

Full Length Research Paper

Etiology of children's diarrhoea in Southern India: Associated pathogens and usual isolates

Subashkumar R.^{1*}, Tha. Thayumanavan², G. Vivekanandhan¹ and P. Lakshmanaperumalsamy³

¹PG and Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore – 641 029, India.

²School of Biotechnology, Dr. G.R. Damodaran College of Science, Coimbatore – 641 014, India.

³Karpagam University, Coimbatore – 641 021, India.

Accepted 27 February, 2012

The frequency of diarrhoeagenic bacteria, isolated from all sampling areas showed that *Escherichia coli* was recorded as the predominant bacterium with 58.57%, followed by *Vibrio*, *Salmonella*, *Aeromonas*, *Shigella*, *Pseudomonas*, *Streptococcus*, *Proteus* and *Staphylococcus* with 40.58, 33.47, 30.54, 10.46, 10.04, 10.04, 7.11 and 5.43%, respectively. *E. coli* is the most significant aetiological agent of childhood diarrhoea and represents a major public health problem in many developing countries. *Vibrio* is well known as a causative agent of gastrointestinal, extra intestinal and wound infections leading to fatalities in some cases. Other diarrhoeagenic bacteria may be significant and cause diarrhoeal infection in this study area.

Key words: Gastroenteritis, paediatric infections, diarrhoeagenic bacteria.

INTRODUCTION

Standing in this new millenium, it is interesting to note that a number of exotic infections, unknown previously, and many infectious diseases are emerging and becoming a serious global health concern. Among the infections of the gastrointestinal tract, the severe diarrhoeal scourge accounts for a large number of deaths in several parts of the world (Guarrant et al., 1990). About 61% of bacteria have ability to cause diarrhoea (Gascón, 1993). Of the diarrhoeagenic bacteria *Escherichia coli*, *Vibrio* and *Shigella* spp. are most common and extensively studied, followed by *Salmonella* and *Aeromonas*.

Diarrhoeal illness is well recognized as a major cause of morbidity and mortality in young children in many developing countries (UNICEF, 1992; WHO, 1995; Urbino et al., 2003). About 24.1% of all infant deaths in the first two years of life is due to diarrhoeal disease (Simanjuntak et al., 1998). WHO predicts that there will be about 5 million deaths in children younger than five years by 2025, of which 97% will be in the developing countries and mostly caused by infectious diseases, within which

diarrhoea will continue to play a prominent role (WHO, 1998). In India, approximately 2.5 million children were affected with diarrhoeal illness in every year (Taper and Sanderson, 2004). One of the major challenges in the gastrointestinal diseases is the recent increase in the number of probable aetiological agents. The main causes of diarrhoea are poor personal and food hygiene and lack of clean drinking water (Sanderson and Walker, 1993).

The members of Enterobacteriaceae are the most important aetiologic agent of childhood diarrhoea and represent major public health problems in the world (Taneja et al., 2003). A significant proportion of human infectious diarrhoea is associated with enterotoxigenic bacteria. The best known of these are *Vibrio cholerae* and enterotoxigenic *E. coli* (ETEC) (Robinson et al., 1983). In India, species belongs to the genus *Vibrio* are well known as causative agents of gastrointestinal, extra intestinal and wound infections with a fatal outcome in some cases (Dumontet et al., 2000). *E. coli* is the most important aetiological agent of childhood diarrhoea and represent a major public health problem in developing countries (Nataro and Kaper, 1998). Other bacteria which are reported to cause gastroenteritis include *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas* and *Yersinia* species (Collee and Bradley, 1997).

*Corresponding author. E-mail: rsubashkumar@gmail.com. Tel: +91 (0) 422 2642095, 2642236. Fax: +91 (0) 422 2644452.

Although sporadic studies on the incidence of individual infectious diarrhoeal agents within the study region and the resultant lack of base-line data, it is reasonable to assume that the consumption of contaminated foods and water may increase the frequency infection among the human population and especially children. It is an important threat to the public health and childhood disease. Attention has been given to the incidence of diarrhoeagenic bacteria from the four selected locations of Tamil Nadu, South India. In the above juncture, a detailed assessment and methodology has been undertaken.

MATERIALS AND METHODS

Samples

Diarrhoeal stool samples were collected from children (below 5 years) suffering from acute diarrhoea and gastroenteritis infection from January to December, 2003. The samples were obtained from the hospital based clinical laboratories and village primary health centres at Coimbatore, Kovilpalayam, Pattukottai and Tirupur. They were examined for cyst and bacteria prior to the transportation to the laboratory. The stool specimens suspected of having bacteria as the aetiological agent for diarrhoea alone were processed in the present investigation.

