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### Emerging Trends in the Etiology and Antimicrobial Susceptibility Pattern of Enteric Pathogens in North India

Rumpa Saha, Priyamvada Roy\*, Shukla Das and Iqbal Rajinder Kaur

University College of Medical Sciences & Guru Teg Bahadur Hospital, Dilshad Garden; Delhi-110095; India

\*Corresponding author

#### KEYWORDS

Antimicrobial susceptibility pattern, Bacteria, Gastroenteritis, North India, Parasites.

#### A B S T R A C T

Gastroenteritis is serious public health problem in India. A 15-year retrospective study was conducted to analyze etiological agents of gastroenteritis and to establish antimicrobial resistance pattern of bacterial pathogens. Fecal samples from patients with diarrhoea were received prior to antibiotic administration and processed for identification of various pathogens. Out of 10,446 stool samples collected during study period, 1990 (19.05%) and 538 (5.15%) yielded bacterial pathogens and parasites respectively. The most frequently isolated parasite and bacterial pathogen were *Giardia lamblia* and *Vibrio cholerae* respectively. Majority of enteric pathogens were isolated from the children. Bacteria isolated showed resistance to commonly used antimicrobials.

### Introduction

Global, regional and national estimates place diarrhoeal diseases as a major neglected public health problem. In the Southeast Asian region, almost 48% of the estimated 3.07 million deaths annually are ascribed to diarrhoeal diseases along with acute respiratory infections with the maximum burden in 5 countries: Bangladesh, India, Indonesia, Myanmar and Nepal where these diseases are responsible for 60,000 deaths

annually (Ghimire, 2008). Knowledge of the wide variety of bacteria, viruses and parasites as etiological agents contributing to its epidemiology and changing trends of gastroenteritis is crucial. Arbitrary use of antimicrobial agents has led to the development of multidrug resistant organisms (Deen *et al.*, 2008). Precise understanding of the etiological agent of diarrhoea in a given situation is a gruelling

task that requires systematic monitoring of the various pathogens. We thus analyzed 15 years data to demonstrate the etiological spectrum of diarrhoea and to determine the antimicrobial resistance trend of bacterial pathogens during these years.

### Materials and Methods

This 15-year study (2001-2015) included all patients of gastroenteritis attending an 1800-bed tertiary care hospital in Delhi. A total of 10,446 stool specimens were processed for identification of various pathogens. Routine stool microscopy of saline and iodine preparations was examined for intestinal parasites following the standard formol ether method of concentration (Garcia, 2001). Smears stained with modified acid fast stain from each specimen were examined for Coccidian parasites. A battery of culture media – alkaline peptone water, selenite F broth, Mac Conkey agar, bile salt agar, and xylose lysine deoxycholate agar were used for isolation of bacterial pathogens following standard laboratory techniques (Garcia, 2001). *Salmonella* sp., *Shigella* sp. and *Vibrio cholerae* isolates were serotyped by slide agglutination test using specific antisera. The antimicrobial susceptibility of all isolates was assessed as per NCCLS/CLSI guidelines.

### Results and Discussion

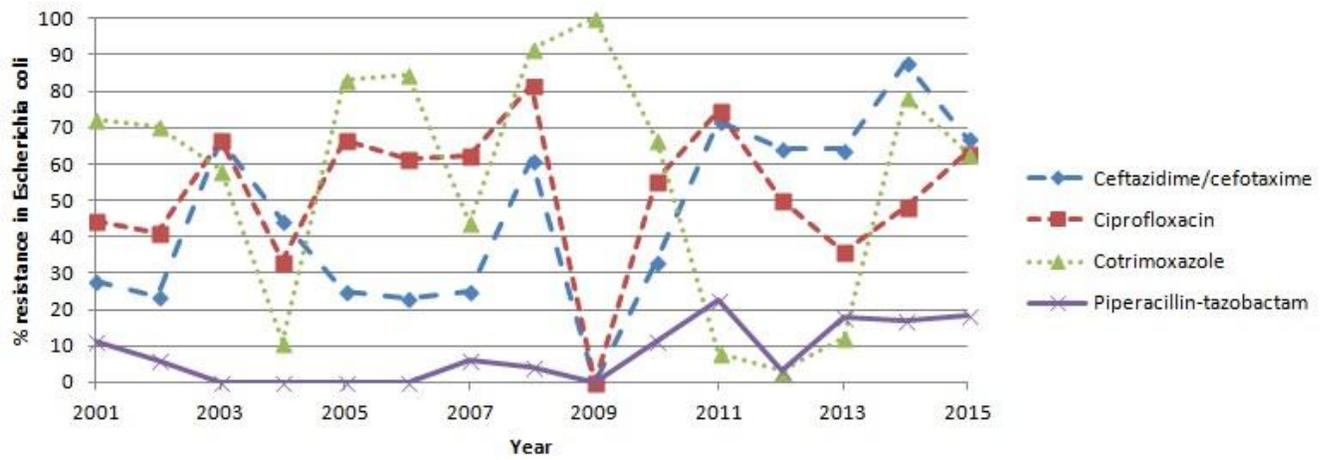
From the 10,446 fecal specimens received, 1990 (19.05%) yielded bacterial pathogens. There was no significant change in overall number of bacterial pathogens year-wise, however bacteria were present in significantly more numbers in children ( $\leq 12$  year) than in adults ( $P=0.000$ ) similar to reports from Costa Rica (Pérez *et al.*, 2010). This is mainly due to high prevalence of diarrheagenic *Escherichia coli* in children,

