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ANTIMICROBIAL RESISTANCE OF ENTERIC PATHOGENS ISOLATED FROM CHILDREN WITH ACUTE DIARRHOEA IN PATTUKKOTTAI, TAMIL NADU, INDIA

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ABSTRACT

Acute gastroenteritis is a common infection among the children. The present study was conducted to study the bacterial pathogens in paediatric diarrhoeas and their antibiotic resistance pattern. Stool samples were collected between January 2012 to December 2012. A total of 118 patients with diarrhoea who were under five years of age. Stool samples were inoculated, isolated and identified using standard bacteriological methods. The majority of the isolates were *E. coli* (36.4%), followed by *Aeromonas spp* (22%), *Salmonella* spp. (18.6%), *Shigella* spp. (14.4%) and *Vibrio* spp (8.5%). All the bacterial isolates were 93.3% resistance to ampicillin. 92.4% to amoxicillin, 47% to cefixime, 42.7% to chlorampenical and 30.9% to nalidixic acid. The antimicrobial profile of all isolated bacteria *Vibrio* (45%), followed by *Shigella* (35.3%), *Salmonella* (32.3%), *E.coli* (30%), and *Aeromonas* (22.31%) showed high resistance rates against the tested 10 antimicrobials. The highest antimicrobial resistance rates were found against Ampicillin (93.3%) and Amoxicillin (92.4%). The isolates showed maximum sensitivity to Amikacin followed by cefotaxime, gentamycin, and ciprofloxacin. High level resistance to first line antimicrobials is due to unselected use of these drugs in low risk patients without complications. Periodic monitoring of drug resistance in enteric pathogens in each geographical area helps in choosing the appropriate antimicrobial agent for empiric therapy.

Keywords: Diarrhoea, Enteric bacterial pathogens, antimicrobial resistance, seasonal variations.

INTRODUCTION

Diarrhoea caused by multidrug-resistant bacteria is an important public health problem among children in developing countries. Global, regional and national estimates clearly place diarrhoeal diseases as a major public health problem worldwide as it is responsible for approximately 4 billion cases of diarrhoea per annum, of which 2 billion cases result in death (UNICEF, 2012). Each year, more than 2 million children don't live to see their 5th birthday because of diarrhoea and pneumonia (Boschi *et al.,* 2008).

Inappropriate prescription of antibiotics prompted resistance and increased infectious disease mortality not only in developing countries but also in developed countries. Aging populations, changes in behavior and a decline in the development of new antibiotics exacerbated a deteriorating situation (Dandekar and Dandekar,

2010). The antibiotic resistance of enteric bacteria has profound clinical implications because it threats the life and causes many of serious diseases such as acute gastroenteritis (Nair *et al.*, 2010).

Acute gastroenteritis is a severe infection of the gastrointestinal tract (WHO, 2009). Sometimes people refer to it as "stomach flu" which is characterized by diarrhoea, stomach pain, nausea, vomiting, fever or feeling unwell. Symptoms may start quite slowly or come on suddenly. Gastroenteritis usually passes in less than 24 hours but can continue for several days. Diarrhoea is defined as having loose or watery stools at least three times per day, or more frequently than normal for an individual. Though most episodes of childhood diarrhoea are mild, acute cases can lead to significant fluid loss and dehydration, which may result in death.

The major cause of death for children is affected by diarrhoeagenic bacteria *E. coli* spp., *Vibrio* spp., *Salmonella* spp., *Aeromonas* spp., *Shigella, Yersinia enterocolitica*, Rotavirus, *Cryptosporidium* spp., *Entamoeba histolytica*, and *Giardia lamblia*. These pathogens can cause potentially serious diseases, which may be fatal, especially in children. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks (Gupta and Gupta, 2009).

Seasonal cycles of infectious diseases have been variously attributed to changes in atmospheric conditions, the prevalence or virulence of the pathogen, or the behavior of the host organism. An understanding of the seasonal variation of enteric pathogens would contribute greatly in focusing healthcare initiatives in a climate of limited resources to a cost-effective reduction in disease morbidity and mortality which is why it has attracted considerable attention from healthcare researchers around the world with several studies having been conducted in both the developing and the developed countries (Alam *et al.,* 2003). This study performed microbiological investigation of some potential pathogens associated with diarrhoea, to characterize the isolates and their antibiotic resistance related to the diarrhoeal disease in children.