Isolation and identification

The diarrhoeal stool specimens were collected using sterile swabs with gentle scrap and put into the Stuart's transport medium (3 mL) in a submerged condition with icebox. A portion of stool sample was aseptically transferred to a sterile selective enrichment broth to study the incidence of bacterial enteric pathogens present in the diarrhoeal stool samples. Further, the enriched culture was transferred to selective agar medium as specified by the manufacturer (HiMedia, India) for the isolation of different bacterial genera. Starch ampicillin agar (SAA), Eosine methylene blue agar, Brilliant green agar (BGA), *Salmonella Shigella* agar (SS), Cetrimide agar, Nutrient agar, Mannitol salt agar (MSA) and KF *Streptococcus* agar medium were used for the selective isolation of *Aeromonas* spp., *E. coli*, *Salmonella* spp., *Shigella* spp., *Pseudomonas* spp., *Proteus* spp., *Staphylococcus* spp. and *Streptococcus* spp. (faecal) respectively. The biochemical identification was performed by oxidase reaction, fermentation of sugars, presence of lysine, gelatine liquefaction, arginine decarboxylation and absence of ornithine decarboxylase, proposed by the method outlined by Garrity et al. (2005).

Statistical analysis

The χ^2 and Fisher's exact tests were used to compare the diarrhoeagenic bacterial genera between the four sampling stations in all the months. A *p*-value < 0.05 was considered as significant. The statistical analysis was performed using SPSS 10.0 for Windows XP.

RESULTS

Incidence of diarrhoeagenic bacterial genera in children diarrhoeal stool samples

A total of 239 diarrhoeal samples of children were

processed for the incidence of different diarrhoeagenic bacterial genera such as *Aeromonas*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus* and *Vibrio* (Table 1). For all samples analysed, 30.54% (*p* < 0.05) were found to harbour *Aeromonas* spp. Among the four sampling areas (Coimbatore, Kovilpalayam, Pattukottai and Tirupur), the maximum level of incidence of *Aeromonas* spp. was recorded at Coimbatore with 34.4% (*p* < 0.05). It was a significant rate of incidence in all four sampling areas. The diarrhoeal stool samples were found to have significantly higher numbers (58.57%) of *E. coli* than other genera (*p* < 0.05). A significant rate of incidence of *E. coli* was observed when compared to other bacterial genera. The incidence of *Proteus* spp. was recorded as 7.11% (*p* < 0.05). In all sampling areas, the number of *Proteus* spp. isolated from the children's diarrhoeal samples was of less significance. None of the samples collected from Kovilpalayam and Tirupur showed the presence of *Proteus* spp. Samples collected from Pattukottai and Coimbatore exhibited 11.3% (*p* < 0.01) and 7.4% (*p* < 0.05) of incidence of *Proteus* spp. respectively.

The results revealed that 10.04% (*p* < 0.05) of the children stool samples collected from all the four sampling areas tested positive for *Pseudomonas* spp. in all four sampling areas. The maximum incidence was recorded in the samples collected at Tirupur with 28.5% (*p* < 0.05) followed by Pattukottai, Kovilpalayam and Coimbatore with 15% (*p* < 0.05), 12.5% (*p* < 0.01) and 9.5% (*p* < 0.05) respectively. Significant number of samples (33.47 %) was found to harbour *Salmonella* spp. with highest (47.2%) at Pattukottai and lowest (16.6%) at Kovilpalayam. In all four sampling areas, the isolation rate was significant (*p* < 0.05). About 10.46% of *Shigella* spp. was isolated from the diarrhoeal samples. The incidence of *Staphylococcus* and *Streptococcus* spp. was observed as 5.43 and 10.04% (*p* < 0.05) in all sampling areas respectively. A significant number of diarrhoeal samples with *Staphylococcus* spp. and *Streptococcus* spp. was recorded. Of the four sampling areas, 40.58% of *Vibrio* spp. was recorded in the diarrhoeal samples. The maximum occurrence of *Vibrio* spp. was observed in the samples collected from Coimbatore with 41.2% (*p* < 0.05).

Month-wise incidence of diarrhoeagenic bacteria in diarrhoeal samples

Of the 148 diarrhoeal stool samples collected from the hospital based clinical laboratories in Coimbatore (Figure 1), the maximum incidence of *Aeromonas* spp. was recorded in June (62.5%) and the minimum was recorded during November (14.3%). The incidence of *E. coli* was 78.2% during May and a minimum of 22.2% during August. *Proteus* spp. was recorded in the month of April (35.3%) and followed by 13, 7.6 and 5% during May, July and October, respectively. The incidence of *Pseudomonas* spp.

