International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 3; Issue 1; January 2018; Page No. 306-311

Prevalence and antimicrobial resistance pattern of diarrheagenic *escherichia coli* isolated from actue diarrhoea children

[1] Vijayalakshmi Selvakumar, [2] Panneerselvam A, [3] Subashini G, [4] Bhuvaneswari S, [5] Arockia Suganya S
[1, 3, 4] Assistant Professor, PG& Research Department of Microbiology, Shrimati Indira Gandhi College, Trichy, Tamil Nadu, India
[5] PG & Research Department of Microbiology, Shrimati Indira Gandhi College, Trichy, Tamil Nadu, India
[2] Head &Associate Professor , PG& Research Department of Botany &Microbiology, AVVM Sri Pushpam College, Poondi, Thanjavur, Tami Nadu, India

Abstract
Diarrhoea is one of the causes of the uppermost mortality and morbidity in children, predominantly in children younger than 5 years. In world, 6 million children die each year from diarrhoea, where the common deaths come about in developing countries. In the present study Prevalance and Antimicrobial resistance Pattern of Diarrheagenic *Escherichia coli* Isolated From Acute Diarrhea Children were studied. A total of 27 under-five years old children who included in this study the minimum age of cases was less than 12 months and the maximum age was 60 months. Among 27 isolates, all were resistant to amphicillin/claxacillin, cefdinir, cefixime, ceftazidime, cefuroxime, cephalexin and co-trimoxazole. The MDR isolate AS-13 was selected for further investigations and characterization. Most of the *E. coli* isolates showed multiple drug resistance and measures such as observation of appropriate personal hygiene by children, mother's behavior and environmental condition, use of effective disinfectants in reducing the potential pathogenic organisms in house and so forth. Prescribers should be well-known with local antibiotic sensitivity profiles and should conform to the local antibiotic guide-lines. A hospital antibiotic policy should be formulated based on local antimicrobial resistance data. Prescribers should be educated about the use of antibiotics, when not to use them and also the infection control strategies.

Keywords: *E.coli,* Antimicrobial resistance, actue diarrhea

Introduction
Casualty due to diarrhoea in developing countries is estimated to have declined from 4.6 million deaths in 1982 to 2 million deaths in 2003 (WHO, 2003), which translates to 18% of deaths of children under the age of 5 between 2000-2003 (Usfar, 2010), mostly among young children in developing countries (Kermani, 2010). In mainly diarrhoeal deaths in India were 0.212 million in 2010 (Liu *et al*., 2012). Although mortality due to acute diarrhoea in children has decreased both in developed and developing countries in recent years after the introduction of oral dehydration solution. Those associated with persistent diarrhoea occur in malnourished children and is usually disproportionately high, accounting for up to 45% of diarrhoeal deaths in Brazil, Bangladesh and in several African countries (Prescott *et al*., 2002).
Acute diarrhea remains as one of the most prevalent diseases affecting young children in developing countries in spite of the growing knowledge achieved in recent years. Even though programs sponsored by World Health Organization (WHO) and other improvements on the quality of life of several populations have succeeded in decreasing mortality rates, the incidence of diarrhea in children younger than five years in developing countries remains high, at 3.2 cases per child per year, but rates can be as high as 11 episodes of diarrhea per child per year in extremely poor areas (Guerrant *et al*., 1983). At the end of 20th century, 2.5 million deaths are estimated to have occurred worldwide each year, making diarrhea responsible for 21% of deaths of children younger than five years old (Kosek *et al*., 2003).

Materials and methods
This study entitled "Prevalence and antimicrobial resistance pattern of diarrhegenic *E.coli* isolated from acute diarrhea children" was carried out in Poultech Agro Research Centre, Namakkal during December 2016- March 2017.

Study Design
This was a comparative cross-sectional study that examined socioeconomic and environmental factors of children as exposure variables and *Escherichia coli* in diarrhoeal stool samples as an outcome variable. The study attempted to compare these variables in the children below the age group of 5 years in Mettutheru, Namakkal district, Tamil Nadu, India and was conducted from Dec-2016 to Jan-2017.

Sampling Technique and Sample Size
The data obtained by conducting interviews and diarrhoeal sample of respondents who have been determined (Questionnaire in Table - 1). The sample size of diarrhoeal sample was 27 for the isolation of *Escherichia coli*.

