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PREVALENCE AND ANTIBIOTIC RESISTANCE OF DIARRHEAGENIC *ESCHERICHIA COLI* ISOLATED FROM ACUTE GASTROENTERITIS PATIENTS IN COIMBATORE, INDIA

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ABSTRACT

The present study assessed the antibiotic susceptibility patterns of diarrheal isolates of *E.coli*. A total of 144 isolates were confirmed with selective media and biochemical test. The isolates were analyzed for antimicrobial susceptibility to 16 types of antibiotics using disc diffusion method. Among them, Tetracycline (92.3%) showed the highest resistance and Chloramphenical (20.5%) showed lowest resistance to *E. coli* isolates. Presently 3% of isolates were resistance to all 16 antibiotics, 39% of where resistance to 12 antibiotics. Totally 19 types of resistance patterns were observed from 9 groups of antibiotics, among them highest resistance were observed from Tetracycline and followed by Carbapenems. This study revealed that the highest antibiotic resistance was observed in not only betalactam antibiotics, which was highest in non betalactam antibiotics also. 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: Acute gastroenteritis, *E.coli*, Antibiotic resistance; Diarrheagenic pathogens, betalactam antibiotics.

INTRODUCTION

Gastroenteritis is the most common type of illness which is commonly called "food poisoning," because it mainly occurs due to food and water contamination. In India, nearly 10 million people are affected in each year. The illness occurs by the infection and inflammation of the digestive system. This diarrheal infection in developing countries is largely due to low hygiene, or sanitation as well as low budgets of primary and secondary health care.

In India, around 23% of childhood mortality has been reported due to diarrhea.[1] Among the bacterial agents, *Escherichia coli* is the most common etiologic pathogens of diarrhea in human and animal gut. Treatment in severe cases is typically consists of a broad-spectrum antimicrobial, although antimicrobial resistance in *E.coli* is widely prevalent in both developed and developing countries.[2]

From the last decades, this pathogen slowly has developed strains independently resistant to several antibiotics even commonly used drugs for the treatment of enteric infections, including ampicillin, tetracycline and co-trimoxasole has increased among DEC. The overuse and misuse of antibiotics in the treatment of diarrhea could lead to increased antibiotic resistance.[3]

In India, few studies have investigated the prevalence of diarrheagenic *E. coli* and their MDR patterns against various groups of antibiotics, where awareness and understating of MDR is still limited. Having known the development of drug resistance and seriousness of antimicrobial resistance among *Escherichia coli*, the present study was undertaken to assess the antibiotic resistance pattern of *Escherichia coli* isolated from acute gastroenteritis patients in Coimbatore.

METHODOLOGY

Microorganisms

This study was conducted from Jan 2011 to Dec 2014. Totally 155 *E. coli* isolates were obtained from the Microtech Diagnostic Centre, Coimbatore, Tamilnadu. All isolates of *E.coli* were isolated from acute gastroenteritis patient's stool samples. Diarrhea was defined as a history of more than one liquid stool per day or three or more stools of loose consistency during the previous 24 hours. The following information was provided by the submitting laboratories: sex, age, date of collection and patient location. One isolate per patient was included.

Confirmation of the isolates

All the bacterial cultures were tested for viability and purity once again by sub-culturing on MacConkey agar. The identification of the isolates were re-confirmed by performing biochemical test such as Indole test (I), Methyl red test (MR), Voges Proskauer test (VP), Citrate utilization test (C), Urease production test (U), Oxidase test, Catalase test, TSI agar test and Carbohydrate fermentation test.

Determination of Antibacterial activity

The disc diffusion method was followed to determine the antibacterial activity. Petri plate containing 20 ml of Mueller Hinton agar was seeded with 4 hours old fresh culture of clinical isolates and referral strains. By making use of template drawn discs were dispensed on the solidified Mueller Hinton agar with test organisms. This was incubated at 37^0C for 24 hours in an incubator.[4] The zone of inhibition was measured by making use of Antibiotic zone scale (Hi - media). The resistance patterns were interpreted as per CDC recommendations.