Table 1. Percentage of incidence of bacterial genera in four sampling areas.

Genera	% incidence				Total (n = 239)
	Coimbatore (n =148)	Kovilpalayam (n = 24)	Pattukottai (n = 53)	Tirupur (n = 14)	
<i>Aeromonas</i> spp.	34.4 (51)*	20.8 (5)	26.4 (14)	21.4 (3)	30.54 (73)
<i>E. coli</i>	55.4 (82)	66.6 (16)	60.3 (32)	71.4 (10)	58.57 (140)
<i>Proteus</i> spp.	7.4 (11)	0 (0)	11.3 (6)	0 (0)	7.11 (17)
<i>Pseudomonas</i> spp.	9.5 (14)	12.5 (3)	15 (3)	28.5 (4)	10.04 (24)
<i>Salmonella</i> spp.	31 (46)	16.6 (4)	47.2 (25)	35.7 (5)	33.47 (80)
<i>Shigella</i> spp.	6 (9)	0 (0)	24.5 (13)	21.4 (3)	10.46 (25)
<i>Staphylococcus</i> spp.	4 (6)	4.1 (1)	9.4 (5)	7.1 (1)	5.43 (13)
<i>Streptococcus</i> spp.	5.4 (8)	4.1 (1)	22.6 (12)	21.4 (3)	10.04 (24)
<i>Vibrio</i> spp.	41.2 (61)	16.6 (4)	35.8 (19)	21.4(3)	40.58 (87)

*Number of positive samples

peaked during October (25%) and the minimum was recorded during March (11.1%). *Salmonella* spp. were recorded in January (66.6%) with the lowest incidence recorded in May (8.6%). *Shigella* spp. was recorded 20% in February and lower incidence (4.3%) was recorded during May. During July, 15.4% of *Staphylococcus* spp. was recorded as the maximum. The percentage frequency of *Streptococcus* spp. was recorded during March, April, May, June and December with 11.1, 17.6, 4.3, 12.5 and 13.3%, respectively. The highest percentage of prevalence of *Vibrio* spp. was recorded in July with 69.2 %.

In Kovilpalayam, 24 children diarrhoeal stool samples were collected from village primary health care centre and the results are represented in Figure 2. It recorded that all samples (100%) were positive for *Aeromonas* spp during summer month of March, while the minimum (40%) was recorded in August. Similarly all (100%) samples persists *E. coli* in the month of March, May, June and September. None of the samples collected from this sampling area tested

positive for *Proteus* spp. and *Shigella* spp. In July, *Pseudomonas* spp. was recorded with 66.6 %. It was also observed that all the samples collected during October contained *Salmonella* spp. *Staphylococcus* spp. and *Streptococcus* spp. were observed only during May (33.3%) and August (20%) respectively. *Vibrio* spp. were observed only in April (25 %), June (50 %), July (33.3 %) and September (50 %).

A total of 53 children diarrhoeal samples, a significant percentage of *Aeromonas* spp. was recorded in May (43%) from Pattukottai (Figure 3). All the samples contaminated with *E. coli* during August and December. The maximum percentage of incidence of *Proteus* spp. was observed during March with 40%. The highest percentage of *Pseudomonas* spp. was encountered during November (66.6%). During August, the entire samples positive for *Salmonella* spp. tested and the lowest incidence (25%) was recorded in September. It was also observed that all samples collected during February and August tested positive for *Shigella* spp. About 60 % of the incidence was

observed for *Shigella* spp. during October and 40% in March and May. The incidence of *Staphylococcus* spp. was recorded only during May, October and December with 20, 40 and 33.3% respectively. A significant point of (50%) incidence of *Streptococcus* spp. was observed during April and September. All the samples collected during July and August tested positive for *Vibrio* spp.

A total of 14 diarrhoeal stool samples were collected from Tirupur and the incidence of the bacterial genera were tested (Figure 4). The maximum incidence of *Aeromonas* spp. was recorded in March with 50%. All the samples were positive for *E. coli* during August, October and November. It was also observed that none of the sample has been recorded *Proteus* spp. in Tirupur. All the samples were *Salmonella* spp. and *Pseudomonas* spp. contaminated during February and November respectively. Only one sample had *Staphylococcus* spp. among the four stool samples. Species of *Shigella* and *Vibrio* were recorded only during the months of April (25%) and October (66.6%) respectively. The incidence of *Streptococcus*