MATERIALS AND METHODS

Specimen collection: The surveillance was conducted for a period of 1 year from January 2012 to December 2012 among diarrhea patients in three hospitals at Pattukkottai. A total of 118 stool samples were collected from children with diarrhoea. The samples were collected from hospitalized diarrhea patients before the administration of antibiotics and carried to the laboratory in Cary-Blair transport medium.

Bacteriology: The stool samples were cultured on Thiosulphate-citrate-bile salt sucrose (TCBS) agar for the isolation of *Vibrio spp.,* MacConkey agar for the isolation of *E. coli,* Hektoen enteric agar and SS agar for the isolation of *Shigella* and *Salmonella spp.* and Rimler-Shotts agar for the isolation of *Aeromonas spp.* (Hi-Media, Mumbai, India). Identification of bacterial isolates involves the use of biochemical screening media are usually used.

Antibiotic susceptibility test: Antibiotic susceptibility testing of *Salmonella, Shigella, Vibrio,* pathogenic *E. coli,* and *Aeromonas spp.,* was carried out by the disk diffusion technique using a commercially available disc (Hi-Media). The antimicrobial sensitivity of the test strains to ten antibacterial drugs was done using the Kirby-Bauer disk diffusion method (Bauer *et al*., 1966). The antibiotics used were Amikacin (AK, 30µg), Ampicillin (AM, 10µg), Amoxacillin (AX, 10µg), Cefixime (FX, 5µg), Cefotaxime (CF, 30µg), Chloramphenicol (CH, 30µg), Ciprofloxacin (CP, 5µg), Gentamycin (GM, 10µg), Nalidixic acid (NA, 30µg), and Ofloxacin (OF, 05µg).

A lawn of test pathogen (1ml of an 18 hours peptone broth culture) was prepared by evenly spreading 100µl inoculums with the help of a sterilized spreader onto the entire surface of the agar plate. The plates were allowed to dry before applying antibiotic disc. Then, some commercially available antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 h to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 37°C for 24 hours. If an antimicrobial activity was present on the plates, it was indicated by an inhibition zone. The diameter of the inhibition

zones was measured in millimeter at 24 hours using a scale. An organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if the diameter was less than 13 mm. The intermediate readings were considered as sensitive in the assessment of the data.

RESULTS

Whole-stool samples were collected from 118 individuals in three hospitals at Pattukkottai. All patients were under five years of age. The stool samples were analyzed by conventional biochemical methods and antimicrobial susceptibility. The results showed that bacterial isolates were present in all patients. Among these isolates, pathogenic *E. coli* comprised 43 (36.4%), *Aeromonas* 26 (22%), *Salmonella* 22 (18.6%), *Shigella* 17 (14.4%) and *Vibrio cholera* 10 (8.5%).

There were high rate of diarrhoea patients from October to November, the rainy season in this area. It gradually decreased towards the month of December and continued at a low rate through January with prevalence reappearing from April through June. A high prevalence of infection due to *E.coli, Aeromonas and Vibrio* were observed from winter and summer (Table 1).

Aeromonas was highly sensitivity to AK, CF, CP, GM, NA and OF (Table 2), but was highly resistant to AM, AX, FX and CH respectively. The *E.coli* were highly resistant to AX, AM and NA, but was highly sensitivity to AK followed by GM, OF, CP and CF. The *Salmonella* was highly resistant to AX, AM, FX, CH, and NA respectively. The pathogenic *Salmonella* spp. strains were 100% sensitivity to AK, CF, CP and GM. Similarly, the diarrhea producing *Shigella spp*. were 100% resistant to AM and it was highly resistant to AX, FX, NA and CH. The *Shigella spp*. strains were 100% sensitive to AK, CF, CP and GM. All *Vibrio spp* isolates were 100% resistant to AX, AM, CH and moderate resistant to FX, NA and OF, but it was 100% sensitive to AK, CF, CP and GM.

All the bacterial isolates from diarrhoeal patients (Figure 1) were 100% completely susceptible to amikacin, 98.1% to gentamycin, 97.2% to cefotaxime, ciprofloxacin, 83.5% to ofloxacin, 69.1% nalidixic acid, 57.3% to chlorampenical, 53% to cefixime, 7.6% to amoxicillin and 6.7% to ampicillin. All the bacterial isolates were resistant to ampicillin (93.3%), amoxicillin (92.4%), cefixime (47%), chlorampenical (42.7%), nalidixic acid (30.9%), ofloxacin (16.5%), cefotaxime, ciprofloxacin (2.8%), gentamycin (1.9%), but 0% resistant to amikacin. The antimicrobial profile of all isolated enteropathogenic bacteria showed high resistance rates against the tested 10 antimicrobials. *Vibrio* (45%), followed by *Shigella* (35.3%), *Salmonella* (32.3%), E.coli (30%), and *Aeromonas* (22.31%). Almost all isolates were resistant to Ampicillin and Amoxacillin.

