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Antibiotic Resistance Pattern Of Diarrhoegenic *Escherichia coli*

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ABSTRACT

Antibiotic resistance of microbes is an emerging problem throughout the world. Periodical checking of drug resistance is essential to overcome this problem. *Escherichia coli* is the major pathogen of diarrhoea in children and travelers. To assess the antimicrobial resistance pattern of diarrhoeogenic *Escherichia coli*, stool samples were collected from diarrhoeal patients attending Annal Ghandhi Memorial Government Hospital and two private hospitals of Tiruchirappalli. A total of 368 Es*cherichia coli* strains were isolated by making use of selective and differential media and identified by various biochemical tests. The isolates were confirmed by serotyping. 32 commercial antibiotics were subjected to look for their activity by disc diffusion method. Results revealed that all the *Escherichia coli* strains tried were resistant to antibiotics tested. The percentage of activity varies between 11 to 97%. 89% of *Escherichia coli* isolates were sensitive to oxytetracyline . All the *Escherichia coli* isolates were found to be multiple drug resistant.

KEYWORDS

Escherichia coli;
Multiple drug resistance;
Diarrhoea;
Antibiotics.

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INTRODUCTION

Diarrhoea caused by *Escherichia coli* is one of the major health problems for children in developing countries and travelers[14]. Concept of *Escherichia coli* as normal flora was ruled out because 60% of diarrhoeal pathogens were found to be *Escherichia coli* in China[19]. *Escherichia coli* was categorized into different groups based on their pathogenicity and serological nature. They are enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC), entero invasive *Escherichia coli* (EIEC), enteroaggregative *Escherichia coli* (EAggEC), and enteroha emorrhagic *Escherichia coli* (EIEC)[19].

Antibiotics are used as chemotherapeutic agents to treat various bacterial infections. Antimicrobial therapy for diarrhoea reduces severity and duration of illness and also prevent lethal complications[13].

Drug resistance is one of the emerging clinical and public health problem[7]. Resistance has emerged even to newer and more potent antimicrobial agents[6]. Antimicrobial resistance in enteric pathogen is of great importance in developing countries, where the rate of diarrhoeal diseases is high[16]. Routine monitoring of antibiotic resistance provide data for antibiotic therapy and resistance control.

World Health Organization, Center for Disease Control and other disease prevention agencies have recognized the importance of studying the emergence and determinants of resistance as well as the need for control strategies[7].

Having known the development of drug resistance and seriousness of antimicrobial resistance among diarrhoeogenic pathogens, particularly *Escherichia coli*, the present study was undertaken to assess the antibiotic resistance pattern of *Escherichia coli* isolated from diarrhoeal patients of Tiruchirappalli.

MATERIALS AND METHODS

A total of 1259 stool samples were collected from the patients suffering from diarrhoea and admitted in diarrhoeal wards of Annal Ghandhi Memorial Government Hospital, Tiruchirappalli and two private hospitals of Tiruchirappalli. Study group comprises patients below 15 years. Samples were collected from the patients for a period of one year i.e., from June 2001 to July 2002 and processed at the Department of Microbiology, Srimad Andavan Arts & Science College, Tiruchirapalli-620 005, Tamil Nadu, India.

Isolation of *Escherichia coli*

For proper recovery of *E.coli*, stool samples were enriched by keeping in gram negative broth for 3-5 hours at 37^0C and inoculated on selective and differential media like hektoein enteric agar, XLD medium and EMB agar. After 24 hours incubation, plates were looked for the growth of microorganisms.

Identification of *Escherichia coli*

Bacterial isolates were identified by making use of various biochemical tests[8] other than routine macroscopy, microscopy and staining techniques.

Isolated and identified *Escherichia coli* were further confirmed by serotyping by making use of monovalent and polyvalent O antiserum obtained form M/s Denka Seikan Co Ltd Tokyo, Japan.

Test organism

368 *Escherichia coli* were isolated from the stool of diarrhoeal patients and were used as the test organism. *Escherichia coli* MTCC 46 obtained from Microbial Type Culture Collection Center, Chandigarh was used as a standard referral strain.

Test antibiotics

Both board spectrum and narrow spectrum antibiotics were used to asses the sensitivity pattern of the clinical isolates of *Escherichia coli*, *Salmonella* sp. and *Shigella* sp. Antibiotics like Amikacin (30μg), Azithromycin (15 μg), Azlocilin (75 μg), Aztreonam (30μg), Carbencilin(100μg), Cefdinir (5 μg), Cefe pime(30μg), Cefixime(5μg), Cefpodoxime(10μg), Cefprozil(30 μg), Chloramphenicol(30μg), Cipro floxin (5 μg), Doxtcyclin (30 μg), Fosfomycin (200 mg), Levoflaxin (5 μg), Meropenem (10 μg), Methicillin (5 μg), Minocyclin (30 μg), Nalidixicacid (30 μg), Novobiocin (30 μg), Ofloxacin (5 μg), Rifamycin (5 μg), Sparfloxacin (5 μg), Ticarcilin (75 μg), Vancomycin (30 μg), Kanamycin (30 μg), Clarithro mycin (15 μg), Trimethoprim (5 μg), Gentamycin(10 μg), Spectinomycin (100 μg), Amoxycillin (30 μg),

and Oxytetracycline (30 μg), were used in the present study to screen the sensitivity pattern of enteric pathogens.

