Circulating Rotavirus Types and Drug-Resistant Diarrheagenic *Escherichia coli* Causing Enteric Infection in Under-Five Children in Rural West Bengal, India

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Received Date: October 5, 2018; Accepted Date: October 20, 2018; Published Date: October 29, 2018

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**Abstract**

Acute gastroenteritis is an important cause of global morbidity and mortality. In this study, we collected 486 fecal specimens from diarrheal patients during January 2014 to December 2014. We have found G1[P8] as highest prevalent (85%) genotype of rotavirus followed by G9[P4] (9%). Furthermore, group A rotavirus (54.52%) was found as the primary causative agent of childhood diarrhea and diarrheagenic *Escherichia coli* (31.89%) as the second causative agent. A significant number of rotavirus-bacteria mixed infection was found. The antibiotic susceptibility study reveals the prevalence of multi-drug resistance diarrheagenic *Escherichia coli*. This study suggests a particular preventive measure for diarrhea during the first two years of life.

**Keywords:** Acute gastroenteritis; Diarrheagenic *Escherichia coli*; Rotavirus; G [P] typing

**Introduction**

Acute gastroenteritis due to enteropathogens is a leading cause of hospitalization of young children in developed countries and one of the significant causes of mortality in developing countries [1]. In developing countries, each child experience 3.5 to 7.0 diarrheal episodes during first two years of life and 2 to 5 diarrheal episodes up to 5 years of life [2]. The global morbidity and mortality due to single rotavirus infection were estimated about 110 million cases out of which 4 million children die annually [3,4]. In poorest countries, childhood death due to rotavirus gastroenteritis accounts for 8% [5]. In India, approximately 22% of the 453,000 deaths among children below five years of age are because of rotavirus gastroenteritis [6] and about 20 to 70% of hospitalizations are attributable to rotavirus [7]. There are two surface antigens of group A rotavirus called as VP7 (G-type) and VP4 (P-type) and they have a role in neutralization of host defense mechanism [8]. However, the etiological agents of acute gastroenteritis include a broad range of causative agents that differ significantly with geographical variation [9]. Among other causative agents of acute gastroenteritis, viral agents account for 75% out of which rotavirus account for 50% [10], and diarrheagenic *Escherichia coli* (DEC) is responsible for 30%-40% of all diarrheal illness among children <5 years of age [11]. The DEC has six major pathotypes including enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIPEC), and diffusely adherent *E. coli* (DAEC). The pathotypes of DECs differ in their virulence mechanism and clinical manifestation [12]. They are the primary cause of persistent diarrhea due to their high prevalence both in the hospital, and community setting [13]. In a hospital setting in-patients, diarrhea due to EPEC found in 25.4% of cases [14]. Worldwide, about 600 million cases of diarrheal illness are attributable to DECs out of which one million children (<5 years of age) died annually, and ETEC infections account for about 75% of it [15]. In a recent report by WHO, EPEC and EAEC are enlisted in top priority pathogens that require immediate vaccine development after rotavirus [16]. Thus in the present time, DECs become a leading cause of the global health burden of diarrheal illness [17]. The financial burden that India has for treating rotavirus infection has been estimated to be 41-72 million US$ annually [18]. In India about 2.2% to 5.8% of the household’s annual income incurred directly per diarrheal episodes [19]. It indicate that the economic burden that India has presently to combat diarrheal illness, is also a severe issue in providing and maintaining quality health care services.

In human, 10 G-serotypes and 11 P-genotypes of rotavirus were identified [20]. Several studies have been suggested severity of rotavirus diarrhea due to different combinations of G and P type. Moreover, a common G and P type had never been identified with same or different geographic regions on a different time scale [21]. Thus, it is essential to monitor the prevalent circulating strain to create vaccine development strategies. The present study aimed to determine the predominant genotype of rotavirus and other causative agents of childhood diarrhoea such as Diarrheagenic *Escherichia coli* (DEC), *Vibrio cholerae*, *Vibrio sp.*, *Shigella* sp. and *C. jejuni* among children under five years of age admitted with diarrheal symptoms in rural areas of West Bengal, India. We also determined the antibiotic susceptibility patterns of enteropathogenic bacteria and prevalence of co-infection among enrolled diarrheal cases.

