International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 2 Issue 6‖ June2013 ‖ PP.43-46

Antimicrobial Resistance Pattern Of Diarrheagenic *Escherichia Coli* Isolated From Acute Diarrhea Patients.

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ABSTRACT: *Acute diarrhea is a public health problem and an important cause of morbidity and mortality, especially in developing countries. The etiology is varied, and among pathotypes - Escherichia coli are the most important.Our objectives were to determine drug susceptibility pattern to different conventional and commonly available antibiotics of diarrheagenic E. coli (DEC) in fecal samples from children under five years old. DEC are detected by multiplex PCR. Among 68 DEC, 16 samples contain more than one pathogenic genes of DEC. Most of the strains are resistant to ampicillin, erythromycin, nalidixic acid and cephalexin. Azithromicin, Co-trimoxazole and Ciprofloxacin are relatively more sensitive and the sensitivity pattern of mecillinam, ceftriaxone and gentamicin is quite satisfactory. Sample containing more than one pathogenic genes are resistant to multiple antibiotics. Differentiation between the diarrheagenicE. coli pathotypes is of great importance since they are involved in acute diarrheal diseases and may require specific antimicrobial chemotherapy. The high antimicrobial resistance observed in our study raises a broad discussion on the indiscriminate or improper use of antimicrobials, besides the risks of self-medication.*

KEYWORDS- *antimicrobial* resistance, *diarrheagenic E. coli, multiplex PCR.*

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I. INTRODUCTION

Diarrhea caused by *E. coli* infection is one of the major public health concerns in many developing countries and has contributed exceedingly to morbidity and mortality and increase in health costs. [1, 2]

Resistance of antibiotics is very common in bacterial isolates all around the world.[3] Information on antimicrobial resistance patterns is important in choosing the appropriate antibiotic therapy.

Supportive anti-dehydration treatment is the cornerstone of therapy and must be promptly started. Antimicrobial therapy is indicated to treat the moderate to severe disease to reduce the duration of illness for diarrhea [4] and on risk of complication.

Escherichia coli are common members of the normal flora of the human intestine. [5, 6] *E. coli* is an important opportunistic pathogen that has shown an increasing antimicrobial resistance to most antibiotics. [5,7] Antimicrobial resistance in *Esch. coli* have been reported world-wide but susceptibility pattern vary region to region. [8]

Many diarrheal cases are not diagnosed, either because they are mild and self-limiting, in which the patient does not seek medical attention, or because, especially in developing countries, the medical and laboratory resources are not available [9]. The aim of this investigation was to determine susceptibility pattern of DEC to different conventional and commonly available antibiotics.

II. MATERIALS AND METHODS

Stool samples from children (aged below 5 years) were collected from January 2011 to December 2011 from outdoor and indoor patient department of Dhaka Medical College Hospital and Dhaka Shishu Hospital. Stool samples from patients who had not received antibiotic treatment at the time of collection of samples were collected using clean, dry, plastic, wide-mouth containers and taken to the department of Microbiology, Dhaka Medical College for bacterial analyses within 1 - 2 hours of collection. Characterization and identification of *E. coli* cultures were made on the basis of morphology, cultural characteristics and biochemical reactions. All the stool samples were cultured into MacConkey agar for primary isolation of common intestinal pathogens and incubated at 37°C for 24 hours. All colonies on MacConkey agar plates suspected to be *E. coli* (lactose fermenter, non-mucoid, 2-3 mm diameter, circular, smooth and convex) were further sub-cultured onto nutrient agar and incubated for another 24 hours. The cultures on nutrient agar plates were subjected to Gram's-staining, motility, urease production, glucose, oxidase, sucrose, mannitol, lactose, indole and citrate utilization tests. All Gram-negative, rod-shaped, motile, indole-positive, urease-negative isolates that produced acid on Triple Sugar Iron agar slants were identified as species of the genus *E. coli* following standard procedures. [10]