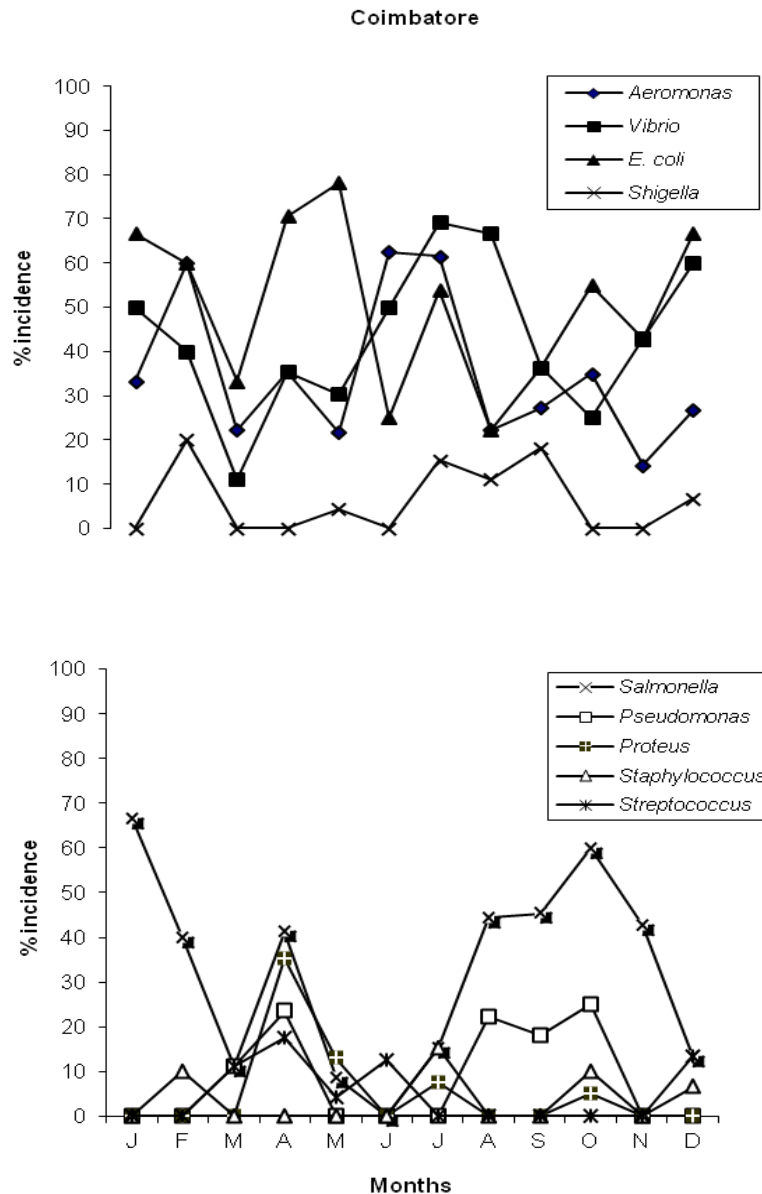


Figure 1. Incidence of diarrhoeagenic bacterial genera in Coimbatore

spp. was recorded only during March and October with 50 and 66.6% respectively.

DISCUSSION

Diarrhoeagenic bacteria in different sampling areas

In developing countries, pathogens have been identified in 65% of stool samples from children with acute diarrhoea. Many viruses and bacterial pathogens in the intestine have been identified, of which rotavirus and enteropathogenic *E. coli* (EPEC) are the most common and the dominant normal flora in the intestinal tract of humans and animals. Also the

following group of bacteria; viz., *Salmonella*, *Shigella*, *Aeromonas*, *Vibrio*, *Proteus*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Morganella*, *Serratia*, *Yersinia*, *Pseudomonas*, *Edwardsiella*, *Plesiomonas* and *Providencia* have been reported to cause diarrhoea (Bravo et al., 2003).

In the present study, the dominant diarrhoeagenic organisms were isolated from the stool samples were *E. coli*, species of *Aeromonas*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus* and *Vibrio*. Occurrence of these aetiological agents remains a serious public health concern. Among the bacterial genera isolated, *E. coli* was recorded as the most prominent, followed by the species of *Vibrio*, *Salmonella*, *Aeromonas*, *Shigella*, *Pseudomonas*, *Streptococcus*, *Proteus* and