RESULTS AND DISCUSSION

Acute gastroenteritis accounts for millions of deaths each year in young children, mostly in developing countries. It causes diarrhoea or vomiting (or both) in seven days duration and may be accompanied by fever, abdominal pain, and anorexia. Diarrheal illnesses are a severe public health problem and a major cause of morbidity and mortality in infants and young children. In India, nearly 10 million cases of gastroenteritis are recorded per year.

The gastroenteritis has been frequently a result of poor sanitation, the lack of safe drinking water, or contaminated food-conditions common in developing nations.

Gastroenteritis by themselves has varying forms with different etiologies and pathologic mechanisms. However, clinical symptomatologies have invariably helped the clinician to characterize the disease and to determine the management strategies. The clinical symptoms appear in different combinations at varying time durations throughout the course of the disease. Analysis of this clinical pattern is the monitoring device to assess the progress of the disease, the efficacy of the treatment and the level of prognosis for eventual recovery from the disease. Several pathogens such as bacteria, viruses and parasites are the causes of diarrhea in human. Among them, *Escherichia coli* is the most important etiologic agents of diarrheal diseases.[5]

The illness is triggered by the infection and inflammation of the intestinal tract. It is not a life-threatening condition and does not require treatment in all the cases as it is self-limiting. In severe cases, it may lead to severe dehydration that ultimately results in shock or coma. According to symptoms, physicians insist the sample collection for microbial evolution. Initially stool samples were collected and subjected for identification of diarrheal causing isolates. Acute gastroenteritis, occurred by bacteria and parasites, are one of the most seen diseases in our region. Our aim was to determine the antibiotic resistance and virulence characterization in collecting stool isolates of *E. coli*.

In this current study, stool isolates of *E.coli* were obtained from the Microtech Diagnostic Centre, Coimbatore, Tamil nadu. A total of 155 *E.coli* isolates were collected from clinically diagnosed cases of acute gastroenteritis (AGE) admitted in a nearby hospital from clinical laboratory. The following information was provided by the submitting laboratories: sex, age, date of collection and patient location. Among the 155 isolates, 144 of were confirmed as *E.coli*. Apart from selective media and biochemical characterization, Chromogenic media were utilized for the confirmation of isolates.

The findings in this study indicate that age remains major risk factors in diarrheal disease, children between the ages of 0 to 10 years are highly affected with *E. coli* infection. The male infant and children with *E.coli* was 34.2%, while it was 28.1% in female infants and children. The rate of prevalence was showed in Fig.1 and Fig.2. Totally, a prevalence of 31.1% *E.coli* in respondent's 0 to10 years in this work, this was lower than the 41 % obtained by Ifeanyi[6] and Sweta et al.,[7] and higher than the 2.6% obtained by Yilgwan and Okolo.[8] These differences might be due to improper sanitation and unhygienic infrastructure of the respondents from these cities.

The high occurrence rate of diarrhea among children 0 to10 years in this study may be due to the fact that children within this age group on their own cannot differentiate between what to eat and what not to eat; they have not knowledge about the hygienic practices and also weaker immunity. In this study, it was observed that the number of *E.coli* gotten from adults was quite small compared to that obtained from children and this might not be unrelated to the fact that, adults in the locality rarely visit health institutions when they have diarrhea.

The use of traditional versus improved media formulation containing chromogenic substrate is currently an important topic in the field of microbiology. The focus behind such

developments was to produce media that would make the detection and identification of microorganisms more rapid and more reliable. Chromogenic substrates, such as ONPG, X-Gal, or X-Glu together with a specified selectivity of the medium are the simple principle behind Chromogenic media. The target organisms are characterized by enzyme systems that metabolize the substrates to release the chromogen. The chromogen can then be visually detected by direct observation of a distinct colour change in the medium. Direct confirmation of the target organism without further testing is sometimes possible. In 2013, Kalchayanand *et al.*,[9] observed *E.coli* from food samples with Chromogenic media. Our studies were agreed with previous study of Maya.[10] They were observed the different species of bacteria from clinical samples.