Overall, 538 (5.15%) showed presence of parasites, with significant change in number of cases seen year-wise ( $P=0.038$ ), but there was no significant difference in no. of cases between adults and children. However, on considering bacteria and parasites together, significantly more numbers were also present in children. The most frequently isolated parasite (1.30%) was *Giardia lamblia*, followed by *Ascaris lumbricoides* (0.09%). Overall the most frequently identified enteric bacterial pathogen was *Vibrio cholerae* (53.92%), followed by *Escherichia coli* (38.24%). *Shigella* sp. was isolated from stool of 0.85% cases, with *Shigella flexneri* type II being the commonest (83.15%), comparable to other reports from India (Taneja and Mewara, 2016). Among the nontyphoidal *Salmonella* sp., 17 cases of *Salmonella typhimurium* were isolated, besides 1 case of *Salmonella cholerasuis*.

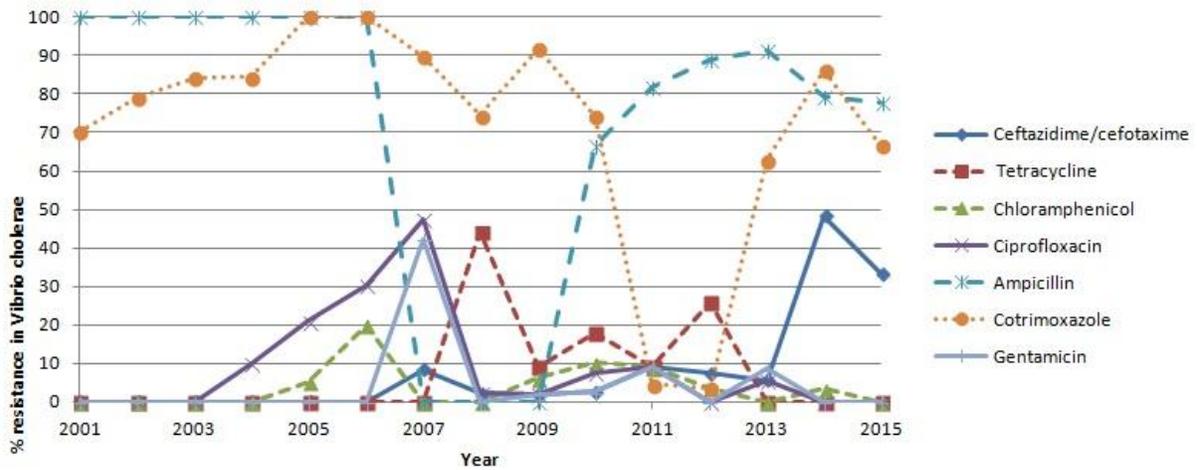
The entire 15-year period was divided into 3 periods of 5-year: 2001-2005, 2006-2010, 2011-2015, for the ease of analyzing the antibiotic resistance pattern. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 13.0 (SPSS Inc., Chicago, USA). Statistical significance was defined as  $P < 0.05$ .

In case of *E.coli* significant change in resistance to ciprofloxacin was encountered with a sudden rise followed by a sudden fall ( $P=0.017$ ) as also indicated by nalidixic acid ( $P=0.000$ ). Similar changes were also seen for gentamicin ( $P=0.017$ ) and cotrimoxazole ( $P=0.000$ ). However specific falling trend was seen only with cotrimoxazole and ciprofloxacin (Figure 1). Taneja *et al* have also reported low resistance levels of *E. coli* strains against nalidixic acid and cotrimoxazole (Taneja *et al.*, 2006).