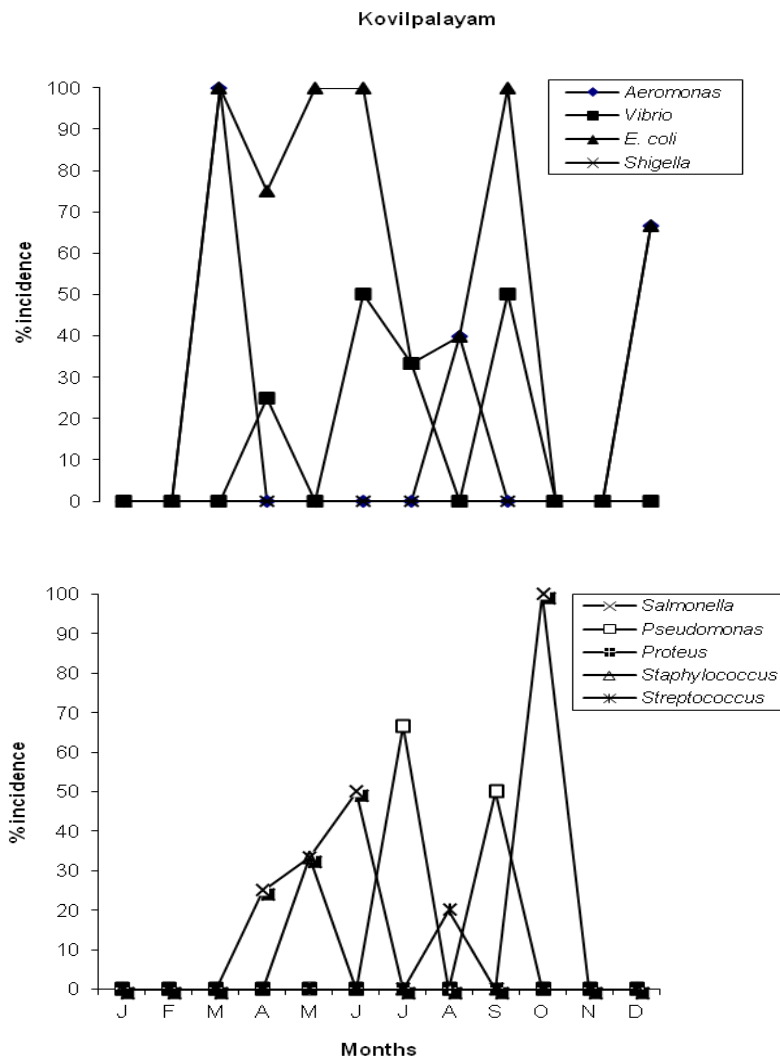


Figure 2. Incidence of diarrhoeagenic bacterial genera in Kovilpalayam

Staphylococcus. Of these diversified bacterial pathogens, *E. coli* was deemed to be the most common causative agents of diarrhoea. The maximum percentage of *E. coli* recorded was 58.57% ($p < 0.05$), while the minimum incidence of *Staphylococcus* spp. was 5.43%. The higher incidence of *E. coli* along with the diarrhoeagenic organisms showed the significant health threat to the children in the study area. In many instances the isolation of *E. coli* from stool samples of asymptomatic individuals was similar to that of the diarrhoeal cases (Cravioto et al., 1979). However, its association with several outbreaks and in volunteer studies has unquestionably confirmed the role of *E. coli* as a critical aetiologic agent leading to diarrhoea (Okeke et al., 2003).

Another predominant bacterial genera was *Vibrio*, which has been proven to be a major cause of epidemic diarrhoea (Fasano, 2000). A significant level of *Vibrio* spp.

was recorded in all the sampling areas ($p < 0.05$). This may be due to the tendency of this organism to cause severe diarrhoea, thus making infected individuals more likely to seek medicinal treatment (Oyofe et al., 2002). In a recent outbreak of cholera in Chandigarh, India, was recorded that 58.5% of children had been affected with acute diarrhoea (Taneja et al., 2003). *Aeromonas* spp. can now be considered relatively common enteropathogens (Bottarelli and Ossiprandi, 1999). Recently, a report of Jalal et al. (2010) showed that multiple antibiotic resistant aeromonads isolated from mangrove soil, it was originated from high risk source of contamination. In the present study, a significant proportion (30.54%) ($p < 0.05$) of *Aeromonas* spp. was observed and hence, it may be considered as one of the causative agents of diarrhoea in children. Maltezou et al. (2001) revealed that *Aeromonas* (33%) was the third frequently isolated bacterial agent from children