Table 1: Questionnaire

Socioeconomic and demographic characteristics of the respondents			
1	Age category	1	Less than 12 months
		2	13-24 month
		3	25- 36 month
		4	37- 48 month
		5	48- 60 month
2	Gender	1	Male
		2	Female
3	Literacy Status of parents	1	Illiterate
		2	Literate
4	Economic Status (Annual income)	1	Low (< 50000)
		2	Middle (1 to 2 Lakhs)
		3	High (>2 Lakhs)
5	Number of children living in the house	1	More than 2 children
		2	2 children
6	Drinking water	1	Unprotected
		2	Protected
7	Latrine in the house	1	No
		2	Yes
8	Disposal of the child's stool	1	Throw away in open surroundings
		2	Put in the latrine
9	Buying food for children from street vendors	1	Yes
		2	No
10	*Escherichia coli*	1	Yes
		2	No

Variables

Outcome (Dependent) variable

Include occurrence of *Escherichia coli* from diarrhoeal stool samples at the time of the survey.

Explanatory (Independent) variable

1. Socioeconomic status- includes age category, gender of children and literacy status and economic status (Annual income) of parents in the Mettutheru, Namakkal district, Tamil Nadu.
2. Study of environmental factors of children in house type, number of children living in the house, drinking water, latrine in the house, disposal of the child's stool and buying food for children from street vendors.

Data Management and analysis

The data entry was performed using IBM SPSS version-20. Frequencies were used to check for missed values and variables.

Collection of Sample

Stool sample was collected from children at the age group below 5 years and subjected to microbiological analyses. The samples were collected in sterile plastic container and then swabs were immediately transported to the laboratory for culture.

Isolation of Diarrhoea Causing *E. coli*

Each sample was inoculated on Eosin methylene blue agar (Tariq *et al.,* 2016). The culture Fig s were incubated at 37°C for 24 hours and observed for growth through the formation of colonies. The isolates were identified by their morphology and biochemical characteristics.

Preliminary Examination of culture

Morphological Test

Gram's staining and motility tests were carried for identification.

Biochemical Test

Biochemical tests of selected isolates were done according to Bergey's manual of determinative Bacteriology (Krieg and Holt, 1984).

Antibiotic Susceptibility test using disc diffusion method

Antibiotic and essential oil sensitivity testing was done for all the isolates on Mueller-Hinton agar by modifying Kirby-Bauer disc diffusion technique (Bauer *et al.*, 1966). In this study 13 antibiotics were used. Each belongs to different groups. Some of the standard antibiotics are: Amikacin (10mcg), Amphicillin/Claxacillin (10 mcg) , Amoxyclav (30 mcg), Cefdinir (5 mcg), Cefixime (5 mcg), Cefotaxime (30 mcg), Ceftazidime (30 mcg), Ceftriaxone (30 mcg), Cefuroxime (30 mcg), Cephalexin (30 mcg), colistin (50 mcg), Co-Trimoxazole (25 mcg), Floxidin (30 mcg), Gentamycin (30 mcg), Norflexacin (10 mcg) and Streptomycin (25 mcg).

Molecular Ribotyping of AS-13

Molecular ribotyping of the selected strain was carried out using the partial sequence of 16S rRNA. Ribotyping was performed using universal primer pair for 16S rDNA. A portion of the 16S rRNA gene was amplified from the genomic DNA. The sequence of forward (16SF) and reverse (16SR) primers used for amplifying 16S rDNA were obtained from Sigma, India.PCR was performed in a thermal cycler (Genei, Bangalore) according to Kumar *et al.,* 2002 with some modification under the following standardized conditions. Amplified DNA was sequenced and submitted to NCBI and got accession number (Altschul *et al*., 1990).

Phylogenetic tree construction

Phylogenetic tree was constructed using the neighbour joining method implemented in CLUSTAL W software. Tree was constructed using nucleotide evolutionary model for estimating genetic distances based on synonymous and non-synonymous nucleotide substitutions. Tree was visualized using the CLUSTAL W N-J tree.

Restriction Site Analysis

The restriction sites in 16S rDNA gene was analysed by using restriction mapping program (nc2.neb.com/NEBcutter2).

Secondary structure prediction

The secondary structure of 18S rDNA gene in fungi was predicted using gene bee tool (www.genebee.msu.su/service/ma2-reduced.html).

