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Occurrence of Extended Spectrum Beta-lactamases Producing Alpha Hemolytic *Escherichia coli* in Neonatal Diarrhea

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Abstract: *E. coli*, often highly pathogenic in neonatal and immuno-compromised patients, are usually supposed susceptible to a variety of chemotherapeutic agents, however with the time and also due to the extensive use of chemotherapeutic agents irrespective of their susceptibility, have evolved drug resistant strains. Moreover, the production of extended spectrum β-lactamases producing enteric pathogens is a serious issue. In this context the present study was conducted to find out occurrence of extended spectrum Beta-lactamases producing alpha hemolytic *Escherichia coli* in neonates, of less than one month of age, suffering from diarrhea. Fecal samples were collected from various private hospitals in Mathura and Agra districts of Uttar Pradesh, India. With the help of hospital nursing staffs sterilized stool samples were collected and processed for isolation of E. coli. The double disk diffusion method was applied to assess the ESBL production. *E. coli* organisms were isolated from 39 kids out of 120 samples. The assessment of isolates revealed alpha hemolytic nature of 23 isolates on 5% sheep blood agar. As usual when drug sensitivity was performed that revealed their multi drug resistance pattern which on further examination with double disk method showed 17 of them to be extended spectrum β-lactamases producing *E. coli*. The presence of enterohemorrhagic extended spectrum β-lactamases producing *Escherichia coli* in kids is a matter of concern and public health importance as it may be due to the transmission from hospital itself during the birth time or post birth admission period.

Key words: Antibiotic sensitivity, extended spectrum β-lactamases, *Escherichia coli*, neonatal diarrhea

INTRODUCTION

Recently, researchers are focusing on infections, which are zoonotic in nature like salmonellosis (Verma *et al*., 2007, 2008, 2011a , b), campylobacteriosis (Kumar *et al*., 2012), colibacillosis (Malik *et al*., 2012), leptospirosis (Verma *et al*., 2012), swine flu (Dhama *et al*., 2012) etc. These pathogens cause serious complications in animals as well as humans particularly newborns. Among these role of in neonatal and immuno-compromised patients is well described (Pruss, 1998). *E. coli* are mainly enteric pathogens but bacteremia, wound infections, urinary tract infection, neonatal meningitis along with gastrointestinal infections are often fatal in newborns (Raina *et al*., 1999). On the basis of their pathogenesis *E. coli* has several types that are involved in different types of gastroenteritis and enterohemorrhagic *E. coli* (EHEC) are one of them. These enterohemorrhagic *E. coli* (EHEC) are also known as Verotoxigenic *E. coli* (VTEC) (Riley *et al*., 1983) and similar to shiga toxin-producing *E. coli* (STEC). These have been found predominantly associated with diarrhea in humans (Mainil and Daube, 2005). In general as a commensal of intestine *E. coli* are usually supposed susceptible to a variety of chemotherapeutic agents, however with the time and also due to the extensive use of chemotherapeutic agents irrespective of their susceptibility, have evolved drug resistant strains. Moreover, the production of extended spectrum β-lactamases (ESBL) producing enteric pathogens has been a serious issue. As Mathur *et al*. (2002) have reported 68% ESBL positivity rate in their enterobacteriacae isolates and this increase in ESBL mediated resistance amongst *E. coli* isolates has made it worldwide a major public health threat. In this context, ESBL study was also initiated along with assessment of

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their antibiogram profile to screen the presence of ESBL producing *E. coli* in neonatal diarrhea.

MATERIALS AND METHODS

Sample collection: A total of 120 samples were collected from the cases of kids admitted in various private hospitals in Mathura and Agra Districts of Uttar Pradesh. All the kids were suffering from diarrhea and were less than one month of age. With the help of hospital nursing staffs sterilized paper napkins were fixed inside the diaper and immediately after deposition of stool it was transferred in sterilized screw capped bottles for further laboratory examinations.

Microbiological culture and identification: Samples were inoculated onto MacConkey Lactose agar (MLA) (Himedia, Mumbai) and incubated aerobically/ microaerobically at 37°C for 24 h (Quinn *et al*., 2002). After 24 h incubation, the plates were observed for presence of bacterial colony. All the pink colonies suspected to be of *E. coli* were further restreaked on MLA plates and after incubation at 37°C for 24-48 h pink colonies from each MLA plate were picked up and streaked on Eosine Methylene Blue (EMB) agar (Himedia, Mumbai) for observing the metallic sheen. Bacterial isolates suggestive of *E. coli* on MLA and EMB were studied on the basis of their cultural, morphological and motility characteristics as per the method of Cruickshank *et al*. (1975).

