8] As the ß- lactam drugs are the most common treatment for bacterial infections, so persistent exposure of bacterial strains to a number of ß-lactams has induced continuous production & mutation in ß- lactamases. As a result there is an expansion of activity of ß- lactamases even against the newer ß- lactam drugs. ESBL strains have been associated with resistance to other non ß-lactam drugs like Aminoglycosides and Chloramphenicol.[8] This leads to increased patient mortality and morbidity when antibiotics inactive against ESBL producing organisms are used. Also ESBL producing organisms are threat to infection control and there is a potential for transfer of such organism to other patients. So control of outbreak of infection by ESBL producing organisms is very important. For this

detection of ESBL producing organisms from clinical samples like urine is essential.[8] The present study was therefore conducted with a view to find out the occurrence of ESBL producing E. coli in urine and at the same time to see the antimicrobial susceptibility profile of isolated *E.coli* to formulate effective antibiotic strategy and to plan a proper hospital infection control policy to prevent the spread of these strains.

Materials:

This prospective observational study was carried out in the Department of Microbiology, Dhaka National Medical College, Dhaka. Urine samples from all patients (indoor & outdoor) with suspected urinary tract infection were evaluated from 1st January 2014 to 30th June 2015. Early morning mid stream urine specimens were collected aseptically in pre-sterile dried containers from all patients. Urine were cultured on Mac Conkey agar and Blood agar media. The plates were incubated overnight at 37°c. *E.coli* isolates with significant bacteruria (>10^5 colony/ml of urine) were identified based on standard laboratory procedures; namely colony morphology, gram staining, motility, biochemical tests.[9]

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing of the isolates was carried out using various antimicrobial disks

(shown in table I) by Kirby-Bauer disk diffusion method.[10] Inoculum of 0.5 McFarland standards turbidity was prepared in a nutrient broth from isolated colony of *E.coli* selected from 18-24 hour agar plates. Within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension. The swab was rotated several times and pressed firmly against the inside wall of the tube above the fluid level and inoculated on the dried surface of Mueller - Hinton agar plate by streaking the swab over it. For even distribution of the inoculum, the swab was streaked two more times at 60° angle over the surface. After 3-5 minutes antibiotic disks were applied and pressed down to ensure complete contact with agar surface. The disks were distributed evenly to ensure a minimum distance of 24 mm from centre to centre. The plates were then inverted and incubated aerobically at 37°c within 15 minutes. The diameter of zone of inhibition for

Table I: Antimicrobial disc used & their zone diameter interpretative for *E.coli*

Antimicrobial disc	Disc potency	S	I	R
Imipenem	10µg	≥23	20-22	≤19
Amikacin	30µg	≥17	15-16	≤14
Gentamicin	10µg	≥15	13-14	≤12
Nitrofurantoin	50µg	≥17	15-16	≤14
Nalidaxic Acid	30µg	≥19	14-18	≤13
Ciprofloxacin	1µg	≥21	16-20	≤15
Cephradine	30µg	-	-	-
Ceftazidime	30µg	≥21	18-20	≤17
Ceftriaxone	30µg	≥23	20-22	≤19
Cefuroxime	30µg	>20	17-19	<16
Cefixime	5µg	≥21	18-20	≤17
Trimethoprim-Sulphamethoxazole	25µg	≥16	11-15	≤10
Doxycycline	30µg	≥16	13-15	≤12

Note: S= Sensitive, I= Intermediate, R= Resistant individual antimicrobial agent was measured in millimeter with the help of a ruler and described as sensitive, intermediate & resistant according to CLSI 2012 guideline.[11]

Detection of ESBL: All *E.coli* isolates were tested for ESBL production by Double Disk Synergy test (DDST), which is a phenotypic confirmatory test of ESBL production. For this, a lawn culture of isolated bacteria was made on Mueller- Hinton Agar and disks containing 30 µg Ceftriaxone and 30 µg Ceftazidime were placed with a disk of Amoxicillin-Clavulanic acid (20 µg /10 µg) in between. The distance between the disks was 20mm centre-to-centre. The plate was incubated overnight. A clear extension of the edge of any Cephalosporin inhibition zone toward the disk containing Clavulanic acid was interpreted as synergy, indicating the presence of ESBL.[11]

Result:

Among all the isolates producing significant bacteruria from urine, 377 were *E.coli* (Table:II). Among them 165 (44%) isolates were ESBL producer. 58% of *E.coli* isolated from indoor specimens & 37% from outdoor specimens were ESBL producers (Table:III). Sensitivity of isolated *E.coli* to Imipenem (96%), Nitrofurantoin (91%) and Amikacin (89%) was very good. But sensitivity to 3rd generation cephalosporin was between 37% to 51% which is quite low. Even only 49% isolates were sensitive to Ciprofloxacin. Better sensitivity was observed to Gentamicin (77%) (Table: IV).