Results

Children's and Parent's Socio-Demographics Characteristics

A total of 27 under-five years old children who included in

this study the minimum age of cases was less than 12 months and the maximum age was 60 months. Children aged less than 12 months had 8 cases, making up 29.6 % of the total; 13-24 month had 7 cases (25.9%); 25-36 months had 4 cases (14.8%), 37-48 had 6 cases each (22.2%) and 48-60 month had 2 cases (7.4%) which shown in table 2. The number of males (16) was higher than females (11) in almost all age groups.

Eleven (40.7%) and 16 (59.3%) parents were illiterate and literate respectively. Figure- 2 shown that there were 29.6% of parents were low income group and 51.9 % were middle income group and 18.5% were high income group.

Environmental conditions of the study households

The environmental condition of children living area was tabulated in table-3. About 48.1 % of the households had more than 2 children while51.9 % have only two children. Eleven (40.7%) households used unprotected water sources while 66 (59.3%) households only used protected drinking water. 15 houses (55.6%) had latrine while 12 houses (44.4%) had no latrine. Even latrine in houses, 66.7% peoples throw away the child's stool in open surrounding while only 33.3% put in the latrine. The 66.7% of children brought food items from street vendors and 33.3% cases not had street foods.

Isolation and identification of *E.coli*

E.coli was isolated from diarrheal stool samples and morphological; biochemical characterization was noticed in table- 3 and Fig -1 (Table4 and Fig -1).

Table 2: Socioeconomic characteristics of Children and parents by place of residence, Mettutheru, Namakkal

Socioeconomic factors (n=63)		Frequency	Percent
Age category of Children	Less than 12 months	8	29.6
	13-24 month	7	25.9
	25- 36 month	4	14.8
	37 - 48 month	6	22.2
	48- 60 month	2	7.4
Gender of Children	Male	16	59.3
	Female	11	40.7
Literacy status of parents	Illiterate	11	40.7
	Literate	16	59.3
Economic Status of parents	Low (< 50000)	8	29.6
	Middle (1 to 2 Lakhs)	14	51.9
	High (>2 Lakhs)	5	18.5

Table 3: Environmental conditions of the study households by place of residence, Mettutheru, Namakkal District

Environmental factors (n=63)		**Frequency**	**Percent**
Number of children living in the house	More than 2 children	13	48.1
	2 children	14	51.9
Drinking water type	Unprotected	11	40.7
	Protected	16	59.3
Latrine in the house	No	12	44.4
	Yes	15	55.6
Disposal of child's stool	Throw away in open surroundings	18	66.7
	Put in the latrine	9	33.3
Buying food for children from street vendors	Yes	18	66.7
	No	9	33.3

Table 4: Morphological and biochemical characteristics of *E. coli* isolates from diarrheal stool samples

S.No	**Biochemical**	***E. coli***
1	Gram's staining	Gram negative
2	Motility	Motile
3	Oxidase test	Negative
4	Catalase test	Positive
5	Selective media for identification	Eosin methylene blue
6	Colonies colour on selective media	Metallic sheen
7	Urease test	-
8	Indole test	+
9	Methyl red test	+
10	Voges Proskauer test	-
11	Citrate utilization test	-
12	TSI	A/A G
13	Sugar fermentation test	
	1. Glucose	AG
	2. Sucrose	AG
	3. Lactose	AG
	4. Mannitol	AG
	5. Maltose	AG

- Negative; + positive; A = acid; K = alkaline; NC = no change; g = gas; w = weak