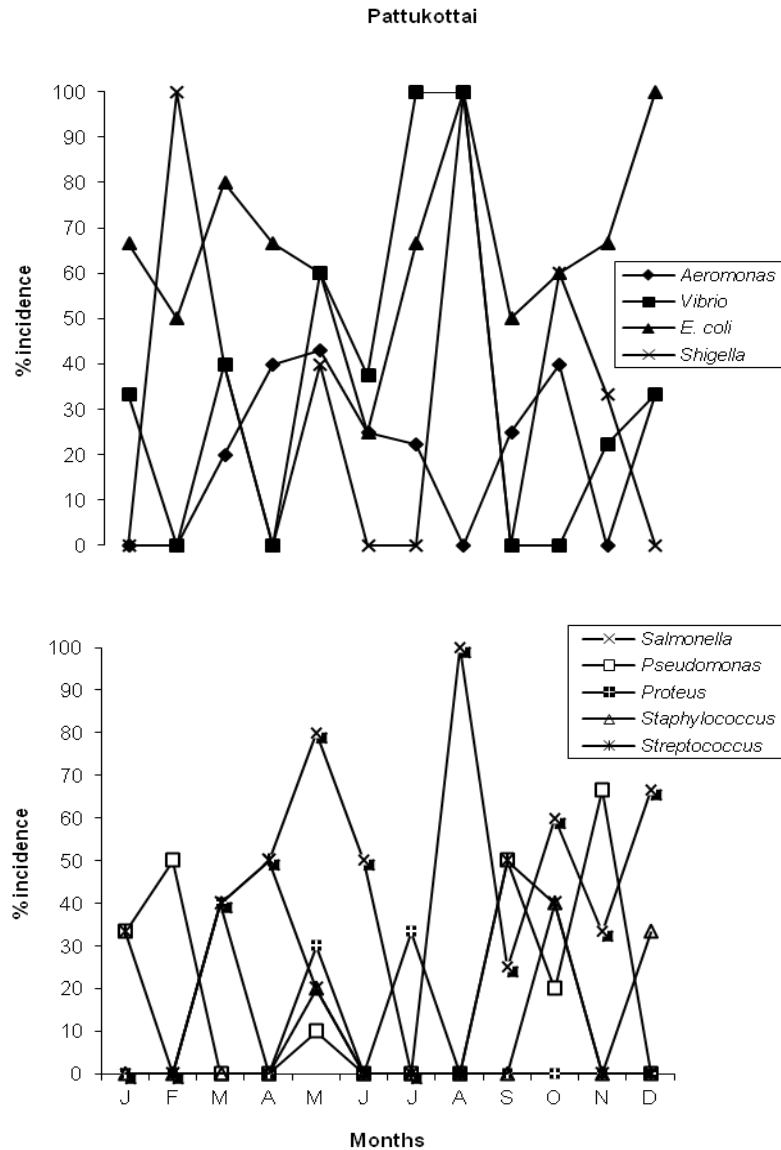


Figure 3. Incidence of diarrhoeagenic bacterial genera in Pattukottai.

with acute diarrhoea in the area of Athens, in a proportion comparable to that of *Salmonella* and other agents. In this study, *Aeromonas* was also considered a major diarrhoeagenic causative agents leading to diarrhoea in children.

Incidence of *Salmonella* and *Shigella* species were recorded as 33.47% ($p < 0.05$) and 10.46% ($p < 0.05$) respectively. WHO reported that 15% of the deaths due to diarrhoea in children younger than 5 years were the result of *Salmonella* (WHO, 1995). Maltezou et al. (2001) reported that 47% of the children diarrhoeal episodes caused by *Salmonella*. Nzeako and Okafor (2002) reported that 11 and 1.8% of *Salmonella* and *Shigella* species respectively were recorded from children infected with diarrhoea in Nigeria. In the present investigation a significant percentage of *Salmonella* spp. was observed in

the study area. It may persist as a major public health threat in all the conditions. In this study, we have also recorded the incidence of *Shigella* in a minor elevation in all the study areas. No significant range of these pathogens has been observed. However, no incidence of *Proteus* and *Shigella* has been noticed in Kovilpalayam. *Proteus* species are most common flora of the human intestinal tract, along with *E. coli*, *Klebsiella* and etc. *Proteus* has been isolated from multiple environmental habitats, including long-term care facilities and hospitals. Similarly the incidence of one of the diarrhoeagenic bacterial genus *Pseudomonas* was considerably lower. In this study, there was no significant level of *Proteus* and *Pseudomonas* spp. observed in all the four sampling areas. The usual clinical presentation of infection due to the above agents is diarrhoea, abnormal pain, low grade

