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Original Article

Study of Extra intestinal *E.coli* and their resistant pattern in a tertiary care hospital of Bangladesh.

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Abstract

E.coli is one of the most important gram negative bacterial pathogen in human and can be subdivided into the following; (i) intestinal non-pathogenic, commensal variants (ii) Intestinal pathogenic isolates and (iii) extra intestinal pathogenic *E.colior* ExPEC isolates. ExPEC causes the vast majority of urinary tract infections (UTIs), is a leading cause of adult bacteremia and is the second most common cause of neonatal meningitis. The clinical specimens obtained from patients admitted in Holy Family Red Crescent Medical College Hospital (HFRCMCH) from January 2014 to December 2016. All laboratory works were performed in department of microbiology and immunology of HFRCMCH. The infection rate was maximum in urine (89%) followed by blood (5%), pus (5%) and others (1%). Most of the ExPEC were isolated from outdoor department 62.6%. ExPEC displayed higher resistance to many antibiotics like 75% resistant to Ampiciline , 55% resistant to Cotrimazole, 22% resistant to Gentamycin, 17% resistant to Nitrofurantoin, 55% resistant to Ciprofloxacin, 70% resistant to Ceftriaxone, 82% resistant to Cefalexin, 50% resistant to Azithromycin, 10% resistant to Amikacin, 48% resistant to Tetracycline, 7% resistant to Imipenem and 8% resistant to Meropenem. In conclusion, there were high resistant rate to commonly available antibiotics. It seems that appropriate and judicial use of antibiotics may help to control the evolving problem of drug resistance towards ExPEC.

Key words: Extra intestinal *E.coli*, Resistance.

Introduction:

Escherichia coli (*E. coli*) is one of the most common gram-negative bacterial pathogen in humans[1]. Escherichia coli (*E. coli*) exhibits considerable physiological and metabolic versatility and includes a variety of non-pathogenic, commensal variants, which belong to the normal gut flora of humans and animals[2]. Additionally, several pathogenic variants have been identified which cause various types of intestinal or extra intestinal infections. In contrast to intestinal pathogenic *E. coli* (IPEC), which are obligate pathogens, extra intestinal pathogenic *E. coli* (ExPEC) are facultative pathogens which belong to the normal gut flora of a certain fraction of the healthy population where they live as commensals[3]. ExPEC causes various range of clinical diseases of all age group[1]. It possesses virulence traits that allow it to invade, colonize, and induce disease in bodily sites outside of the gastrointestinal tract[4]. It is the most common cause of UTI,[5] *E. coli* is also the most common cause of community-acquired bacteremia and sepsis. It is one of the leading causes of neonatal meningitis and neonatal sepsis, which often lead to serious complication to death[6]. *E.coli* also shows feature of other extra intestinal infections such as osteomyelitis, cellulitis, and wound infections[7].

Uropathogenic *E. coli* are the primary causes of community-acquired UTIs with an estimated 20% of women over the age of 18 years suffering from at least one UTI in their lifetime[8]. UPEC is responsible for 70-95% of community-onset UTIs and approximately 50% of nosocomial UTIs, hence accounting for substantial morbidity, mortality, and medical expenses[8]. *E. coli* infections also result in a heavy economic burden[9]. Global morbidity and mortality rates due to ExPEC infections are substantial and increasing[1].

The use of an inappropriate antibiotic will delay effective treatment[10] and also cause rising of antibiotic resistant strains. Resistance in ExPEC to majority of commercially available antibiotics like aminoglycosides, cephalosporin, and quinolones raises an important therapeutic problems.

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The aim of the present study was to highlight the antibiotic resistance pattern of extra intestinal *E.coli* infection in Holy Family Red Crescent Medical College and Hospital.

Methodology:

It was a retrograde study. Clinical samples were received from outdoor and indoor patients in Holy Family Red Crescent Medical College Hospital. Sample were collected from the patients age limit 0 to 100 years admitted in holy family Red Crescent medical college from January 2014 to December 2016. Samples included urine, blood, pus, tracheal aspirate and sputum. All laboratory works were performed in department of microbiology and immunology in HFRCMCH.