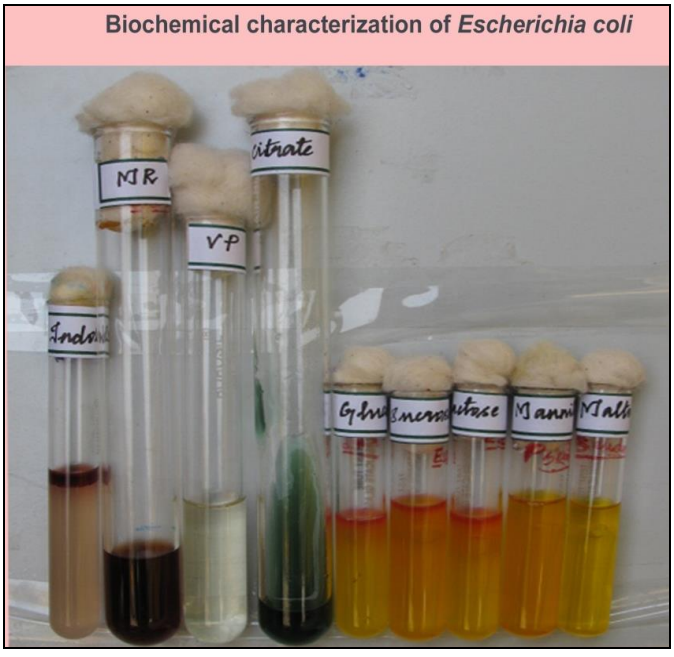

Fig 1: Biochemical characterization of *Escherichia coli*

Frequency of *E.coli* infection among the children below 5 years old

Among the 27 cases, 14 children (51.9%) were infected with *E. coli* while remaining 13 (48.1%) cases did not infect with *E. coli* (Figure-1).

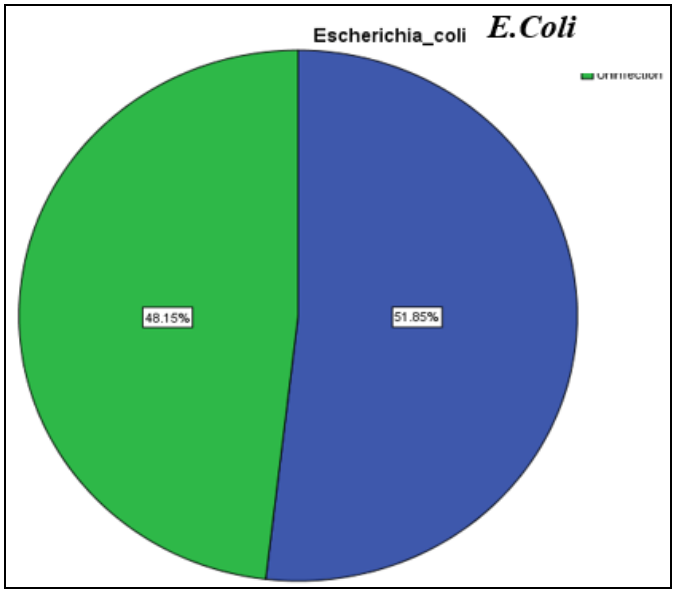

Fig 2: Frequency of *E.coli* infection among diarrhea children

Distribution of Respondents with *E. coli* infection

Distribution of respondents with *E. coli* infection was tabulated in table-5.

Table 5: Distribution of respondents with *E. coli* infection in stool samples

Socioeconomic and demographic characteristics of the respondents			*Escherichia coli*					
			Infection		Uninfection		Total	
			No	%	No	%	No	%
1	Age category	Less than 12 months	4	14.8%	4	14.8%	8	29.6%
		13-24 month	3	11.1%	4	14.8%	7	25.9%
		25- 36 month	2	7.4%	2	7.4%	4	14.8%
		37- 48 month	3	11.1%	3	11.1%	6	22.2%
		48- 60 month	2	7.4%	0	0.0%	2	7.4%
2	Gender	Male	8	29.6%	8	29.6%	16	59.3%
		Female	6	22.2%	5	18.5%	11	40.7%
3	Literacy Status of parents	Illiterate	8	29.6%	3	11.1%	11	40.7%
		Literate	6	22.2%	10	37.0%	16	59.3%
4	Economic Status (Annual income)	Low (< 50000)	6	22.2%	2	7.4%	8	29.6%
		Middle (1 to 2 Lakhs)	6	22.2%	8	29.6%	14	51.9%
		High (>2 Lakhs)	2	7.4%	3	11.1%	5	18.5%
5	Number of children living in the house	More than 2 children	12	44.4%	1	3.7%	12	44.4%
		2 children	2	7.4%	12	44.4%	2	7.4%
6	Drinking water	Unprotected	10	37.0%	1	3.7%	11	40.7%
		Protected	4	14.8%	12	44.4%	16	59.3%
7	Latrine in the house	No	10	37.0%	2	7.4%	12	44.4%
		Yes	4	14.8%	11	40.7%	15	55.6%
8	Disposal of the child's stool	Throw away in open surroundings	13	48.1%	5	18.5%	18	66.7%
		Put in the latrine	1	3.7%	8	29.6%	9	33.3%
9	Buying food for children from street vendors	Yes	10	37.0%	8	29.6%	18	66.7%
		No	4	14.8%	5	18.5%	9	33.3%