**Materials and Methods**

**Study population, study site, and sample collection**

Fecal samples from 486 children <5 years of age hospitalized with acute diarrhea were collected from Medinipur Medical College and
Hospital (MMC&H), Medinipur West Bengal, India. The samples were collected for 12 month period from January 2014 to December 2014. The patients were predominantly from rural areas of Paschim Medinipur and Jhargram (Jangamahal area) to take health care services at MMC&H. Written consents from each patients' guardians were taken before enrollment in this study.

**Clinical data collection**

The clinical records from each patient with matched selection criteria were collected with direct supervision of the medical officer. The Vesikari clinical method of diarrheal severity scoring was used for screening the clinical data [21].

**Selective isolation and preliminary identification of pathogenic bacteria**

Selective media based method was used for preliminary identification of enteropathogenic bacteria. Eosin methylene blue (EMB) agar and MacConkey agar were used for identification and isolation of *E. coli*, *Shigella sp.*, *Thiosulfate-citrate-bile salts-sucrose* (TCBS) agar was used for *V. cholerae* and *Vibrio* sp. Campylobacter agar was used for *Campylobacter jejuni*. All selective media and Luria-Bertani (LB) were purchased from HiMedia Laboratory, India.

**Nucleic acid extraction and polymerase chain reaction**

Extraction of genomic DNA from each isolate was carried out by genomic DNA kit (QIAGEN, India). The extracted genomic DNA from each strain was then quantified at 260/280 nm using spectrophotometer (UV-1800, Shimadzu, Japan) and used as template DNA (1 µg ml⁻¹ as final conc.) in the PCR. The PCR was developed by using 2X PCR TaqMixture (HiMedia, India). The final reaction volume was 25 µl. The primer sequences and PCR program that was used is described in Table 1. The PCR products were checked in 1.0 g% (w/v) agarose gel electrophoresis and photographed using Gel Doc XR (Gel Doc, Bio-Rad, USA).

**Detection of rotavirus antigen by enzyme immunoassays (EIA)**

The detection of rotavirus antigen was performed by using Premier™ Rotaclone kit (Fisher Scientific). Viral suspension (VS) was prepared by adding 1 ml of sample diluent (provided in the kit) to 1.5 ml microfuge tube to prepare a 10% suspension or dilution of the fecal specimen by the addition of approximately 0.1 g of solid feces (small pea-sized portion) or approximately 100 µl of liquid feces. After thoroughly mixing the suspensions were centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was collected carefully in a new tube and was used for enzyme immunoassay (EIA). Rest of the protocol was followed as described by the original kit manufacturer.