Preparation of inoculum

Clinical isolates and referral strains were inoculated in nutrient broth and incubated at 37°C for 4 hours in an incubatory shaker (Orbitek). This 4 hour culture was used for determining antibacterial activity.

Determination of antibacterial activity

To look for antibacterial activity disc diffusion method was followed[10]. Petri plates containing 20 ml of Mueller Hinton agar were seeded with 4 hours old fresh culture of clinical isolates and referral standard. By making use of template drawn, commercial antibiotic discs were dispensed on the solidified mueller hinton agar. This was incubated at 37°C for 24 hours in an incubator (Rands SBC) and the results were observed.

RESULTS AND DISCUSSIONS

Escherichia coli happened to be one of the frequent causes of diarrhoeal morbidity in children. In this study *Escherichia coli* was isolated in 368 out of 1259 (58%) acute gastroenteritis cases tested (Figure 1). Xu et al.,[19] in China reported that 60% of the fecal samples of diarrhoeal diagnosis showed *Escherichia coli* as a major pathogen. Incidence of *Escherichia coli* vary among different countries. The reason could be environmental factors like temperature, rainfall, sanitation and economic status and personnal hygene of an individual.

Though *Escherichia coli* was considered to be a normal flora, in recent times it is considered to be pathogenic. This study also confirmed the same ie., 147 strains out of 368 were of EPEC (40%) followed by ETEC (17%), EIEC (8%), EAEC (4%) and 1% is of EHEC (Figure 2). Kain et al.,[17] of China also showed variable nature of serotypes. According to them ETEC was the most frequently detected pathogen in children with diarrhoea. Other *Escherichia coli* like EIEC and EHEC accounted for 7% each.

Historical evidences indicated that diarrheogenic

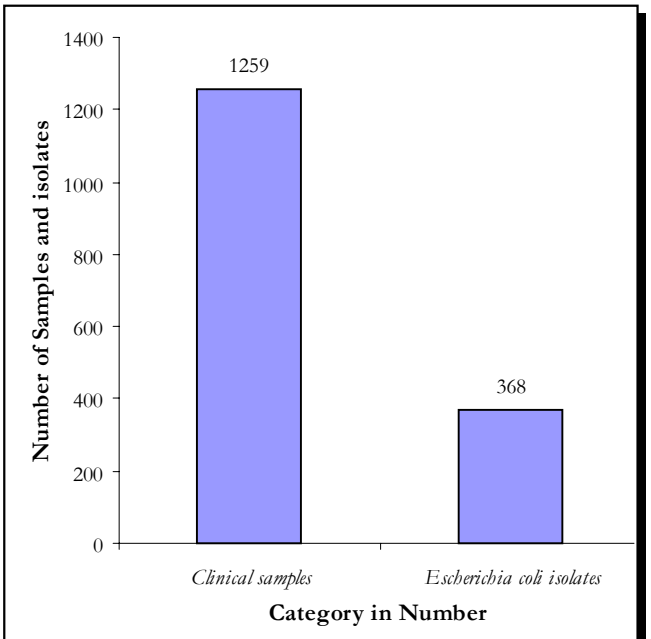

Figure 1: Incidence of *Escherichia coli* among AGE in Tiruchirappalli

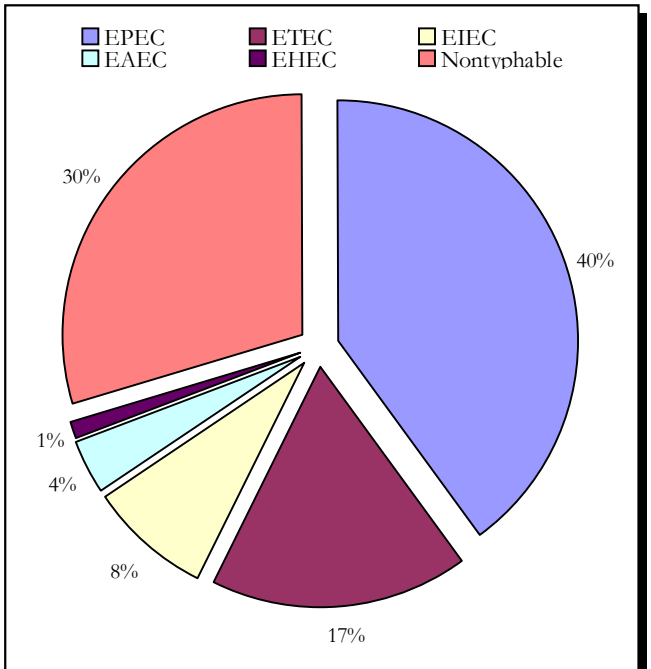

Figure 2: Different serogroups of *Escherichia Coli*

Escherichia coli belongs to certain serotypes that had been associated with outbreak of infantile gastroenteritis[4].