In recent years, biotechnology advance has led to changes in food test technique, and today, we benefit from methods that are more specific, faster and often more sensitive compared to conventional method.[11 and 12]

Antibiotic susceptibility assay

Determining the prevalence of gastroenteritis causing pathogens should save the way for better control of the disease. Most of the acute gastroenteritis is occurred by contaminated food and water. Hence, continued monitoring of the safety of food, health education of food handlers, and close attention to hygiene and sanitary conditions can provide an effective barrier against the spread of diarrheal infection.

The antimicrobial resistance may be as a result of inappropriate and wide use of different antibiotics to treat infection. In sample collected area, antibiotics are often dispensed without any prescription, and often by those who lack formal qualifications as pharmacists. This phenomenon was leading to high rate of antibiotic resistance against enteric pathogens. In most of the economically poor countries, trimethoprim-sulfamethoxazole, ampicillin and tetracycline are widely used to treat diarrhea because of their low cost and easily available. The boundless use of these antibiotics has resulted in an expended prevalence of resistance to these antibiotics by pathogenic bacteria.

In this current study, *E. coli* isolates were less susceptible to first line of antibiotics such as Amoxicillin, Tetracycline and ampicillin, this phenomenon will have very bad consequences for the peoples. Totally 16 antibiotics were utilized in this study, among them, Tetracycline (92.3%) showed the highest resistance and Chloramphenical (20.5%) showed lowest

resistance to *E.coli* isolates. This report is slightly different from the report given by Desenclos.[13] According to them 53% *E.coli* was resistant to ampicillin, 47% to chloramphenicol and 67% to tetracyline.

Presently 77% of isolates were resistant to ceftazidime, 75.5% for Cefoxitin and 75% for Imipenem, Gentamycin. The Cefpodoxime and Erythromycin were showing resistance to 74.3% and 74.1% respectively. This result was contrasted with previous study of Thirunalasundari et al., 2007. They were observed 26% of isolates were resistant to Cefpodoxime. Among the 16 types of antibiotics, lowest resistance was shown by Chloramphenical (20.5%) and followed by Amoxycillin (42.3%). This was lower than the Thirunalasundari[14] reports. According to them, 81% of *E.coli* was resistance to Amoxycillin and 26% to Chloramphenical (Table.1).

To our knowledge, this is the first study, which describes the prevalence of antibiotic resistance among *E. coli* isolated from Coimbatore area. The study shows high rates of resistance to individual antibiotics, by *E. coli* isolates from most isolates (72%) resistant to at least one antibiotic. Among the 144 isolates, 3% of isolates were resistance to all 16 antibiotics, 39% of where resistance to 12 antibiotics (Table.2).

Totally 19 types of resistance patterns were observed, among them, C, COT,AMC,TE,IPM, CEF,NX,CFM,CAZ pattern was observed in 16% (n=23) of isolates and followed by COT,AMC,P,GEN,TE,E,CX,AMP,CIP,CPD,CFM,CAZ, which were observed in 15.2% (n=22) of the isolates. In this study, no one isolates were sensitive to all antibiotics (Table.3).

In this present study, 9 groups of antibiotics were utilized, among them Tetracycline showed highest resistance and with regard 75% of isolates were resistance to Carbapenems, Macrolide and Aminoglycoside group of antibiotics. The Cephalosporin and betalactam group of antibiotics were resistance to 70% and 56.1% of the isolates respectively. Among the 9 group of antibiotics, lowest resistance was observed against to phenical (Table 4). This study revealed that the highest antibiotic resistance was observed in not only betalactam antibiotics, which was highest in non betalactam antibiotics also. This was agreed with Ivan et al.,[15] reports, they were also showing highest resistance against betalactam group of antibiotics.