### Table 1: Primer sequences and PCR amplicon size.

<table>
<thead>
<tr>
<th>Enteropathogenic Bacteria</th>
<th>Primer name</th>
<th>Primer Sequences 5' to 3'</th>
<th>Product size</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EPEC</strong> (bfpA)</td>
<td>F</td>
<td>AATGGTGCTTGCGCTTGCTGC</td>
<td>326 bp</td>
<td>Gómez-Duarte et al. [22]</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GCGCGTTTTACCAACCTGTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EAEC</strong> (aggR)</td>
<td>F</td>
<td>GTATAACAAAGAAGGAGGC</td>
<td>254 bp</td>
<td>Gómez-Duarte et al. [22]</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>ACAGAATGTCAGCATACGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EIEC</strong> (ipaH)</td>
<td>F</td>
<td>CTCGGCAGCTTTAATAGCTGG</td>
<td>933 bp</td>
<td>Gómez-Duarte et al. [22]</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GTGGAGAGCTGAATGTTCTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ETEC</strong> (ST)</td>
<td>F</td>
<td>GCTAAACCAGTAGGCTTCT</td>
<td>147 bp</td>
<td>Gómez-Duarte et al. [22]</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>CCGGATACAGAGCGAGGTTAAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EHEC</strong> (eaeA)</td>
<td>F</td>
<td>GTGGCAGGAAATTGCGGGG</td>
<td>890 bp</td>
<td>Fagan et al. [23]</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>CCCCATTCTTTTTCACCTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vibrio spp.</strong> (16sRNA)</td>
<td>F</td>
<td>CAATGGGCAGGAGCAGGTAGG</td>
<td>889 bp</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GCTGCCCTCTGTAATACGGCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vibrio cholerae</strong> (16sRNA)</td>
<td>F</td>
<td>AGCAAAAGCGGAGGACCTTC</td>
<td>870 bp</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>CAGCCATGCGACCACTGCTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Shigella sp</strong> (16sRNA)</td>
<td>F</td>
<td>TTGGCTTTCCGCTGACAGG</td>
<td>472 bp</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TTAACGTTTGACCCCTCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Campylobacter jejuni</strong> (16sRNA)</td>
<td>F</td>
<td>ATACGTGGGTTGCGCTGG</td>
<td>559 bp</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>ATACGTGGGTTGCGCTGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Extraction of viral dsRNA

Viral dsRNA was extracted using QIAamp® Viral RNA Kit (Qiagen, USA). A stool suspension in sterile Dulbecco's phosphate-buffered saline by adding 0.3 g of solid faeces or approximately 300 µl of liquid faeces in 700 µl of PBS. After thoroughly vortexing for 1 min centrifugation was performed at 10000 rpm for 10 min at 4°C. The clear supernatant was transferred into the sterile 1.5 ml micro centrifuge tube and stored at 4°C until use. Rest of the protocol was followed as described by the original kit manufacturer.

Reverse transcriptase (RT) PCR

Total RNA was extracted using the PureLink™ RNA Mini Kit (Invitrogen, Carlsbad, CA) and quantified using a UV-Vis spectrophotometers (Mecasys Co. LTD, Korea). Total RNA was then converted to cDNA by using Verso cDNA synthesis kit (Thermo Scientific, USA). The standard reaction contained 1x Power SYBR Green PCR master mix (Applied Biosystems), respective primer pairs and cDNA as the template strand. The temperature program for 40 cycles were set to denaturation at 94°C for 1 min, annealing at 55°C for 45 sec. The reaction was conducted in StepOnePlus Real-Time PCR System (Applied Biosystems). The samples were analyzed in triplicate and the recA was used as endogenous control for normalization.

G type and P type PCR (multiplex semi-nested)

The multiplex semi-nested technique was implemented to determine G-P type of the rotavirus strains. The synthesized cDNA was used as the template to determine G-P type of every 3rd rotavirus positive sample. The temperature program and primer sets used are listed in Table 1. The PCR products were then analyzed in 1.2% agarose gel in 1x TBE buffer and photographed using Gel doc (BioRad, USA).

Antimicrobial susceptibility test (AST)

AST of clinical isolates was determined using the Kirby-Bauer disk diffusion method and interpreted by CLSI guidelines [24]. The isolates were tested against the following antibiotics, Amikacin (AK), Ampicillin (AMP), Amoxycillin (AMC), Ceftriaxone (CTR), Cefepime (CPM), Doxycycline HCl (DO), Norfloxacin (NX), Tobramycin (TOB), Gentamicin (GEN), Nitrofurantoin (NIT), Chloramphenicol (C). All antibiotic disks were purchased from HiMedia Laboratory, India.

Statistical analysis

Differences in proportions were assessed by a chi-square test and Fisher’s exact test whichever applicable. The analysis of variance (ANOVA) was performed for quantitative variables. P values < 0.05 were considered statistically significant. All statistical test was performed using Graphpad Prism® 6.01 (Santiago, USA) software packages.