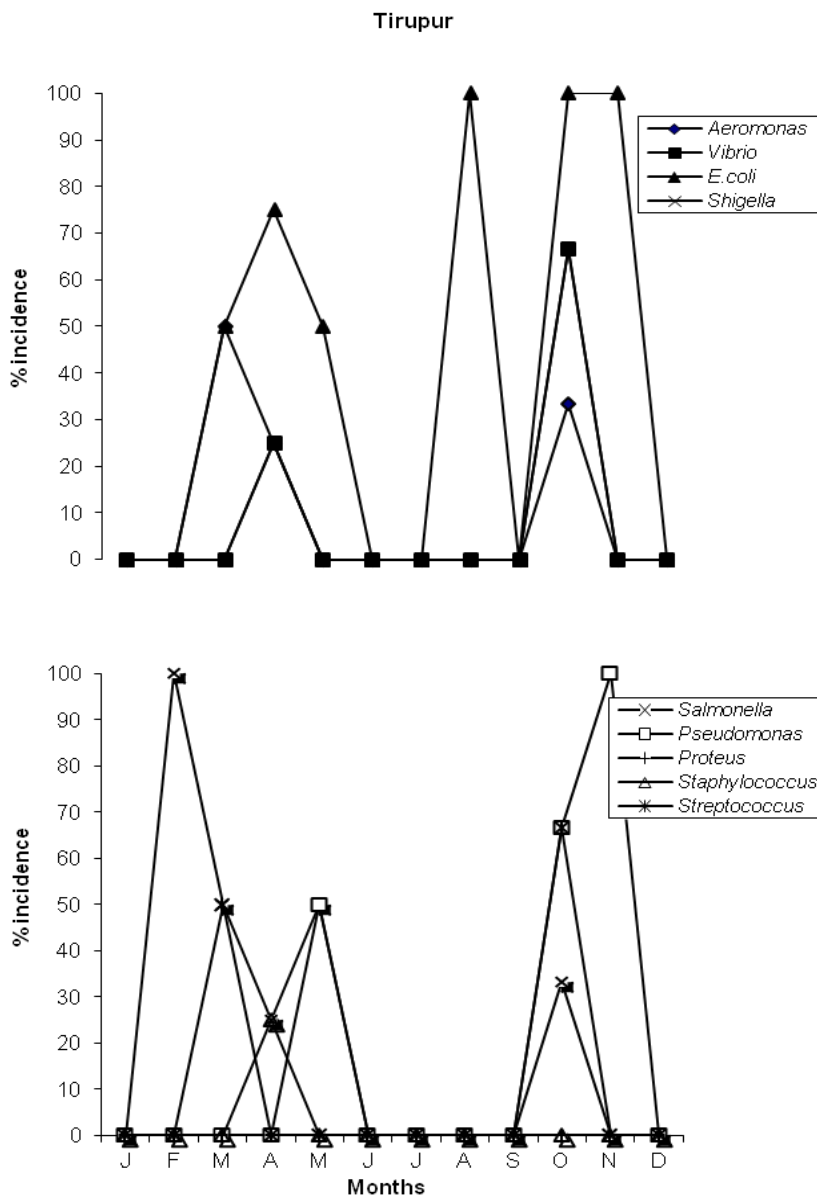


Figure 4. Incidence of diarrhoeagenic bacterial genera in Tirupur.

fever and vomiting (Quadri et al., 1991). *Staphylococcus* species is a bacterium whose aetiological role in diarrhoea has not been well established (Gascón et al., 2003). In the present study the incidence of *Staphylococcus* spp. was recorded as considerably lower with 5.43 %.

The proportions of incidence the bacterial genera were predominantly higher in the months of March to October. Also the prevalence of the diarrhoeagenic bacterial species were observed in significant levels in all the four sampling areas. Understanding the cause of less severe forms of diarrhoea, within a specific geographic area may enable primary care paediatricians to make the appropriate treatment decision which allow a better distribution of monetary and medicinal resources including the application of

preventive measures. This study provides information about the occurrence and prevalence of bacteria associated with diarrhoeal episodes and the significant incidence was recorded among children living in urban and rural area of Tamil Nadu, South India.

REFERENCES

Bottarelli E, Ossiprandi MC (1999). *Aeromonas* infections: An update. Available from <http://www.unipr.it/arpa/facvet/annali/1999/bottarelli2/bottarelli.htm>.
 Bravo LL, Morier LL, Castaneda TN, Ramirez DM, Silva DM, Castro-Escarpulli G (2003). *Aeromonas*: An emerging pathogen associated with extraintestinal infection in Cuba. *Rev. Cubana Med. Trop.*, 55: 208-209.