The conclusion of the present study was that importance of notifying diarrheal disease. The highest antibiotic resistance was observed against to first line antibiotics. Continuous efforts are required to reduce the resistance burden in human by strict monitoring of antibiotic resistance of *E. coli* from diarrheal samples.

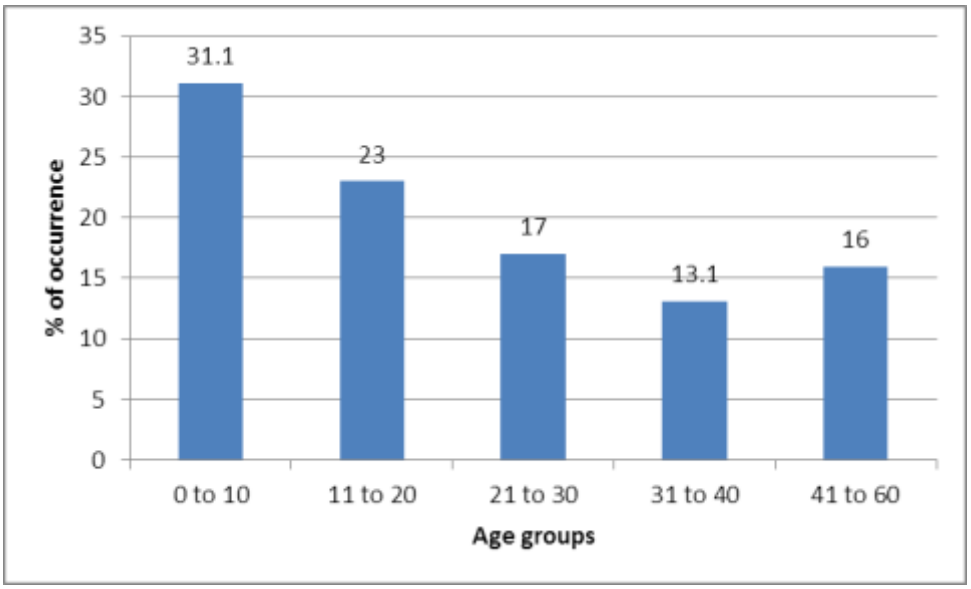

Figure 1: Percentage of occurrence on different age groups.

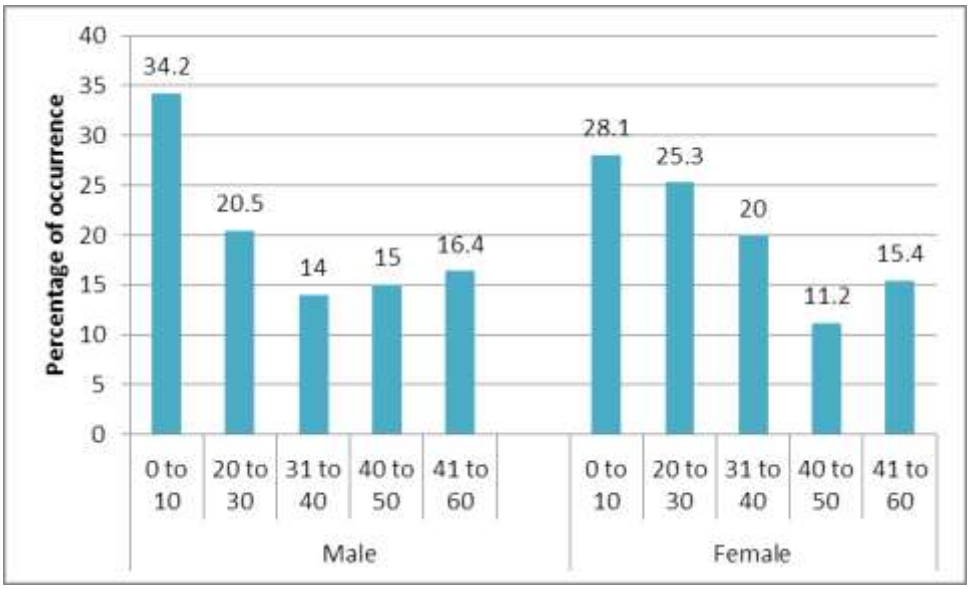

Figure 2: Prevalence of *E.coli* on different age groups.