Isolation and identification of *E.coli:* Typical colonies were enumerated, selected and examined further. *E.coli* was identified by Gram staining, oxidase, citrate utilization and glucose oxidation in Krigler Iron Agar (KIA) media. Theses identification scheme were done as per standard technique.[11, 12]

Antimicrobial susceptibility tests: All the *E.coli* isolates were tested for antimicrobial susceptibility testing by disc diffusion method using Kirby-Bauer technique. Antimicrobial disks used for sensitivity tests were Ampicillin, Cotrimazole, Gentamycin, Nitrofurantoin, Ciprofloxacin, Ceftriaxone, Cefalexin, Amikacin, Azithromycin, Tetracycline, Imipenam and Meropenam[13].

Results:

In the 3 year study period (from January 2014-december 2016), total 28801 samples were analyzed. The positive samples from all isolates were 4810. Out of these 1467 were *E.coli* positive sample.

During the period January 2014- Dec 2014, 607 samples were studied which included: urine (508), blood (55) and pus (36). During the period January 2015- Dec 2015, 418 samples were studied which included: urine (380), blood (19) and pus (15). During the period January 2016- Dec 2016, 445 samples were studied which included: urine (400), blood (15) and pus (26). Rate of number in different samples are shown in Table I.

Table I- ExPEC isolates from different sample

Samples	2014 (Jan-Dec) N%	2015 (Jan-Dec) N%	2016 (Jan-Dec) N%
Urine	508(84%)	380(90%)	400(90%)
Blood	55(9%)	19(5%)	15(3%)
Pus	36(6%)	15(4%)	26(6%)
Others	8(1%)	4(1%)	5(1%)
Total	607	418	445

Table II shows ward distribution of *E.coli* positive samples in different places of hospital. In these period, most of the samples were from outdoor department. From January 2014 to December 2014 period 56.2% samples, from January 2015 to December 2015 period 85.4% samples, from January 2016 to December 2016 period 49.9% samples were isolated from OPD. From January 2014 to December 2014 period 41% samples, from January 2015 to December 2015 period 13.2% samples, from January 2016 to December 2016 period 46.2% samples were isolated from general wards and cabins.

Table II- Distributions of *E. coli* in Different places of HFRCMCH

Places in the hospital	2014 (Jan-Dec) N (%)	2015 (Jan-Dec) N (%)	2016 (Jan-Dec) N (%)	Total N (%)
OPD	341 (56.2%)	357 (85.4%)	222 (49.9%)	920 (62.6%)
Cabins	102 (16.8%)	20 (4.8%)	95 (21.3%)	217 (14.8%)
General Wards	147 (24.2%)	35 (8.4%)	111 (24.9%)	293 (19.9%)
ICU	17 (2.8%)	6 (1.4%)	17 (3.8%)	40 (2.7%)
Total	607 (100)	418 (100)	445 (100)	1470 (100%)

In these three study periods, higher level of resistance was recorded for Ampicillin which was (79, 72, 72%) in 2014, 2015 2016, Cephalexin (82, 88, 72) % resistance, Ceftrixone (62, 78, 68) % resistance, Ciprofloxacin (52, 56, 57) % resistance, Cotrimoxazole (52, 59, 55)% resistance, Tetraclycine (50, 50, 43) % resistance and Azithromycin (45,55,49) % resistance. Resistance against Nitrofurantoin (19, 17, 17) %, Imepenem (7,7,9)% and Meropenem (6, 7, 12) %. was low in 3 year study.

Table III- Antibiotic resistance pattern of E.coli species isolated from different site of infection (Number of percent resistant)

Name of drugs	2014 (Jan-Dec) N=607 (%)	2015 (Jan-Dec) N=418(%)	2016(Jan-Dec) N=445(%)
Ampicilline	79	72	72
Cotrimazole	52	59	55
Gentamycin	25	19	22
Nitrofurantoin	19	17	17
Ciprofloxacin	52	56	57
Ceftriaxone	62	78	68
Cefalexin	82	88	72
Azithromycin	45	55	49
Amikacin	14	9	8
Tetracycline	50	50	43
Imipenem	7	7	9
Meropenem	6	7	12

Discussion:

In this study most of the *E.coliwere* isolated from urine sample (90%). In USA one study showed (2016) they isolated *E.coli* from 52% of UTI infection. Another study done in USA (2005) found 33.5% infection was caused by *E.coli*[14]. They are highly developed country which may reduce incidence of *E.coli* but still the leading cause of UTI (14). In blood stream our results showed 9% of infection was due to *E.coli*. In USA they found (2016) 25% of infection due to *E.coli*[14]. In pus we found 6% of infection was due to *E.coli*. In USA their study showed (2016) 14% infection due to *E.coli*[15].