Results

Clinical severity of studied population

The clinical record analysis according to Vesikari clinical severity score [21], revealed that out of 453 children, 355 children were admitted with moderate diarrheal symptoms and 131 children were admitted with severe diarrheal symptoms, and the rotavirus antigen was detected positive in 197 and 68 fecal samples respectively (Table 2). The clinical manifestations and sign of dehydration, the immediate treatment recommended by the medical officer are summarized in Table 3.

Table 2: Difference and relationship between Vesikari clinical severity score and prevalence of rotavirus at MMC&H.

<table>
<thead>
<tr>
<th>Rotavirus detection</th>
<th>Total no. patient (n=486)</th>
<th>Positive (n=265)</th>
<th>Negative (n=221)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Disease</td>
<td>(0-5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Moderate</td>
<td>(6-10)</td>
<td>355</td>
<td>197</td>
</tr>
<tr>
<td>Severe</td>
<td>(11-15)</td>
<td>131</td>
<td>68</td>
</tr>
<tr>
<td>Very severe</td>
<td>(16-20)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rotavirus detection</th>
<th>Stool appearance*</th>
<th>Sign of dehydration on admission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Watery</td>
<td>Semisolid</td>
</tr>
<tr>
<td>Total no. of patients</td>
<td>191</td>
<td>97</td>
</tr>
<tr>
<td>Positive (n=265)</td>
<td>147</td>
<td>50</td>
</tr>
<tr>
<td>Negative (n=221)</td>
<td>84</td>
<td>43</td>
</tr>
</tbody>
</table>

* Statistically significant

Table 3: Clinical manifestation, sign of dehydration, immediate treatment in children admitted with acute diarrhea at MMC&H.

Molecular identification of rotavirus

The GP typing results shows that in this region G1P[8] (85%), most prevalent strain followed by G9P[4] (5%), G2P[4] (3%), G9P[8] and G2[6] were detected only 1% among the rotavirus positive strains (Figure 1). In our study, G9P[6] was not detected.
Prevalence of enteropathogenic bacteria

The selective media based isolation followed by multiplex polymerase chain reactions show that Enteropathogenic Escherichia coli (EPEC) was the prevalent group. Eighty-one isolates (19.66%) belonged to this group. The second prevalent group detected was Enteroaggregative Escherichia coli (EAEC), and 44 isolates (10.67%) belonged to this group. The third prevalent pathogen was Vibrio cholerae and 34 isolates (8.25%) were identified by PCR (Table 4). Enterotoxigenic Escherichia coli (ETEC), Shigella sp. and Vibrio sp. were next to V. cholerae and 21, 27, 14 strains were identified, respectively. A very negligible number of Campylobactor jejuni (n=6), Enterohemorrhagic Escherichia coli (EHEC) (n=3), Enteroinvasive Escherichia coli (EIEC) (n=2) were detected in this study. Three fecal specimens did not belong to any of the target enteropathogenic bacteria group (Table 4).

Table 4: Prevalence of rotavirus and diarrheagenic E. coli (DEC) among children <5 years of age admitted with diarrheal symptoms at MMC&H.

Co-infection with multiple enteropathogens and rotavirus

One hundred and fifty-five (34.21%) cases of co-infections were detected among 453 fecal specimens (Table 5). The co-infection were prevalent between rotavirus and enteropathogenic bacteria (83.22%). EPEC, EAEC, EIEC, ETEC, Vibrio cholerae, Vibrio sp., Shigella sp. and Campylobacter jejuni were detected respectively, 47.28%, 17.05%, 0.77%, 5.42%, 14.72%, 2.32%, 9.30%, and 3.10% with rotavirus infection. Bacteria-bacteria (DEC-DEC) co-infection were found positive in very few cases (16.77%). Whereas, Diarrheagenic Escherichia coli (DEC) and Vibrio cholerae co-infection were found in 11 fecal specimens.
Antibiotic susceptibility test (AST)

The AST was conducted to identify antibiotic susceptibility patterns among enteropathogenic bacterial isolates (Figure 2). The data obtained from AST revealed that CTR, CTX, AMP, and NX were highly resistant among all studied enteropathogens and they revealed 82.089% (V. cholerae), 80.12% (EPEC), 71.64% (V. sp.), and 55.26% (EAEC) respectively. AK was found highest sensitive for EPEC (97.51%) among all tested enteropathogens.