Table 1. Seasonal distributions of isolated bacterial pathogens from children.

Organisms	Spring	Summer	Autumn	Winter	Total	Percentage
Aeromonas spp.	1	8	0	17	26	22
E. coli	7	8	4	24	43	36.4
Salmonella spp.	5	2	10	5	22	18.6
Shigella spp.	3	9	1	4	17	14.5
Vibrio spp.	0	1	3	6	10	8.5
Total	16	28	18	56	118	100

Table 2. Number of Bacterial species (%) Resistant to Antibiotics tested.

Organisms	**AK**	**AM**	**AX**	**FX**	**CF**	**CH**	**CP**	**GM**	**NA**	**OF**
Aeromonas spp. (N=26)	0(0)	22(84.6)	22(84.6)	10(38.5)	0(0)	4(15.4)	0(0)	0(0)	0(0)	0(0)
E. coli (N=43)	0(0)	39(90.7)	39(90.7)	6(14)	6(14)	10(23.3)	6(14)	4(9.3)	15(34.9)	4(9.3)
Salmonella spp. (N=22)	0(0)	20(90.9)	20(90.9)	14(63.6)	0(0)	10(45.5)	0(0)	0(0)	4(18.2)	3(13.6)
Shigella spp. (N=17)	0(0)	17(100)	16(94)	10(58.8)	0(0)	5(29.4)	0(0)	0(0)	7(41.2)	5(29.4)
Vibrio spp. (N=10)	0(0)	10(100)	10(100)	6(60)	0(0)	10(100)	0(0)	0(0)	6(60)	3(30)

KEY: Amikacin (AK 30µg), Ampicillin (AM, 10µg), Amoxacillin (AX, 10µg), Cefixime (FX 5µg), Cefotaxime (CF, 30µg), Chloramphenicol (CH, 30µg), Ciprofloxacin (CP, 5µg), Gentamycin (GM, 10µg), Nalidixic acid (NA, 30µg) and Ofloxacin (OF, 05µg).

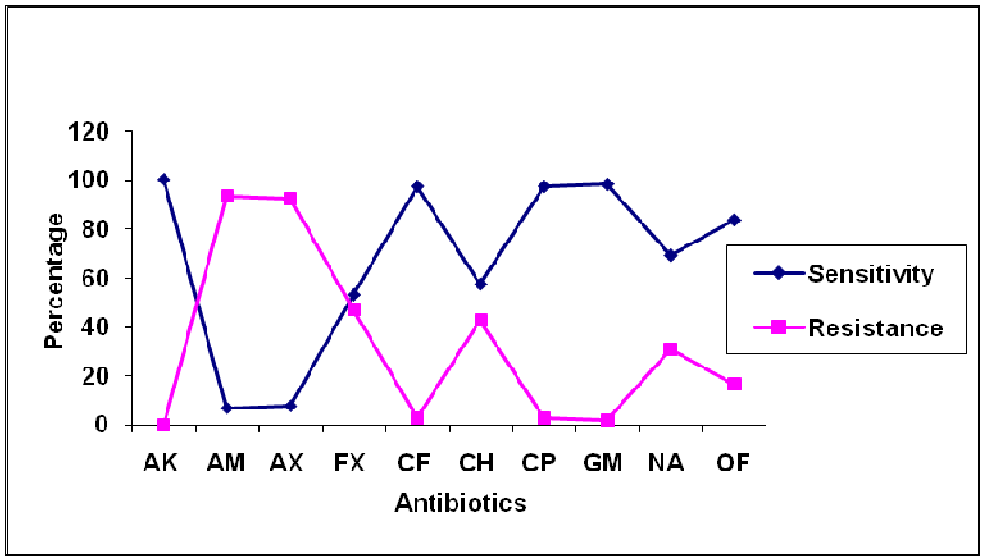

Figure 1. Sensitivity and Resistant pattern of all the organisms isolated from children with diarrhoea.

DISCUSSION

This is the first report of a systematic surveillance study on the prevalence of different bacterial enteropathogens isolated from hospitalized diarrhoea patients from pattukkottai area. In this investigation five bacterial enteropathogens were isolated from hospitalized diarrhea patients.

According to our results, children less than 5 years old are more susceptible to infectious diarrhoea and enteropathogenic bacteria were isolated with a higher frequency from patients belonging to this age group. Many studies are in agreement with our results that children age less than 5 years old are more susceptible to infectious diarrhea (Das *et al*., 2009).