Table II: Distribution of *E.coli* isolated from urine

	No. of urine specimen N	Culture positive specimen n (% of N)	E. coli isolated n1(% of n)
Indoor	688	241(35)	120 (50)
outdoor	1870	549 (29)	257 (47)
Total	**2558**	**790 (31)**	**377 (48)**

Table III: ESBL pattern of *E.coli* isolated from urine

	ESBL producer n(%)	Non ESBL producer n(%)
Indoor	70 (58)	50 (42)
Outdoor	95 (37)	162 (63)
Total	**165 (44)**	**212 (56)**

Table IV: Antimicrobial susceptibility of isolated *E.coli*

Antimicrobial agents	Sensitive n (%)	Resistant n (%)
Imipenem	361 (96)	16 (4)
Amikacin	335 (89)	42 (11)
Gentamicin	290 (77)	87 (23)
Nitrofurantoin	343 (91)	34 (9)
Nalidaxic Acid	79 (21)	298 (79)
Ciprofloxacin	184 (49)	193 (51)
Cephradine	33 (9)	344(91)
Ceftazidime	139 (37)	238 (63)
Ceftriaxone	192 (51)	185 (49)
Cefuroxime	49 (13)	328 (87)
Cefixime	143 (38)	234 (62)
Trimethoprim-		
Sulphamethoxazole	56 (15)	321(85)
Doxycycline	177 (47)	200 (53)

Discussion

The discovery and development of antibiotics was one of the greatest advances of modern medicine. But antibiotic resistant bacteria has emerged as a threat to this advancement. In this study an attempt was made to understand the antimicrobial sensitivity pattern and epidemiology of ESBL production of *E.coli* isolates in urine . The present study was based on laboratory findings and includes both indoor and outdoor patients. All the isolated *E.coli* were tested for ESBL production and antimicrobial sensitivity pattern. Highest sensitivity was found to Imipenem (96%). Nitrofurantoin (91%) and Amikacin (89%). Near about similar sensitivity was found in various studies.[3,12,13] Nitrofurantoin is a widely available antibiotic which can be administered orally and is very effective in uncomplicated LUTI. Resistance to Nitrofurantoin is rarely reported among *E.coli* though a study in India reports 34% resistant *E.coli* isolates.[1] In the present study a low sensitivity was observed with 3rd generation Cephalosporins

(Ceftazidime 37%, Ceftriaxone 51% & Cefixime 38%). A study in India showed 33% of their isolated *E.coli* were sensitive to Ceftazidime and Ceftriaxone.[1] Conventionally ESBL producers are also multidrug resistant organisms. They are usually less sensitive to ß-lactams as well as to other classes of antimicrobials including Trimethoprim- Sulfamethoxazole, Fluroquinolones and Aminoglycosides as all these resistant encoding genes share same plasmid.[8] In our study we found that 49% and only 15% of *E.coli* strains were sensitive to Ciprofloxacin and Trimethoprim-Sulfamethoxazole respectively. But moderate degree of sensitivity was found to Gentamicin (77%). High level of resistance to Ciprofloxacin and Trimethoprim-Sulfamethoxazole has been reported in other studies.[3,14,15] Bamford et al who studied antimicrobial susceptibility pattern of uropathogenic *E.coli* from the year 2007 to 2011 demonstrated a significant decline in sensitivity to fluroquinolones, while sensitivity to Amikacin and Gentamicin remained significantly high.[16]

In the present study out of 377 *E.coli* isolates, 165 (44%) were ESBL producers. It varies from country to country & from time to time. A previous study in Bangladesh shows 54% of uropathogenic E. coli were ESBL producer[2]. In studies from India[1] & Nepal[16] it was 54.67% & 13.51% respectively. ESBL production by *E.coli* is important due to the fact that they are normal flora of intestine and can transfer this ESBL gene to other bacteria by plasmid, so serve as reservoir of infection. In our study, prevalence of ESBL among indoor patients and outdoor patients was 58% and 37% respectively. A study from India also show more ESBL producing *E.coli* in indoor patients.[1] This result means that ESBL is also common in community which proves the assertion of Pitout et al that they are as much a problem in community as in hospital.[17] ESBL production by organisms restricts the use of ß- lactam drugs which have got an extended spectrum of activity against Gram negative bacteria and have low toxicity. Various studies have reported about emergence of CTX-M type of ß- lactamases among the uropathogens, the occurrence of which is linked to prior antibiotic therapy within one month preceding the current episode.[18] Also in conditions like pregnancy where the choice of antimicrobials is limited to ß- lactams like Ampicilin & Cephalosporins, infections due to ESBL strains make the treatment difficult.[13] This study along with various other studies[13,14,15] indicate a widely prevalent MDR pattern of ESBL producing organisms to commonly used antibiotics & an urgent need to reconsider antibiotic prescribing pattern. Good infection control practice & proper antibiotic management are main factors to prevent the outbreak of infection by ESBL producing organisms. Educational programs to increase awareness among medical stuffs should also be developed.

As antibiotic susceptibility pattern of organisms and prevalence of ESBL production among them differ geographically, so such institutional studies will help in formulation of antibiotic policy for a particular geographical area.[19]

Conclusion:

ESBLs have evolved greatly over the last several years. Their presence along with the plasmid mediated Quinolone and Aminoglycoside resistance will create significant therapeutic problems in future. So it will be very difficult to control infection by such multi-resistant organisms. Therefore, enhanced infection control coupled with appropriate antibiotic management for infections should be practiced to limit the spread of ESBL producing organisms.

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