Prevalence of rotavirus and seasonal variation

Two hundred and eight fecal specimens were found rotavirus positive, which is 61.81% out of 453 fecal samples. The high susceptible age group was found to be 6-12 month (P<0.05), 43.92% samples belonged to this group (Figure 3). The second susceptible age group was found to be 12-24 month (P<0.05), 38.21% samples belonged to this group (Table 4). The enrolled case data analysis revealed that rotavirus is prevalent in the winter season (December-February) and during onset of summer (March-April) was highest (Figure 4). The same phenomenon was also observed for prevalent G and P types of rotavirus (Figures 5 and 6). In March 2014 significantly (P<0.0001) high prevalence (25.71%) of rotavirus was detected and in December 2014, February 2014 and January 2014 the prevalence rate of rotavirus was 18.92%, 14.28%, and 10.35%, respectively.
diarrheal symptoms. We observed 40.53% (197/486) children were admitted with severe diarrheal symptoms (AGE with vomiting or AGE with some dehydration) out of which 16.99% children were <12 years of age. This severity may be primarily due to lack of proper parental care, poor maternal diet, malnutrition, lack of health education, poor socioeconomic status and irregular breast-feeding [25]. We have observed that children less than 24 months of age were susceptible to rotaviral diarrhea, comprising 48.14% of all diarrheal cases and 88.30% of all positive rotavirus cases (P<0.05). However, this prevalence rate was lower in other countries [26]. This difference is due to much-influencing factors including geo-demographic variations, the measure was taken during sample collections, and most importantly socioeconomic status and mother-child nutrition [27]. We have observed that group A rotavirus was prevalent (54.52%, 265/486) among all diarrheal cases. Furthermore, enteropathogenic bacteria was prevalent (48.35%) in children (<5 years) with diarrheal symptoms. Thus, this study confirming group A rotavirus as a main etiological agent in pediatric diarrhea [28]. However, the prevalence of enteropathogenic bacteria was low in the previous study [29]. Several lines of documents have suggested that the prevalence of diarrhea is strongly associated with sanitation [30]. The isolated bacteria are indicators of fecal contamination, indicating low hygiene in the intake of foods [31,32]. Moreover, young children those were taking health care services at MMC&H, primarily belonged to the low socioeconomic group and had a poor living standard, which is the main reason for this high prevalence of diarrheagenic bacteria [33]. In our study, G1P[8] (85.0%) was most prevalent genotype followed by G9[P4] (5%), G2[P4] (3%), G2[P6] (2%), others variants were detected only 1% among the rotavirus positive cases. Several other studies also suggested that G1P[8] genotype to be predominant genotype of group A rotavirus worldwide [9]. Taken together, our study suggests the region-specific emergence of rotavirus strains and it is important to develop vaccine strategies. The spectrum of enteropathogenic bacteria detected during this study, shows that EPEC, EAEC, EIEC, ETEC, EHEC, Vibrio cholerae, Vibrio sp., Shigella sp., and C. jejuni were 17.88%, 9.71%, 0.44%, 4.63%, 0.66%, 7.50%, 3.09%, 5.96% and 1.32%, respectively. In 7.06% cases, target enteropathogenic bacteria were not detected. We have also found EPEC to be prevalent of 49.38% (40/81) among children <6 months of age and EAEC was prevalent at 59.09% (26/44) among children 6-12 months of age. Similar results were observed in the previous study [34]. Thus, indicating a marked age group for DECs infection among children (<5 years) in this area. The demonstrating role of different pathotypes of diarrheagenic E. coli in diarrheal diseases had also been reported elsewhere [12]. It may be due to decline protection by maternal antibodies, changing food habits [35]. The younger children are usually less infected by DECs pathotypes due to their natural acquired immunity [36]. The DECs detection rates have been reported to correlate with the seasons [37]. We observed that the seasonal spectrum of etiological agents causing diarrhea among children (<5 years) is similar to other study [37]. In our study, we have found that during the winter season (Nov 2014-February 2014) and in early summer (March 2014-April 2014) occurrence of rotavirus infection was 52.87% and 33.45% respectively. However, early summer occurrence rate was high in the previous study [38]. In the summer EPEC, EAEC, ETEC, Shigella sp. and C. jejuni were prevalent by 77.77%, 86.36%, 90.47% respectively and Shigella sp. and C. jejuni both were prevalent by 100%. Whereas, Vibrio cholerae and Vibrio sp. prevalent by 88.23% and 85.71% in the rainy season [11,39]. A higher prevalence of diarrheagenic E. coli was detected during summer whereas, Vibrio cholerae and Vibrio sp. were prevalent during the rainy season (p<0.05). The prevalence of Vibrio sp. in rainy