Table 1: Percentage of antibiotic resistance on *E.coli.*

S.No	Antibiotics	Resistance (%)
1.	Chloramphenical	20.5
2.	Trimethoprim -sulfamethoxcin	54
3.	Amoxycillin	42.3
4.	Piperacillin	72.1
5.	Gentamycin	75
6.	Tetracycline	92.3
7.	Erythromycin	74.1
8.	Cefoxitin	75.5
9.	Ampicillin	54.2
10.	Imipenem	75
11.	Ceftriaxone	52
12.	Nalidixic acid	64
13.	Ciprofloxacin	58.3
14.	Cefpodoxime	74.3
15.	Cefixime	70
16.	Ceftazidime	77

Table 2: Isolation of multidrug resistance isolates of *E.coli.*

S.No	Number of antibiotics	Number of isolates
1.	16	4
2.	13	12
3.	12	56
4.	10	16
5.	9	41
6.	7	3
7.	5	1
8.	4	8
9.	3	1
10.	2	2

Table 3: Antibiotic resistance patterns of stool isolates of *E.coli.*

S.No	Resistance Patterns	No. of Isolates	Percentage
1.	P,GEN,TE,E,CX,IPM,CTR,NX,CPD,CAZ	15	10.4
2.	COT,P,GEN,TE,E,CX,IPM,NX,CIP,CPD,CFM,CAZ	15	10.4
3.	P,GEN,TE,E,CX,AMP,IPM,NX,CP,CPD,CFM,CAZ	18	12.5
4.	COT,AMC,P,GEN,TE,E,CX,AMP,CIP,CPD,CFM,CAZ	22	15.2
5.	C,COT,AMC,TE,IPM,CEF,NX,CFM,CAZ	23	16
6.	P,G,TE,T,E,CX,AMP,IPM,CEF,CPD	18	12.5
7.	COT,AMC,P,GEN,TE,E,CX,AMP,IPM,CEF,NX,CIP,CFM	8	5.5
8.	CIP,CPD,CFM,CAZ	8	5.5
9.	COT,AMC,P,GEN,TE,E,CX,AMP,IPM,CEF,NX,CIP,CPD	4	3
10.	GEN,TE,R,CX,AMP,IPM,CAZ	1	0.69
11.	C,COT,AMC,GEN,TE,CX,NX,CPD	1	0.69

12.	COT,AMC,GEN,TE,E,CX,AMP,CEF,NX,CIP,CPD,CFM,CAZ	1	0.69
13.	CIP,CPD,CFM,CAZ	1	0.69
14.	C,COT	1	0.69
15.	C,COT,AMC,P,GEN,TE,E,CX,AMP,IPM,CEF,NX,CIP,CPD,CFM,CAZ	4	3
16.	TE,E,CX,NX,CIP	1	0.69
17.	P.GEN,TE,CX,IPM,NX,CIP	1	0.69
18.	GEN,TE,E,CX,AMC,IPM,CEF,NX,CIP,CPD	1	0.69
19.	CFM,CAZ	1	0.69

Table 4: Resistance on group of antibiotics.

S.No	Group of antibiotics	Antibiotic name	% of resistance
1.	Betalactam	Ampicillin Piperacillin Amoxycillin	54.2 72.1 42.3
2.	Cephalosporin	Cefoxitin Cefixime Cefpodoxime Ceftriaxone ceftazidime	75.5 70 74.3 52 77
3.	Folate pathway inhibitor	Trimethoprim sulfamethoxcin	54
4.	Aminoglycoside	Gentamycin	75
5.	fluoroquinolone	Norfloxacin Ciprofloxacin	64 58.3
6.	Tetracycline	Tetracycline	92.3
7.	Chloramphenical	Chloramphenical	20.5
8.	Carbapenems	Imipenem	75
9.	Macrolide	Erythromycin	75