Detection of *E. coli* toxin (hemolysin): Isolates of *E. coli* were tested for hemolysin production on 5% sheep blood agar according to Beutin *et al*. (1989). Blood agar plates streaked with *E. coli* were incubated at 37°C and examined at 6 h of interval up to 24 h for the presence of zone of haemolysis.

Antibiogram: All the isolates were examined for their drug sensitivity pattern by disc diffusion method (Bauer *et al*., 1966) using 20 commonly used antibiotic discs (Hi-Media, Mumbai) viz., amikacin (30 mcg), ampicillin (10 mcg), aztreonam (30 mcg), cefadroxil (30 mcg), Cefdinir (5 mcg), ciprofloxacin (30 mcg), co-trimoxazole (25 mcg), cloxacillin (5 mcg), erythyromycin (15 mcg), gentamicin (10 mcg), kanamycin (30 mcg), lincomycin (15 mcg), norfloxacin (10 mcg), nitrofurantoin (300 mcg), pefloxacin (5 mcg), penicillin (10 IU), rifampin (5 mcg), tetracyclin (30 mcg), tobramycin (10 mcg), vancomycin (30 mcg). For the preparation of bacterial lawn on plates, six h young broth culture (4.8×10^{10} CFU mL^{-1}) of each isolate was smeared over the nutrient agar medium by sterilized cotton swab. Then inoculated plates were allowed to dry at room temperature for 10-15 min. Following this the antibiotic discs were placed on the surface of inoculated medium by sterile forceps with uniform spacing between two discs and pressed gently to ensure full contact. Plates were incubated at 37°C for overnight. The zone of inhibition was recorded in millimeters and compared with the chart provided by the manufacturer (HiMedia, Mumbai) for assessing the sensitivity of the antibiotics. The interpretation of results was performed as per the guidelines of NCCLS (2002).

ESBL determination by double disk method: The disk approximation method was used with the antimicrobial disks of cefpodoxime and clavulanic acid. A Mueller-Hinton agar plate was inoculated with a suspension made from an overnight blood agar culture of the isolates as recommended for a standard disk diffusion susceptibility test. Disks containing the standard cefpodoxime (10 μg) are placed 15 mm apart (edge to edge) from an amoxicillin-clavulanic acid disk containing 10 μg of the latter compound as per the recommendation of Coudron *et al*. (1997) to have greater sensitivity. Following incubation for 16-20 h at 35°C, any enhancement of the zone of inhibition between a beta-lactam disk and that containing the beta-lactamase inhibitor is indicative of the presence of an ESBL was observed with all the precautions (Moland and Thompson, 1994).

RESULTS AND DISCUSSION

After the incubation of prescribed conditions, out of total 120 samples 49 MLA plates revealed bacterial growth with pink color colonies. When these pink color colonies were transferred on EMB media only 39 isolates produced metallic sheen, characteristic of *E. coli*. These isolates were further confirmed on the basis of cultural, morphological and biochemical characters (Table 1). It revealed 32.5% occurrence rate of *E. coli* in neonatal diarrhea (Table 2). On blood agar 23 isolates revealed a hazy wider zone of haemolysis depicting 58.97% incidence of haemolytic *E. coli* in total cases of diarrhea due to *E. coli* (Table 2). The double disk diffusion method showed the presence of 17 ESBL producing isolates representing 43.59% (Table 2). Antibiogram showed that *E. coli* isolates had 100% resistance against most of the commonly used antibiotics such as ampicillin, Cefdinir, co-trimoxazole, cloxacillin, erythyromycin, lincomycin,

Table 1: Cultural, morphological and biochemical characteristics of isolates

	Cultural		Morphological	Biochemical
Organisms	MLA	EMB	Gram's staining	IMViCpattern
E. coli	Pink colony	Metallic sheen	Gram Negative coco bacilli	+ + - -

IMViC: Indole utilization, Methyl red reduction, Vogus proskaure, Citrate utilization tests, +: Positive test result, -: Negative test result

Table 2: Occurrence of *E. coli* in neonatal diarrhea

Total No. of samples	Occurrence of *E. coli*	Occurrence of *E. coli* (%)	α hemolytic *E. coli* (%)	ESBL producing *E. coli* (%)
120	39	32.5	19.16 (58.97)*	14.16 (43.59)*

*Figure in parenthesis represents values against total No. of isolates (39)

Table 3: Results of antibiotic sensitivity test of *E. coli* isolated from neonatal diarrhea cases