Antibiotic susceptibility patterns of *E. coli* isolate

Antibiotic Susceptibility Patterns of ***E. coli*** from the diarrheal stool sample of child below 5 years old was studied. Among 27 isolates, all were resistant to amphicillin/claxacillin, cefdinir, cefixime, ceftazidime, cefuroxime, cephalexin and co-Trimoxazole. 85.71% isolates resistant to cefotaxime and floxidin, 78.57% isolates to ceftriaxone, 71.43% isolates to amoxyclav, 50.0% isolates to colistin, 42.86% to streptomycin, 35.71 % isolates to gentamycin, 28.57% to norflexacin and 14.29% to amikacin antibiotic (Fig 2).

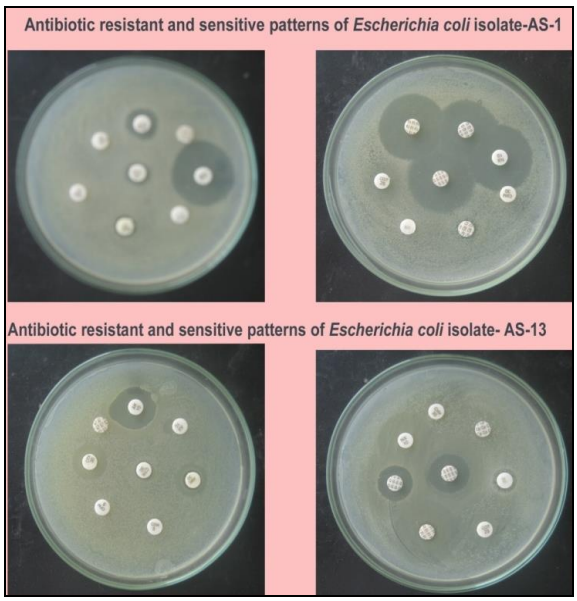

Fig 3

16s rRNA based identification and phylogenetic relationship

The multi drug resistant *E.coi* AS-13 was selected based on antibiotic resistant pattern. *E.coi* AS-13 genomic DNA was isolated and PCR amplified with 16S rDNA. Electrophoretical analysis of PCR products obtained from the amplification of 16S rDNA genes confirmed that full length (1207bp) genes were amplified for *E.coi* AS-13 (Fig -3).

The amplified product was sequenced and sequence of DNA fragment was compared to the sequences available in GenBank, NCBI. Sequence analysis of these isolates was also performed using BLAST (blastn) search tool (http://www.ncbi.nlm. nih.gov) available on the NCBI homepage. The MDR *E.coi* AS-13 strains used in the study exhibited 96 to 98% sequence similarity to the *E.coi* available in NCBI database. Theses sequence data has been deposited in the GenBank (Submission number : SUB2484782).

The phylogenetic tree generated by a weighted neighbor-joining (Figure -2) method clearly revealed the evolutionary relationship of the strain AS-13 to a group of *E.coi*. Thus, this strain was designated as *E.coi* AS-13.

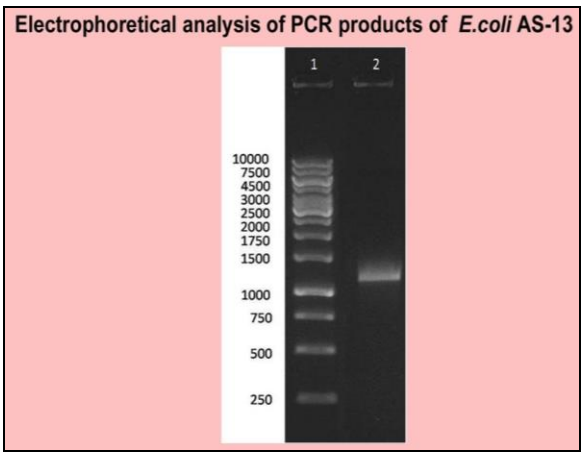

Fig 4: Electrophoretical analysis of PCR products of *E.coli* AS-13

Restriction site analysis

Totally 48 restriction sites were identified. 56% GC contents were recorded and 44% AT contents were recorded (Figure 3).