In the present study enteropathogenic bacteria were isolated from (118) dirrahoael patients *E. coli* (36.4%)*, Aeromonas* (22%)*, Salmonella* (18.6%)*, Shigella* (14.4%) and *Vibrio* (8.5%). This finding is nearly congruent with other local study conducted by Abu Elamreen *et al*., (2007) where they reported that (10%) of their samples had enteropathogenic bacteria which screened by conventional culture method.

In this study multidrug resistance was common among enteropathogenic bacteria showed high resistance rates against to the *Vibrio* (45%), followed by *Shigella* (35.3%), *Salmonella* (32.3%), E.coli (30%), and *Aeromonas* (22.3%) The highest antimicrobial resistance rates were found against Ampicillin (93.3%) and Amoxicillin (92.4%). In the other hand, some of isolated enteropathogenic bacteria were completely sensitive to antibiotics such as amikacin (100%), Gentamycin (98.1%), Cefotaxime and ciprofloxacin (97.2%). Amikacin is a widest spectrum of activity. It is recommended as a reserve drug for hospital acquired gram-negative bacillary infection (Tripathi, 2008). High prevalence of multiple drug resistance amongst the *Aeromonas* isolates was noticed (Cefixime, 38.5%, Ampicillin and Amoxacillin, 84.6%). Multiple drug resistance among *Aeromonas spp* has been reported from many parts of the world (Subashkumar *et al.,* 2012). In the current study, 30% of the *E.coli* isolates were multidrug resistant (MDR). The most resistance pattern was ampicillin/ amoxacillin/nalidixic acid. The incidence of diarrhea due to MDR *E. coli* has increased in developing countries in the last decade. Higher rates have been reported from 50 to 70% in recent years (Aslani *et al.,* 2011 and Raju and balla, 2009), and the highest rate up to 75% have been reported from India (Vaishnavi and Kaur, 2003).

The *Salmonella* spp. was resistant 90.9% to AX, AM, 63.6% to FX, 45.5% were resistant to CH, 18.2 % to NA and 13.6% to OF respectively. There seems to be complete resistance to ampicillin and amoxicillin by *Salmonella* organisms in the study which is in disagreement with reports from other parts of the country (Mache *et al.,* 1997 and Asrat, 2008). Another study in Salvador, Bahia, Brazil showed that *Salmonella* presented very low resistance rates to all drugs tested. These data are useful for practitioners and they reinforce the need for continuous microbiological surveillance (Diniz-Santos et *al.,* 2005). In our study the diarrhea producing *Shigella spp*. were resistant 100% to AM, 94% to AX, 58.8% to FX, 41.2% to NA, 29.4% to CH and OF. Similar study in India showed that resistance to antimicrobial agents was common among all *Shigella* an overall resistance of (63.6%), (58.1%), (18.5%) and (16.3%) was observed for nalidixic acid, trimethoprim/ sulfamethoxazole, ciprofloxacin and furazolidone respectively (Taneja *et al.,* 2004). Most of the *Shigella* isolates were susceptible to amikacin, cefotaxime, ciprofloxacin, and gentamycin and resistant to the other antibiotics.

Vibrio spp (8.5%) were isolated in this study. The spread pattern of *Vibrio* spp. suggested water borne infection in rainy seasons, although the quantity of water available is large in rainy season most water sources are contaminated with excreted microorganisms from surface water runoff (Karki *et al.,* 2010). All *Vibrio spp* isolates were 100% resistant to AX, AM, CH, 60% to FX, NA and 30% to OF. A significant level of incidence of *Vibrio* spp. was recorded in all the seasons. This may be due to the tendency of this organism to cause severe diarrhea, thus making infected individuals more likely to seek medicinal attention (Fasano, 2000).

Conclusion

Our results showed that enteric bacterial infections caused by *E.coli, Salmonella, Shigella, Vibrio* and *Aeromonas* are prevalent in Pattukkottai area. All the isolates were highly susceptible to amikacin, cefotaxime, ciprofloxacin and gentamycin antibiotics, and other isolates were produces different resistant spectrum to other six antibiotics. It is necessary to take them into account in microbiological diagnostics and the clinical interpretation of the result of these investigations. Considering the threat of emerging antimicrobial resistance among these enteric bacterial pathogens, it is important to continue surveillance on these organisms in terms of prevalence, clinical epidemiology, and antimicrobial susceptibility patterns obtained from different hospital and community settings throughout the country.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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