Multiplex PCR for categorization of *Esch. coli* into ETEC, EPEC and EAggEC were done using primers for detection of *lt* or *st* gene for ETEC, *bfp* or *eae* gene for EPEC and *aat* gene for EAggEC. The PCR was carried out in various number of genes combinations. PCR assays were performed in a DNA thermal cycler (Eppendorf AG, Mastercycler gradient, Hamburg, Germany). Each PCR was carried out comprised of preheat at 94^0C for 10 minutes followed by 36 cycles of denaturation at 94^0C for 1 minute, annealing at 60^0C for 45 seconds, elongation at 72^0C for 2 minutes and final extension at 72^0C for 10 minutes. Amplified products were run on to horizontal gel electrophoresis on 1.5% (w/v) agarose gel (Bethesda Research Laboratories) at 100 v (50 mV) for 30 to 35 minutes and visualized with a UV transilluminator (Gel Doc, Major science, Taiwan) after ethidium bromide staining. If the pooled DNA template result was negative following gel electrophoresis, the sample was considered negative for DEC. Hundred bp DNA size standard (Bio-Rad, USA) was used as marker to measure the molecular size of the amplified products. Only the presence of the amplified product with correct size was interpreted as a test positive.

Susceptibility to antimicrobial agents of all identified DEC isolates was done by Kirby-Bauer modified disk diffusion technique. [11]

The antimicrobial susceptibility assay was performed on Mueller-Hinton agar (Oxoid) by the disc-diffusion method and growth inhibition zones were interpreted according to the Clinical Laboratory Standards Institute. [12] The identified DEC were tested against ampicillin (10 µg), cephalexin (30 µg), Gentamicin (10 µg), tetracycline (30 µg), cotrimoxazole (25 µg), nalidixic acid (30 µg), azithromycin (15 µg), ciprofloxacin (5 µg), mecillinam (10 µg), ceftriaxone (30 µg) and cefixime (30 µg). *Escherichia coli* ATCC 25922 was tested along with the isolates for quality control purpose. The result was documented according to performance range of inhibition zone of control strain.

III. RESULT

In this cross sectional study, during one-year period, stool samples were collected from 200 patients aged 0 to 60 months and among them 114 (57%) were males and 86 (43%) were females. Among 200 stool samples, *E. coli* was isolated in 135 (67.50%). Among those *E. coli* isolates, 68 (50.37%) were identified as DEC by PCR. Of the 68 detected DEC, 40 (58.82%) were identified as EPEC, 24 (35.29%) as ETEC and 18 (26.47%) as EAggEC. Out of these 68 DEC positive samples, 52 (76.47%) contained one pathogenic type of DEC and rest 16 (23.53%) samples contained more than one pathogenic strains of DEC. The result for antimicrobial susceptibility testing of the different groups of DEC strains is shown in Table 1. The trend of resistance was different among DEC types. The EPEC, ETEC and EAggEC strains were highly resistant to ampicillin, erythromycin and nalidixic acid. Moderately high resistance was detected towards cefalexin, azithromycin, co-trimoxazole and ciprofloxacin. The DEC isolates were least resistant to mecillinam, ceftriaxone and gentamicin. The antimicrobial susceptibility pattern of samples containing more than one pathogenic strains of DEC is depicted in Table 2. Sample containing EPEC+EAggEC combination were higly resistant to ampicillin, erythromycin, nalidixic acid, azithromycin and co-trimoxazole. ETEC+EAggEC containing samples were noticeably resistant to ampicillin, erythromicin, cefalexin, azithromycin, ciprofloxacin and gentamicin. Samples containg ETEC+EPEC combination were predominantly resistant to ampicillin, erythromicin, nalidixic acid and cefalexin. Sample containing ETEC+EPEC+EAggEC combination were 100% resistant to ampicillin, erythromicin and ciprofloxacin. Fig. 1 demonstrates the antibiotic sensitivity pattern of samples containing combinations of pathogenic strains.

Table 1: Antimicrobial susceptibility pattern of different strains of DEC isolates containing one pathogenic strain.