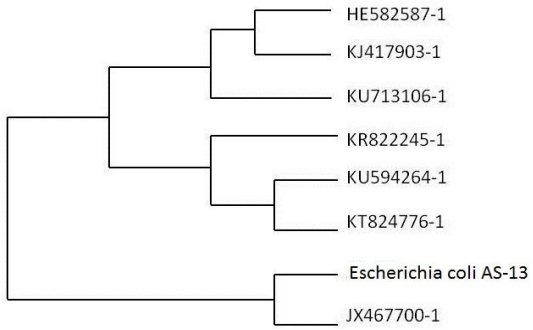

Fig 5: Phylogenetic tree based on weighted neighbor-joining method for the *E. coli* AS-13

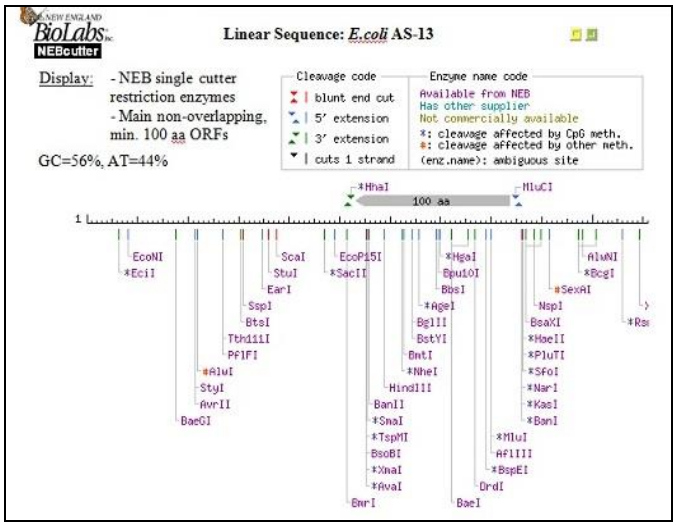

Fig 6: Linear Sequence: *E.coli* AS-13

Discussion

Acute diarrhoeal diseases among the children are one of the most important problems affecting children in the world, reducing their well-being and creating considerable demand for health services. Diarrhoeal diseases are leading cause of preventable death, especially among children aged under five in developing countries. Diarrhoea is defined as a child with loose or watery stool for three or more times during a 24-hour period. The frequency and severity of diarrhoea are provoked by lack of access to enough clean water and sanitary throwing away of human waste, insufficient feeding practices and hand washing; poor housing circumstances and lack of access to sufficient and reasonable health care. *Escherichia coli* (EPEC) is a main cause of diarrhoea in infants and children in addition to pathogens such as *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, *Campylobacter* sp., *Entamoeba histolytica*, and *Giardia lamblia* in developing countries (Ahmed *et al*., 2009). Diarrhoeagenic *E. coli* is the major cause of gastroenteritis in children in the developing world and is associated with high resistance intensity to antibiotics (Ochoa *et al*., 2009).In the present study, less than 12 months old children (4 cases) were the highest rate of *E. coli* infection (14.8%), followed by 13-

24 and 37-38 (3 cases; 11.1%) and 25-36 month old (2 cases; 7.4). This was agreed to study conducted in Thailand (Calistus *et al*., 2009) who showed that children aged from 6-23 months were more endanger of diarrhoeal disease than other age groups. The possible explanation could be due to environmental exposure and increased introduction of solid foods which is unsafe and poor hygiene to children whose their immune not well developed. People in Mettutheru have to spend much time earning for their living, therefore, they do not have enough time to take care of their children. This is especially true of community in the rural areas whose earnings are low and very labor-intensive. In the present investigation, the highest *E.coli* infection occurred in low income group and middle income group followed by high income group. This result was agreed to report of USAID (2010) who stated that the vast majority of these deaths from diarrhoea are among children under-five years of age living in low- and middle-income countries.

Teklemariam *et al.* (2000) observed an inverse relationship between the number of rooms and diarrhoea morbidity. In the present study, the influence of the number of children living in the house directly reflected the *E. coli* infection. 44.4% were infected with more than two children in the house. As the number of children in a family becomes larger, there may be crowding which deteriorate the hygiene condition, which in turn increases the chance of contact with pathogens. There may also be competing for a mother's time and attention and other resources (Woldemichael, 2001).

In the present study showed that antibiotic resistant bacteria are at present all over the place. The pattern of resistance shown by these isolates is in line with the type of antibiotics usually used in all hospitals. Therefore, government at all tiers should make an effort to support research on the development of new molecules that could be appropriate in the treatment of harsh infections caused by antibiotic resistant bacteria.