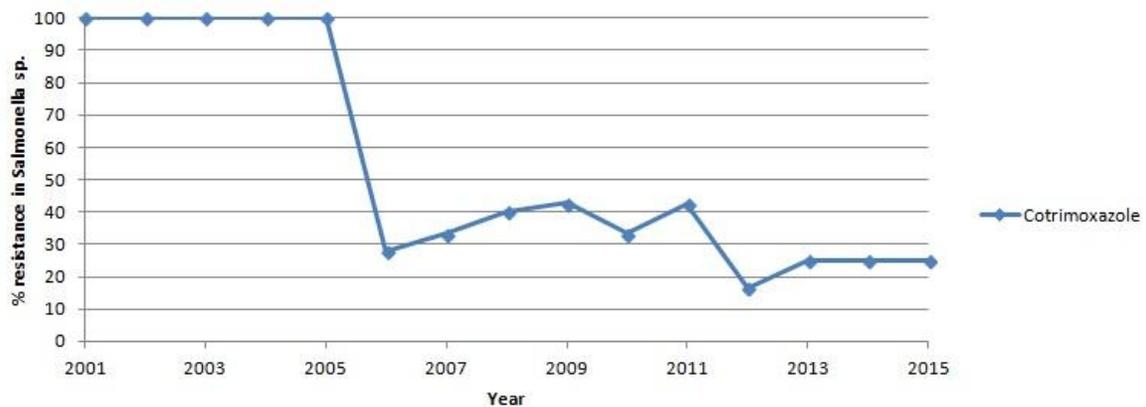
**Fig.1** Antimicrobial resistance pattern of *Escherichia coli* from 2001 to 2015



**Fig.2** Antimicrobial resistance pattern of *Vibrio cholerae* from 2001 to 2015



**Fig.3** Antimicrobial resistance pattern of *Salmonella* sp. from 2001 to 2015



In contrast, Sudershan *et al.*, have found high levels of resistance to cotrimoxazole in case of *E. coli* (Sudershan *et al.*, 2014). Resistance to ceftazidime / cefotaxime as well as piperacillin-tazobactam rose significantly over the years (P=0.000) and displayed an overall rising trend (Figure 1), as also observed by Sudershan *et al* from Hyderabad (Sudershan *et al.*, 2014).

The periodic outbreaks of Cholera in our area illustrate the endemicity of this disease and its appearance as a significant pathogen of acute watery diarrhea in our country.

On analyzing antibiotic resistance to *Vibrio cholerae*, though chloramphenicol, ciprofloxacin and tetracycline resistance markedly increased initially followed by a strikingly significant decrease (P=0.000), an overall rising trend was observed (Figure 2). Similarly ceftazidime/ cefotaxime and gentamicin also displayed a rising trend (P=0.000). Ampicillin and Cotrimoxazole demonstrated an overall a falling (P=0.000) trend (Figure 2). The quick shift in resistance to various antibiotics, as reported in this study, indicate an increased mobility in genetic elements leading to resistance to *Vibrio cholerae* strains. This makes monitoring resistance pattern a crucial need.

*Shigella* sp. displayed a statistically significant change in resistance to ampicillin, ciprofloxacin and cotrimoxazole (P=0.000 in all 3 antibiotics), with a rising resistance to ampicillin and ciprofloxacin, and a falling resistance to cotrimoxazole. Studies have documented high resistance in *Shigella* sp. to ampicillin, cotrimoxazole and fluoroquinolones (Ballal *et al.*, 2014; Mamatha *et al.*, 2007). *Salmonella* sp. demonstrated a statistically significant rise in resistance to ampicillin and ciprofloxacin while a significant falling resistant trend was noticed for cotrimoxazole (P=0.000 in

ampicillin and cotrimoxazole and P= 0.008 in ciprofloxacin) (Figure 3). Fairly high degree of resistance to ampicillin and cotrimoxazole in *Shigella* sp. and *Salmonella* sp. was demonstrated by Ashtiani *et al.*, (2009).

To conclude, this study highlights the high rates of antibiotic resistance to commonly used antimicrobial agents which is of clinical significance. Routine surveillance of drug resistance in enteric pathogens is necessary to detect resistance trends so that an appropriate agent can be chosen for therapy. This would aid in proper formulation of public health guidelines. This will also help in identifying outbreaks of resistant organisms and the data obtained can help in advocating suitable antibiotic regimens.

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