Name of the antimicrobials	EPEC (n= 28)		ETEC (n=11)		EAggEC (n=8)	
	S	R	S	R	S	R
Mecillinam	26 (92.9%)	2 (7.1%)	9 (81.8%)	2 (18.2%)	7 (87.5%)	1 (12.5%)
Ceftriaxone	25 (89.3%)	3 (10.7%)	10 (90.9%)	1 (9.9%)	7 (87.5%)	1 (12.5%)
Gentamicin	24 (85.7%)	4 (14.3%)	8 (72.7%)	3 (27.3%)	6 (75%)	2 (25%)
Ciprofloxacin	17 (60.7%)	11 (39.3%)	7 (63.6%)	4 (36.4%)	5 (62.5%)	3 (37.5%)
Co-trimoxazole	15 (53.6%)	13 (46.4%)	5 (45.5%)	6 (54.5%)	3 (37.5%)	5 (62.5%)
Azithromycin	15 (53.6%)	13 (46.4%)	7 (63.6%)	4 (36.4%)	4 (50%)	4 (50%)
Cefalexin	14	14	5	6	3	5

	(50%)	(50%)	(45.5%)	(54.5%)	(37.5%)	(62.5%)
Nalidixic acid	7	21	4	7	2	6
	(25%)	(75%)	(36.4%)	(63.6 %)	(72.2%)	(27.8%)
Erythromycin	4	24	3	8	6	2
	(14.3%)	(85.7%)	(27.3%)	(72.7%)	(72.2%)	(27.8%)
Ampicillin	1	27	3	8	0	8
	(3.6%)	(96.4%)	(27.3%)	(72.7%)	(00.0%)	(100%)
R – resistant, S - sensitive	Data expressed as (n).					

Table 2: Antimicrobial resistance pattern of samples containing more than one pathogenic strains of DEC (n=16).

Name of the antimicrobials	EPEC+ (n=3) EAggEC		ETEC + (n=4) EAggEC		ETEC+	EPEC (n=6)	ETEC+EPEC (n=3)	+EAggEC
	R	(%)	R	(%)	R	(%)	R	(%)
Mecillinam	1	33.33	1	25	0	0.00	2	66.67
Ceftriaxone	1	33.33	1	25	2	33.33	1	33.33
Gentamicin	0	00	2	50	2	33.33	1	33.33
Ciprofloxacin	1	33.33	2	50	1	16.67	3	100
Co-trimoxazole	2	66.67	1	25	2	33.33	2	66.67
Azithromycin	2	66.67	1	25	2	33.33	1	33.33
Cefalexin	3	33.33	2	50	3	50	2	66.67
Nalidixic acid	2	66.67	1	25	3	50	2	66.67
Erythromycin	2	66.67	3	75	4	66.67	3	100
Ampicillin	3	100	4	100	6	100	3	100

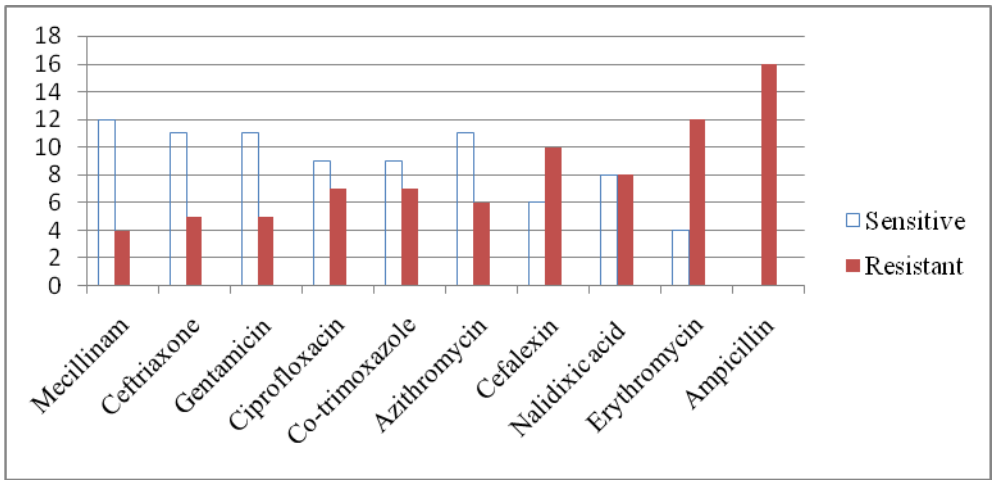

Figure 1: Antimicrobial susceptibility pattern of samples containing more than one pathogenic strains of DEC (n=16).