Conclusion

E. coli isolates showed multiple drug resistance and measures such as observation of appropriate personal hygiene by children, mother's behaviour and environmental condition, use of effective disinfectants in reducing the potential pathogenic organisms in house and so forth. Prescribers should be well-known with local antibiotic sensitivity profiles and should conform to the local antibiotic guide-lines. A hospital antibiotic policy should be formulated based on local antimicrobial resistance data. Prescribers should be educated about the use of antibiotics, when not to use them and also the infection control strategies.

Reference

1. Ahmed B, Shakoori FR, Ali SS, Shakoori AR. Antimicrobial Resistance Pattern and Plasmid Analysis of Escherichia coli from Patients Suffering from Acute Diarrhoea in Azad Kashmir, Pakistan. Pakistan J Zool, 2009; 41(5):371-380.
2. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol, 1990; 215:403-410.
3. Bauer Aw, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disk method. Am J Clin Pathol. 1996; 45(4):493-496.
4. Calistus W, Alessio P. Factors associated with diarrhea among children less than 5 years old in Thailand: a secondary analysis of Thailand multiple indicator cluster survey. J Health Res, 2009; 23:17-22.
5. Guerrant RL, Schorling JB, McAuliffe JF, de Souza MA. Diarrhea as a cause and an effect of malnutrition: diarrhea prevents catch-up growth and malnutrition increases diarrhea frequency and duration. Am J Trop Med Hyg, 1992; 47(Suppl):28-35.
6. Kermani NA, Jafari F, Mojarad HN, Hoseinkhan N, Zali MR. Prevalence and Associated Factors of Persistent Diarrhoea in Iranian Children Admitted to a Paediatric Hospital. Eastern Mediter Health J. 2010; 16(8):831-836.
7. Kosek M, Bern C, Guerrant RL. The global burden of diarrheal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organ, 2003; 81:197-204.
8. Krieg NR, Holt JG. Bergey's Manual of Systematic Bacteriology. The Williams &Wilkins Co., Baltimore and London, 1984.
9. Kumar NR, Arasu VT, Gunasekaran P. Genotyping of antifungal compounds producing plant promoting rhizobacteria, Pseudomonas fluorescens. Curr. Sci., 2002; 82:1463-1466.
10. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, *et al*. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since2000. Lancet, 2012; 379:2151-2161.
11. Ochoa TJ, Ruiz J, Molina M, Del Valle LJ, Vargas M, Gil AI, *et al*. High frequency of antimicrobial drug resistance of diarrheagenic Escherichia coli in infants in Peru. Am J Trop Med Hyg, 2009; 81:296-301.
12. Prescott LM, Harley JP, Donald AK. Microbiology. 4th Edition, McGraw. Hill, Companies USA, 2012; 935-937.
13. Tariq AL, Reyaz AL, Sathiamoorthi T. Isolation and identification of Antibiotic mediated Resistant Betalactum producing Escherichia coli from urinary tract infected patients in Erode District, Tamil Nadu, India. Int.J Drug Dev & Res, 2016; 8:038-042.
14. Teklemariam S, Getaneh T, Bekele F. Environmental determinants of diarrhoea morbidity in under-five children, Keffa-Sheka Zone. Southwest Ethiopia. Ethiop Med. J. 2000; 38(1):27-34.
15. USAID. Integrating sanitation and water supply proprams. Anuual report in Africa. Oadi KO, Kuitunen M. Childhood diarrheal morbidity in the Accra Metropolitan Area, Ghana: Socio-economic, environmental and behavioral risk determinants. J Health Pop Dev Countries, 2010-2005; 33-46.
16. Usfar AA, Iswarawanti DN, Davelyna D, Dillon D. Food and Personal Hygiene Perceptions and Practices among Caregivers Whose Children Have Diarrhea: A Qualitative Study of Urban Mothers in Tangerang, Indonesia. J Nutri Edu Behav, 2010; 42:33-40.
17. WHO. World Health Organization Report; Shaping the future; 1211 Geneva 27, Switzerland, 2003.
18. Woldemichael G. Diarrhoeal morbidity among young children in Eritrea: Environmental and socioeconomic determinants. J Health Popul Nutr. 2001; 19(2):83-90.