REFERENCE

1. Jones G, Schultink W, Babille M. Child survival in India. Indian J Pediatr, 2006; 73: 479–487.
2. Amany El-Sharif and Raghdaa Ali. Molecular detection of TEM-Type β- lactamase producing Escherichia coli from diarrheic Egyptian children. Archives of clinical microbiology, 2012**;** 3(5): 1-4**.**
3. Mohammad Yousef Alikhani, Seyyed Hamid Hashemi, Mohammad Mehdi Aslani, Safar Farajnia. Prevalence and antibiotic resistance patterns of diarrheagenic Escherichia coli isolated from adolescents and adults in Hamedan, Western Iran. IJM, 2013; 5(1): 42-47.
4. A.W. Bauer, W.M. Kirby, C. Sherris, M. Turck Am. J. Clin. Pathol, 1966; 45: 493-496.
5. Ferro TAF, Moraes FC, Da Silva AM, Porcy C, Soares LA, Monteiro CA, Melo Lobao NT, Assis de Mello FA, Monteiro-Neto V, De Maria Silva Figueiredo P. Characterization

of virulence factors in Enteroaggregative and atypical enteropathogenic Escherichia coli strains isolated from children with diarrhea. Adv. Inf. Dis, 2012; 2(4): 1-8.

6. Ifeanyi CIC, Isu RN, Akpa AC, Ikeneche NF. Enteric Bacteria Pathogens Associated With Diarrhoea of Children in the Federal Capital Territory Abuja, Nigeria. New York Science Journal, 2010; 3(1): 62-69.
7. Sweta Muni, Ajay Kumar, Shahi S K, Deepak Pankaj, Aninda Sen, Swati Salila. Diarrhoea with Special Reference to Enteropathogenic *E. coli* in Children below Five Years at a Tertiary Care Centre. American Journal of Food Science and Technology, 2016; 4(5): 125-128.
8. Yilgwan CS, Okolo SN. Prevalence of Diarrhoea Disease and Risk Factors in Jos University Teaching Hospital, Nigeria. Annals of African Medicine, 2012; 11(4): 217-221.
9. Kalchayanand N, Arthur TM, Bosilevac JM, Wells JE, Wheeler TL. Chromogenic agar medium for detection and isolation of *Escherichia coli* serogroups O26, O45, O103, O111, O121, and O145 from fresh beef and cattle feces. J. Food Prot, 2013; 76: 192–199.
10. Angamuthu Shalini Maya, Kesani Prabhakar, Luke Elizabeth Hanna,Yelavarthi Lakshmi Sarayu. Phenotypic and Genotypic Characterization of Extended Spectrum b-Lactamase Producing Escherichia coli Clinical Isolates from Semiurban Area. Journal of Pharmacy Research, 2011; 4(1): 6-10.
11. Stampi S, Caprioli A, De Luca G , Quaglio P, Sacchetti R, Zanetti F. Detection of Escherichia coli O157 in bovine meat products in northen Italy. Int. J. Food Microbiol, 2004; 90(3): 257-262.
12. Ten LN, Im WT, Kim MK, Kang MS, Lee ST. Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. J. Microbiol. Methods, 2004; 56(3): 375-382.
13. Desenclos, Zergaba Chew A, Desve Admassu, G. Ethiopia.J.Trop.Med.Hyg, 1998; 91(6): 296-301.
14. Thirunalasundari, S.Rajan, S.Balakumar. Antibiotic Resistance Pattern Of Diarrhoegenic *Escherichia coli.* BTAIJ, 2007; 1(1): 35-39.
15. Ivan Literak, Radim Petro, Monika Dolejska, Erika Gruberova, Hana Dobiasova, Jan Petr, and Alois Cizek. Antimicrobial Resistance in Fecal Escherichia coli Isolates from Healthy Urban Children of Two Age Groups in Relation to Their Antibiotic Therapy. Antimicrobial agents and chemotherapy, 2011; 3005–3007.