IV. DISCUSSION

Diarrheagenic *E. coli* strains are being recognized as an important pediatric enteropathogens worldwide. [13]In recent years, antibiotic resistance of diarrheagenic pathogens has reached alarming proportions worldwide. The misuse of antibiotics has been found to be the most important selecting force in the generation of bacterial resistance to antimicrobial drugs. [14]

In the present study, resistance to ampicillin and nalidixic acid was shown by 96% and 75% EPEC, 73% and 64% ETEC and 100% and 28% EAggEC respectively. Similarly, in Thailand isolated DEC showed high resistance to commonly used antibiotics such as ampicillin and nalidixic acid. [15]Azithromycin was resistant to 46% EPEC, 36% ETEC and 50% EAggEC. Gentamicin was more effective showing resistance 14% by EPEC, 27% by ETEC and 25% by EAggEC. Regarding the aminoglycosides-gentamicin, low levels of intermediate resistance were found, corroborating data in the literature which suggest a good activity of these antimicrobials against enteric Gram-negative bacilli. Moreover, such drugs are considered as antimicrobials used in hospitals, and resistant bacteria originating from the community are not expected. [16] In present study,

46% EPEC, 55% ETEC and 65% EAggEC were resistant to co-trimoxazole. The levels of resistance observed for trimethoprim-sulfamethoxazole reflect the results from several studies by other authors who demonstrated high rates of resistance towards enteric *E. coli* against this drug. One explanation for this could be its widespread use in the treatment of diseases associated with Gram-negative bacteria, especially in children under two years of age with acute infectious diarrhea. [17] Resistance to ciprofloxacin was shown by 39% EPEC, 36% ETEC and 38% EAggEC. The literature has reported varying rates of resistance against ciprofloxacin, which can be explained by the high prescription of this drug in some countries as a treatment for enteric infections caused by Gram-negative bacteria. [18, 19] Regarding mecillinam and ceftriaxone, 93% and 89% EPEC, 82% and 91% ETEC are sensitive respectively and 88% EAggEC are sensitive to both of these two drugs. The most effective antibiotics are mecillinam and ceftriaxone. The observation may imply that the strains were likely to have originated from the community, which supports the observation of low levels of resistance to such drugs. [20] In present study, sample containing more than one pathogenic strain of DEC, are resistant to more than one antimicrobial drugs. The high antimicrobial resistance observed in our study raises a broad discussion on the indiscriminate or improper use of antimicrobials which becoming an alarming situation in drug resistance.

Monitoring drug resistance patterns of *E. coli* will give vital clues to clinicians regarding therapeutic regimens to be adopted against individual cases and will be an important tool to devise a comprehensive chemo-prophylaxis.The development of newer antibiotics may offer a short term solution to the problem of resistance among diarrheagenic bacteria especially *E. coli* but more effective measures, such as health education and further research on the prevention of infections through quality sanitation.

REFERENCES

[1] J.A. Adachi, Z.D. Jiang, J.J. Mathewson, M.P. Verenkar, S. Thompson, F. Martinez-Sandoval, and H.L. DuPont. EnteroaggregativeEscherichia coli as a major etiologic agent in traveler's diarrhea in 3 regions of the world. Clin. Infect. Dis., 32, 2001, 1706–1709.

[2] K. Ogata, R. Kato, K. Ito, and S. Yamada. Prevalence of Escherichia coli possessing the eaeA gene of enteropathogenic E. coli (EPEC) or the aggR gene of enteroaggregative E. coli (EAggEC) in traveler's diarrhea diagnosed in those returning to Tama, Tokyo from other Asian countries. Jpn J., 2002.

[3] S. Nys, I.N. Okeke, S. Kariuki, G.J. Dinan, C. Driessen, and E.E. Stobberingh. Antibiotic resistance of fecal Escherichia coli from healthy volunteers from eight developing countries. J Antimicrob Chemother, 54, 2004, 552-555.

[4] C.D. Ericsson. Travellars' diarrhea. Epidemiology, prevention and self-treatment. Infect Dis Clin North Am, 12, 1998, 285-303.