	No. of isolates (39)				
Name of antibiotic	Resistant	Intermediate	Sensitive	Sensitivity (%)	Resistant (%)
Amikacin	4	1	34	87.18	10.260
Ampicillin	39	-	-	-	100.00
Aztreonam	5	4	30	76.92	12.820
Cefadroxil	33	-	6	15.38	84.620
Cefdinir	39	-	-	-	100.00
Ciprofloxacin	32	3	4	10.26	82.050
Co-trimoxazole	39	-	-	-	100.00
Cloxacillin	39	-	-	-	100.00
Erythyromycin	39	-	-	-	100.00
Gentamicin	-	17	22	56.41	-
Kanamycin	10	14	15	38.46	25.64
Lincomycin	39	-	-	-	100.0
Norfloxacin	39	-	-	-	100.0
Nitrofurantoin	20	19	-	-	51.28
Pefloxacin	39	-	-	-	100.0
Penicillin	39	-	-	-	100.0
Rifampin	39	-	-	-	100.0
Tetracyclin	39	-	-	-	100.0
Tobramycin	29	10	-	-	74.36
Vancomycin	39	-	-	-	100.0

norfloxacin, pefloxacin, penicillin, rifampin, tetracycline and vancomycin (Table 3). Only three drugs mainly Amikacin (87.18% sensitivity), Aztreonam (76.92%) and Gentamicin (56.41%) were found to be the effective drugs which could prohibit the growth above 50%. Kanamycin showed only 38.46% sensitivity (Table 3).

The occurrence rate (32.5%) of *E. coli* again supports the prior information that *E. coli* are an important pathogens group in community and hospital-acquired infections (Murugan *et al*., 2011). The higher percentage of enterohemorrhagic isolates also augment the gravity of problem. Moreover, the drug resistance pattern is alarming with 100% resistance against many of drugs. These are the drugs which are commonly used for the general treatments in hospitals and thus the continuous exposure to these drugs might be the major reason behind such drug sensitivity pattern. Unfortunately resistance has become increasingly common among gram-negative bacteria making empirical therapy decisions more difficult. The most serious resistance patterns now emerging among gram-negative organisms include resistance to extended spectrum of cephalosporin and penicillin (Motta *et al*., 2003). Recently, Murugan *et al*. (2011) reported difficulties in the treatment of food and water associated gastrointestinal diseases due to *E. coli* and this problem had been compounded by the continued emergence of antibiotic resistance to a growing number of antibiotics. Many reports are available on the drug resistance pattern of *E. coli* isolates from different origin but this appears to be first of its kind as it dealt with neonatal diarrhea. Increase in antibiotic resistance level is now a global problem. Infections with antibiotic resistant bacteria make the therapeutic options for infection treatment, extremely difficult or virtually impossible in some instances (El-Astal, 2004). Goettsch *et al*. (2000) observed resistance against cephalosporines. norfloxacin, amoxycillin, trimethoprim and nitrofurantoin however, in contrast to that Walia *et al*. (2004) reported *E. coli* resistance against carbenicillin, tetracycline, streptomycin but sensitivity to fluroquinolones and third generation cephalosporins. Further more drugs showed resistance as nalidixic acid, gentamicin, cefuroxime (Shehabi *et al*., 2004) and ampicillin, ceftriaxone, ciprofloxacin, ceftazidime and cefotaxime (Patoli *et al*., 2010). These reports and the findings of present study clearly indicate rapid and widening resistance pattern of *E. coli* isolates. Moreover, extended spectrum beta lactamases producing strains of enterobacteriacae have emerged as a major problem in hospitalized as well as community based patients and Infections due to ESBLs-producers range from uncomplicated UTI to life threatening sepsis (Bhattacharya, 2006). Simultaneously the ESBL phenotypes and detection have become more complex due to the diversity of the enzymes produced, emergence of inhibitor resistant ESBL variants plasmid borne resistance genes, concurrent Amp-C production enzyme hyperproduction and porin loss (Sturenburg *et al*., 2004). Although, the present findings with ESBL positivity (43.59%) of isolates are lesser than the Mathur *et al*. (2002), who reported 68% ESBL positivity rate in their enterobacteriacae isolates, even then it is alarming particularly in neonatal diarrhea.

CONCLUSION

The increase in ESBL mediated resistance amongst *E. coli* isolates worldwide makes this a major public health threat. Now the presence of these ESBL producing multi drug resistant α hemolytic *E. coli* in neonatal diarrhea has posed another challenge to public health.

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