The pathogenic factors of diarrhoegenic *Escherichia coli* were intensively studied and identified by Giamanco et al.,[4]. Studies on the relationship between serotyping and pathogenic factors have been reported[4,9,15]. Raj[15] revealed the availability of O

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serotypes like O126, O44, O112, O114, O142, O111, and O157 in indian subcontinent and confirmed its association with infantile diarrhoea. Some *Escherichia coli* serotypes does not correspond to their pathogenic factors[1].

Here also all *Escherichia coli* isolates (368) were subjected to antibiotic sensitivity assay. The results revealed that all the *Escherichia coli* isolates were found to be resistant to more number of antibiotics and are considered as multi drug resistant (MDR) strains (TABLE 2). Among 32 antibiotics tested only few antibiotics showed more than 75 % sensitivity pattern. They are oxytetracycline (83%), spectino mycin (77%), cefodoxime (74%), chloramphenicol (74%), sparfloxacin (72%), gentamycin (72%) and ofloxacin (70%). The resistance of *Escherichia coli* was high for a number of antimicrobial agents. The list includes novobiocin (92%), cefixime (89%), kanamycin (87%), vancomycin (83%), azithromycin (84%), levofloxin (76%), amoxycillin (81%), ticarcilin (72%), and carbenicillin (73%). Other antimicrobial agents also showed variable resistance pattern & the variation was between 20% to 69%.

This report is slightly different from the report given by Desenclos et al.,[3]. According to them 53% *Escherichia coli* were resistant to ampicillin, 47% to chloramphenicol, 30% to co-trimaxazole and 67% to tetracyline. A report given by Watabe et al., (2003) stated that all the *Escherichia coli* strains were completely resistant to tetracycline and sulphonamides (84.6%). Report of Chomvarin et al.,[2] also showed similar kind of result. They have stated that the resistance of *Escherichia coli* was high for nearly all antimicrobial agents particularly ampicillin (96%), tetracycline (70%), cotrimaxazole (69%) and nalidixic acid (44%)

Enteropathogens have developed high level of resistance to all groups of chemotherapeutic agents used for the treatment of diarrhoea. Among the entero pathogens, pathogenic isolates of *E.coli* have a relatively large potential for developing resistance[7]. The reason for increasing resistance among enteric bacterial pathogens is multifactorial. These resistance are influenced by geographic location, year of isolate, class of antimicrobial agent, pressure exerted by antimicrobial agent and source of pathogen[11].

TABLE 2: Antibiotic sensitivity pattern of *Escherichia coli* against various antibiotics

S.No	Antibiotics tested	Quantity used	Number resistant (%)	Number sensitive (%)
1	Amikacin	30µg	184 (50%)	184 (50%)
2	Azithromycin	15µg	309 (84%)	59 (16%)
3	Azlocilin	75µg	184 (50%)	184 (50%)
4	Aztreonam	30µg	192 (52%)	176 (48%)
5	Carbencilin	100µg	269 (73%)	99 (27%)
6	Cefdinir	5µg	136 (37%)	232 (63%)
7	Cefepime	30µg	269 (73%)	99 (27%)
8	Cefixime	5µg	328 (89%)	40 (11%)
9	Cefpodoxime	10µg	96 (26%)	272 (74%)
10	Cefprozil	30µg	221 (60%)	147 (40%)
11	Chloramphenicol	30µg	96 (26%)	272 (74%)
12	Ciprofloxin	5µg	206 (56%)	162 (44%)
13	Doxycyclin	30µg	250 (68%)	118 (32%)
14	Fosfomycin	200µg	232 (63%)	136 (37%)
15	Levoflaxin	5µg	280 (76%)	88 (24%)
16	Meropenem	10µg	173 (47%)	195 (53%)
17	Methicillin	5µg	195 (53%)	173 (47%)
18	Minocyclin	30µg	143 (39%)	225 (61%)
19	Nalidixicacid	30µg	254 (69%)	114 (31%)
20	Novobiocin	30µg	339 (92%)	29 (8%)
21	Ofloxacin	5µg	110 (30%)	258 (70%)
22	Rifamycin	5µg	224 (61%)	144 (39%)
23	Sparfloxacin	5µg	103 (28%)	265 (72%)
24	Ticarcilin	75µg	265 (72%)	103 (28%)
25	Vancomycin	30µg	305 (83%)	63 (27%)
26	Kanamycin	30µg	321 (87%)	47 (13%)
27	Clarithromycin	15µg	121 (33%)	247 (67%)
28	Trimethoprim	5µg	184 (50%)	184 (50%)
29	Gentamycin	10µg	103 (28%)	265 (72%)
30	Spectinomycin	100µg	85 (23%)	283 (77%)
31	Amoxycillin	30µg	298 (81%)	70 (19%)
32	Oxytetracycline	30µg	40 (11%)	328 (89%)

Widespread use of antimicrobial agents in veterinary medicine, growth factor, food and nature has introduced antibiotic resistant in *Escherichia coli*[5].

Strategies to overcome the risk of resistant bacterial spread include the prevention of nosocomial infection and cross contamination. Periodic monitoring of drug resistance in different geographic area,

rational use of antibiotics and identification of correct infection & correct therapy are warrented.

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