Discussion

In this study, we investigated the burden of rotavirus and other enteropathogens among children (<5 years of age) admitted with severe diarrheal symptoms. We observed 40.53% (197/486) children were admitted with severe diarrheal symptoms (AGE with vomiting or AGE with some dehydration) out of which 16.99% children were <12 years of age. This severity may be primarily due to lack of proper parental care, poor maternal diet, malnutrition, lack of health education, poor socioeconomic status and irregular breast-feeding [25]. We have observed that children less than 24 months of age were susceptible to rotaviral diarrhea, comprising 48.14% of all diarrheal cases and 88.30% of all positive rotavirus cases (P<0.05). However, this prevalence rate was lower in other countries [26]. This difference is due to much-influencing factors including geo-demographic variations, the measure was taken during sample collections, and most importantly socioeconomic status and mother-child nutrition [27]. We have observed that group A rotavirus was prevalent (54.52%, 265/486) among all diarrheal cases. Furthermore, enteropathogenic bacteria was prevalent (48.35%) in children (<5 years) with diarrheal symptoms. Thus, this study confirming group A rotavirus as a main etiological agent in pediatric diarrhea [28]. However, the prevalence of enteropathogenic bacteria was low in the previous study [29]. Several lines of documents have suggested that the prevalence of diarrhea is strongly associated with sanitation [30]. The isolated bacteria are indicators of fecal contamination, indicating low hygiene in the intake of foods [31,32]. Moreover, young children those were taking health care services at MMC&H, primarily belonged to the low socioeconomic group and had a poor living standard, which is the main reason for this high prevalence of diarrheagenic bacteria [33]. In our study, G1P[8] (85.0%) was most prevalent genotype followed by G9[P4] (5%), G2[P4] (3%), G2[P6] (2%), others variants were detected only 1% among the rotavirus positive cases. Several other studies also suggested that G1P[8] genotype to be predominant genotype of group A rotavirus worldwide [9]. Taken together, our study suggests the region-specific emergence of rotavirus strains and it is important to develop vaccine strategies. The spectrum of enteropathogenic bacteria detected during this study, shows that EPEC, EAEC, EIEC, ETEC, EHEC, Vibrio cholerae, Vibrio sp., Shigella sp., and C. jejuni were 17.88%, 9.71%, 0.44%, 4.63%, 0.66%, 7.50%, 3.09%, 5.96% and 1.32%, respectively. In 7.06% cases, target enteropathogenic bacteria were not detected. We have also found EPEC to be prevalent of 49.38% (40/81) among children <6 months of age and EAEC was prevalent at 59.09% (26/44) among children 6-12 months of age. Similar results were obtained in the previous study [34]. Thus, indicating a marked age group for DECs infection among children (<5 years) in this area. The demonstrating role of different pathotypes of diarrheagenic E. coli in diarrheal diseases had also been reported elsewhere [12]. It may be due to decline protection by maternal antibodies, changing food habits [35]. The younger children are usually less infected by DECs pathotypes due to their natural acquired immunity [36]. The DECs detection rates have been reported to correlate with the seasons [37]. We observed that the seasonal spectrum of etiological agents causing diarrhea among children (<5 years) is similar to other study [37]. In our study, we have found that during the winter season (Nov 2014-February 2014) and in early summer (March 2014-April 2014) occurrence of rotavirus infection was 52.87% and 33.45% respectively. However, early summer occurrence rate was high in the previous study [38]. In the summer EPEC, EAEC, ETEC, Shigella sp. and C. jejuni were prevalent by 77.77%, 86.36%, 90.47% respectively and Shigella sp. and C. jejuni both were prevalent by 100%. Whereas, Vibrio cholerae and Vibrio sp. prevalent by 88.23% and 85.71% in the rainy season [11,39]. A higher prevalence of diarrheagenic E. coli was detected during summer whereas, Vibrio cholerae and Vibrio sp. were prevalent during the rainy season (p<0.05). The prevalence of Vibrio sp. in rainy
seasons may be due the quality of the water sources being compromised with surface runoff [18]. In this study, we observed 31.89% (155/486) of children were infected with more than one causative pathogen of diarrhea. This higher prevalence of mixed infections may be due to one single causative agent that is facilitating other pathogens to challenge host immune system and vice versa. The rotavirus and diarrheagenic Escherichia coli (DEC) co-infection was 26.54% (129/486) prevalent out of which EPEC + EAEC alone was 21.76% (all positive rotavirus cases) and 13.46% (all diarrheal cases). EAEC+ETEC and Vibrio cholerae were prevalent by 7.85% and 4.85%, respectively (all diarrheal cases). Co-infections among different DECs pathotypes were not detected significantly (p>0.05). Only seven cases (1.54%) EPEC-EAEC and one case (0.24%) of ETEC-EHEC co-infection were detected in this study. However, eleven cases (2.42%) EPEC-Vibrio cholerae and four cases (0.88%) of Vibrio cholerae and Vibrio sp. and one instance of EAEC and Shigela sp. (0.22%) were detected in this study. These findings were found similar with a prior report [40-44]. We observed that the antimicrobial therapy had not been initiated before admission in most of the cases. We have observed highest resistance for CTX (80.24%) in EPEC, (72.72%) in EAEC, VC was found with highest resistance in CTR (82.35%) and 73.52% for AMP, which is the most commonly prescribed antimicrobial treatment for the children admitted to health care center. However, the resistance rates to the newer generation antimicrobials (Amikacin) as detected in our study were still low. Enteropathogens were found sensitive for Amikacin and Chloramphenicol ranging from 83.33 to 100%. However, the use of Chloramphenicol is not recommended in younger children [45-47].