- Collee JG, Bradley R (1997). BSE: A decade on - Part 1. *The Lancet*, 349: 636-641.
- Cravioto A, Gross RJ, Scotland SM, Rowe B (1979). An adhesive factor found in strains of *E. coli* belonging to the traditional infantile enteropathogens. *Curr. Microbiol.*, 3: 95-99.
- Dumontet S, Krovacek K, Stevenson SB, Pasquale V, Baloda SB, Figliuolo G (2000). Prevalence and diversity of *Aeromonas* and *Vibrio* spp. in coastal waters of Southern Italy. *Comp. Immunol. Microbiol. Infect. Dis.*, 23: 53-72.
- Fasano A (2000). Intestinal infections: Bacteria. In: Walker WA, Durie PR, Hamilton JR, Smith JA, Watkins JB (eds) Paediatric gastrointestinal diseases. 3rd ed., Hamilton, Ontario: B. C. Decker, pp 463-485.
- Garrity GM, Bell JA, Lilburn T, Class I (2005). *Alphaproteobacteria* class. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT (eds), *Bergey's Manual of Systematic Bacteriology*, Second Edition, Volume 2, Part C, Springer, New York, 2005, p. 1.
- Gascón J, Vila J, Valls ME, Ruiz L, Vidal J, Corachan M (1993). Etiology of traveler's diarrhoea in Spanish traveler's to developing countries. *Eur. J. Epidemiol.*, 9: 217-223.
- Guarrant RL, Hughes JM, Lima NL, Crane J (1990). Diarrhoea in developed countries: magnitude, special settings, and etiologies. *Rev. Infect. Dis.*, 12: 41-50.
- Jalal KCA, Fatin UTN, Mardiana MA, John BA, Kamaruzzaman YB, Shahbudin S, Omar MN (2010). Antibiotic resistance microbes in tropical mangrove sediments in east coast peninsular, Malaysia. *Afri. J. Microbiol. Res.*, 4 (8): 640-645.
- Maltezou HC, Zafiropoulou A, Mavrikou M, Bozavoutoglou E, Lipai G, Foustoukou M (2001). Acute diarrhoea in children treated in an outpatient setting in Athens, Greece. *J. Infect.*, 43: 122-127.
- Nataro JP, Kaper JB (1998). Diarrhoeagenic *Escherichia coli*. *Clin. Microbiol. Rev.*, 11: 142-201.
- Nzeako B, Okafor N (2002). Bacterial enteropathogens and factors associated with seasonal episodes of gastroenteritis in Nsukka, Nigeria. *Br. J. Biomed. Sci.*, 59: 76-79.
- Okeke IK, Ojo O, Lamikanra A, Kaper JB (2003). Etiology of acute diarrhoea in adults in South-western Nigeria. *J. Clin. Microbiol.*, 41: 4525-4530.
- Oyofe BA, Subekti D, Tjaniadi P, Machpud N, Komalarini S, Setiawan B (2002). Enteropathogens associated with acute diarrhoea in community and hospital patients in Jakarta, Indonesia. *FEMS Immunol. Med. Microbiol.*, 34: 139-146.
- Quadri SM, Zafar M, Lee GC (1991). Can isolation of *Aeromonas hydrophila* from human feces have any clinical significance? *J. Clin. Gastroenterol.*, 13: 537-540.
- Robinson J, Petersen D, Burke V, Gracey M (1983). Identification of enterotoxigenic *Aeromonas* spp. isolated from human and domestic water supplies. *Toxicon Suppl.*, 3: 367-369.
- Sanderson IR, Walker WA (1993). Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). *Gastroenterology*, 104: 622-639.
- Simanjuntak CH, Hardjining S, Hasibuan MA, Pujarwoto T, Koiman I (1998). Gastrointestinal infections in South Asia (V). Technical Meeting of counterparts on Seismic data exchange program on infectious diseases. SEAMIC, Japan.
- Taneja N, Kaur J, Sharma K, Singh M, Kalra JK, Sharma NM, Sharma M (2003). A recent outbreak of cholera due to *V. cholerae* O1 Ogawa in and around Chandigarh, North India. *Indian J. Med. Res.*, 117: 243-246.
- Taper N, Sanderson IR (2004). Diarrhoea in children: an interface between developing and developed countries. *The Lancet*, 363: 641-653.
- United Nations Children's Fund – UNICEF (1992). *The State of the World's Children*. Oxford University Press, New York.
- Urbino D, Arazuza O, Young G (2003). Rotavirus type A and other enteric pathogens in stool samples from children with acute diarrhoea on the Colombian Northern Coast. *Int. J. Microbiol.*, 6: 27-32.
- WHO (World Health Organization) (1995). The management of bloody diarrhoea in young children: WHO/CDD/94.49, World Health Organization, Geneva.
- WHO (World Health Organization) (1998). *World Health Report: Global health situation and trends 1955 – 2025*. World Health Organization: Geneva.