[5] J.P. Nataro and J.B. Kaper. Diarrheagenic Escherichia coli. Clin Microbiol Rev, 11, 1998, 142–201.

[6] P.L. Winokur, D.L. Vonstein, L.J. Hoffman, E.K. Uhlenhopp, and G.V. Doern. Evidence for transfer of CMY-2 AmpC-lactamase plasmids between E. coli and Salmonella isolates food, animals and humans. Antimicrobial Agents and Chemotherapy, 45(10), 2001, 2716-2722.

[7] S. Miranda, M.G. David, and J.C. Peter. Evolution of multiresistance plasmids in Australia clinical isolates of Escherichia coli. Microbiol., 150, 2004, 1539-1546.

[8] T. Estrada-Garcia, J.F. Cerna, L. Paheco-Gil, R.F. Vela´zquez, T.J. Ochoa, J. Torres et al. Drug-resistant diarrhegenic Escherichia coli, Mexico. Emerg Infect Dis, 11, 2005, 1306–08.

[9] F. Quadri, A.M. Svennerholm, A.S. Faruque, and R.B. Sack. Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment and prevention. Clin. Microbiol. Rev., 18, 2005, 465-483.

[10] M. Cheesbrough. District Laboratory Practice in Tropical Countries (Part II). Cambridge University. Editions, Cambridge University press, The Edinburgh Building, Cambridge, United Kingdom 2004: 50-120.

[11] A.W. Bauer, W.M.M. Kirby, J.C. Sherris, and I. Turck. Antibiotic susceptibility by a standardized single method. The Am J Clin Path, 36, 1966, 493-6.

[12] Clinical Laboratory Standards Institute (CLSI). 2009. Performance Standards for Antimicrobial Disk Susceptibility Tests. CLSI document M02-A10, Approved Standard - 10th ed. CLSI, Wayne, PA, USA.

[13] T.J. Ochoa, L. Ecker L, F. Barletta, M.L. Mispireta, A.I. Gil, C. Contreras et al. Age-related susceptibility to infection with diarrheagenic E. coli in infants from peri-urban areas of Lima, Peru.Clin Infect Dis, 49(11), 2009, 1694–1702.

[14] C.S. Yah, and N.O. Eghafona. Plasmids: A Vehicle for Rapid Transfer of Antibiotic Resistance Markers Of Salmonella Species In Animals. J. Am. Sci., 3(4), 2007, 86-92.

[15] S. Kalnauwakul, M. Phengmak, U. Urairat, K. ongmuang, Y. Nakaguchi, and M. Nishibuchi. Examination of diarrheal stools in Hat city, South Thailand for Esch. coliand other DEC using immunomagnetic separation and PCR method. Southeast Asian J Trop Med Public Health, 38, 2007, 871-80.

[16] C.R. Usein, S. Tatu-ChitoiuCiontea, M. Condei, and M. Damian. Escherichia coli pathotypes associated with diarrhea in Romanian children than 5 years of age. J. Infect. Dis., 62, 2009, 289-293.

[17] K.R.S. Aranda, U. Fagundes-Neto, and I.C.A. Scaletsky. Evaluation of multiplex PCRs for diagnosis of infection with diarrheagenic Escherichia coli and Shigellaspp. J. Clin. Microbiol.,42, 2004, 5849-5853.

[18] D.M. Livermore, D. James, M. Reacher, C. Graham, T. Nichols, P. Stephens, A.P. Johnson, and R.C. George. Trends in fluoroquinolone (ciprofloxacin) resistance in Enterobacteriaceae from bacteremias, England and Wales, 1990-1999. Emerg. Infect. Dis., 8, 2002, 473-478.

[19] C.M. Yang, M.F. Lin, C.H. Lin, Y.T. Huang, C.T. Hsu, and M.L.Liou. Characterization of antimicrobial resistance patterns and integrons in human fecal Escherichia coli in Taiwan. J. Infect. Dis., 62, 2009, 177-181.

[20] A. Erb, T. Stürmer, R. Marre,and H. Brenner. Prevalence of antibiotic resistance in Escherichia coli: overview of geographical, temporal and methodological variations. Eur. J. Clin. Microbiol. Infect. Dis., 26, 2007, 83-90.