Urinary tract infections are one of the most common diseases encountered in the medical practices. *E.coli* is the leading cause of both community-acquired and nosocomial UTIs. In table 2 showed 62.6% patient were from outdoor department and 37.4% from indoor. Which indicate community acquired UTI was about two times more than hospital acquired UTI. One study in Midford hospital, Dhaka (2011) showed 259(63.48%) isolates were from in patient department and 155(45.45%) were from outpatient department[16]. One study in Brazil (2003) showed they have about 58% community acquired UTI due to *E.coli*[17]. Our result was slightly dissimilar to that result may be due to their number of indoor patients were comparatively more than our indoor patient. Patients with indwelling urinary catheters, undergoing urological manipulations, long-stay elderly male patients and patients with debilitating diseases are at high risk of developing nosocomial UTI. The organisms responsible usually originate from patients' endogenous intestinal flora, but occasionally from a moist site in the hospital environment [16].

The antibiotic resistant pattern of isolated *E. coli* of the present study reveals that maximum resistance was found in last three years against Cefalexin (82%) followed by Ampiciline (75%), Ceftriaxone (70%) while moderate resistance was shown towards ciprofloxacin (55%) and Cotrimazole (55%) Azithromycin (50%) and Tetracycline (48%) Gentamycin and Amikacin were found to be resistance against 22% and 10% *E.coli* isolates respectively. Resistance of the Nitrofurantoin was low in 3 year study (17-18%). Low resistance was observed to Imipenem (7%) and Meropenem (8%).

A study conducted in Midford hospital, Dhaka (2011) showed Amoxycillin (97.36%) followed by Cotrimoxazole (81.88%), Tetracycline (81.57%) Cephalexin (76.61%) Ciprofloxacin (59.35%) and Nitrofurantoin (55.26%). Gentamicin, Ceftrixone and Imipenem were 33.90%, 13.16% and 4.97% resistance respectively[16]. Another study done in Dhaka medical college (2015) found *E.coli* had been shown highly sensitive (100%) to Imipenem followed by Ceftriaxone (62.50%), Ceftazidime (55.36%) and Azitromycin (35.72%) respectively. Low resistance patterns were shown against Doxycycline (13.39%), Cotrimoxazole (16.07%), Amoxiclav (21.43%) and Ciprofloxacin (23.22%) respectively[18]. A study done in 2003 in Mymensingh showed 97% resistance in Ampiciline, 82% in Cotrimoxasole, 62% in Gentamycine, 62% in Nitrofurantoien, 59% in Ciprofloxacin, 71% in Ceftriaxone, 87% in Tetraclycline, 1% in Imipenam[19]. In another study of Ibrahim Medical College and Birdem ICU also, *E.coli* isolates were highly resistant (>80) to Cephalosporins and Fluoroquinolones and resistance to Imipenem was low (4.6%)[20].

A study done in Ahmabad in 2012 showed 93% resistant in Ampiciline, 65% in Gentamycine, 84% in Ciprofloxacine and Amikacin 12.5%[21]. In a study from the Netherlands (2000), almost similar level of resistance was reported among isolates of *E. coli*, where resistance towards Ampicillin, Sulphamethoxazole- Trimethoprim (SXT), Cephalothin, Ciprofloxacin and Nitrofurantoin was 97.8%, 92.8%, 86.6%, 38.8% and 7.7%, respectively[22]. A study done in Ethiopia showed resistance level were 100% in Ampiciline, 14.3% in Ciprofloxacin, 28.6% in Tetracycline, 0% in Gentamycin, Nitrofurantoin and Ceftriaxone[23]. Their resistant pattern in slightly different from our study may be due to their sample size was very small. Resistant pattern of *E.coli* against various antimicrobial drugs are considerably different among countries, centers and even different wards of the same hospital. So, this type of studies are very important to improve the antibiotic profile for ExPEC infection[23].

Conclusion:

ExPEC is a global pathogen causing a spectrum of diseases that affect all ages. It is an important cause of UTIs, enteric infections, and systemic infections in humans that collectively cause considerable morbidity, lost productivity, and increased health-care costs. Injudicious use of antibiotics leads to a rising of resistance strains. Isolates are becoming increasingly resistant to commonly used antibiotics. Drug resistance was very high with oral agents, so it is very difficult to treat patients in community sector. We should cautious with indiscriminate use of antibiotics. Antibiotics should be prescribed with proper dose and duration after culture and sensitivity reports are available.

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