Conclusion

Vaccination against particular disease directly reduces annual health care cost and ensures better public healthcare services of any nation. However, it is quite tough job for scientist to formulate a common vaccine against disease that is caused by diverse variety of serotypes. In India childhood diarrhoea due to rotavirus and diarrheagenic E. coli cause huge loss of per-capita income. The reduction of this great loss of nation’s economy can only be possible by the formulation of an effective cocktail vaccine for childhood diarrhoea, specifically against rotavirus. Presently no such vaccine available in the market for childhood diarrhoea and the vaccine available for rotavirus is with poor efficacy rate due to diversity of circulating serotypes.

Our study has identified the emergence of G1P[8] rotavirus strain and EPEC in rural areas of West Bengal in particular. It is an important due for the development of an effective cocktail vaccine or region-specific rota-vaccine. Our study also suggests particularly 6-12 year age group of children are vulnerable to DECs and rotavirus infections therefore, we recommend specific preventive measures to control DECs, specifically in the summer season and during winter seasons rotavirus prevention of rotaviral infection and oversight should be emphasized.

Acknowledgement

Indian Council of Medical Research (ICMR), Govt. of India (Project No. 5/8-1 (189)/TF/2011-12 ECD Dated 24.01.2013/05.02.13) is sincerely acknowledged by authors.

Ethical Consideration

The ethical committee at Vidyasagar University approve the study.

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