Research Article

Occurrence and antibiogram of enteric bacterial isolates from stool samples of gastroenteritis children under 5 years of age in district Faisalabad, Pakistan

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Abstract

Acute gastroenteritis is accompanied by severe bloody diarrhea, hemolytic uremic syndrome and acute renal impairment. It is one of leading cause of morbidity and mortality in children, worldwide. Annually, acute gastroenteritis is associated with 2 million deaths in children under 5 years of age. The present study aimed to determine the occurrence and antibiotic susceptibility profiling of enteric bacterial pathogens isolated from stool samples of gastroenteritis patients. A total of 200 stool samples were collected from DHQ hospital Faisalabad followed by enrichment in Selenite F and nutrient broth media. Post 24 hours incubation at 37°C, cultures were streaked on various selective and differential media. Bacterial isolates were first identified by morphological and microscopic examination and then confirmed by biochemical tests. Isolated bacteria were tested for antibiotic sensitivity by Kirby's Bauer disc diffusion method. Our results indicated the presence of three major bacterial isolates with *Escherichia coli* (*E. coli*) showing highest prevalence rate (78%), followed by *Salmonella Typhi* (55%) and *Shigella dysenteriae* (36%). *E. coli* was most sensitive (80%) to enrofloxacin and least sensitive to ciprofloxacin (10%). *S. Typhi* isolates were also most sensitive to enrofloxacin (80%), while most resistant to cefotaxime (90%). About 95% of *Shigella dysenteriae* isolates were resistant to amoxicillin (95%), while highest sensitivity (80%) was found against ofloxacin. *E.coli* was the most prevalent bacteria in enteritis patients. Results of antibiotic sensitivity suggest combination antibiotic therapy (enrofloxacin+ ofloxacin) should be prescribed to treat enteritis infections in children. These findings will be helpful in diagnosing and determining the course of antibiotic therapy to treat children with gastroenteritis.

Keywords: Antibiotic sensitivity; children; enteric bacterial pathogens; gastroenteritis

Introduction

Gastroenteritis is the inflammation of the gastrointestinal tract and its clinical findings include high body temperature (more than 40°C/ 107°F), severe abdominal pain, nausea, vomiting and bloody stools along

with respiratory symptoms [1]. Diarrhea along with rigorous malnutrition produces high danger of complications [2]. Gastroenteritis is caused by many pathogenic microorganisms and is associated with several risk factors. Children with age of five

years are more susceptible to the disease on average. The incidence of death due to diarrheal cases is 70, 0000 per year [3]. According to WHO (World Health Organization) and UNICEF (United Nations International Children's Emergency Fund), in developing countries, annually 2 billion morbidity cases occur due to diarrhea and 1.9 million death occurs in the children with the age under five years old [4]. Pakistan is the fourth one country which is suffering from the major burden of gastroenteritis. In Pakistan diarrhea is a major cause of death in children and one-third population of children is hospitalized due to gastroenteritis [5]. There are a number of infectious microorganisms which are involved in gastroenteritis problems in children. *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter*, *rotavirus*, *Entamoeba histolytica* and *Giardia intestinalis* are the major causative agents of gastroenteritis in children [6]. *Escherichia coli* (*E. coli*) is a gram-negative, rod shaped, motile and lactose fermenter pathogenic bacteria. It belongs to Enterobacteriaceae family and lives as normal microflora of ruminant animals like sheep, cows and human [7]. *Salmonella Typhi* (*S. Typhi*) is a gram-negative, rod shaped, motile and non-lactose fermenting pathogenic bacteria belongs to Enterobacteriaceae family. It is one of the major causes of gastroenteritis in children and primarily transmitted through fecal and oral route [8]. *Shigella* is gram-negative, rod shape, facultative anaerobic, non-motile, non-lactose fermenting pathogenic bacteria and is closely related to *Salmonella*. There are four different groups of *Shigella* i.e. Serogroup A, B, C, and D including *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei* respectively [9]. The present study was aimed to determine the prevalence of major causative agents (*E. coli*, *Salmonella Typhi* and *Shigella dysenteriae*) of gastroenteritis in children and to investigate

antibiotic susceptibility profile of isolated bacteria from stool samples.

Materials and methods

The study was carried out in compliance with rules of local Institutional Bioethics Committee (IBC), University of Agriculture Faisalabad, Pakistan. A written consent was taken from each patient. A total of 200 stool samples were collected in sterilized bottles from pediatric ward of DHQ hospital Faisalabad. Samples were enriched in Selenite F broth and nutrient broth. After 24 hrs incubation at 37°C, a loop full culture from turbid broths was inoculated on *Salmonella Shigella* (SS) and MacConkey agar media. After overnight incubation at 37°C, the pure cultures were further processed for morphological and biochemical characterization [10, 11]. Biochemical confirmation of all presumptive isolates was done using Remel RapID one kit (Oxoid, UK) as per manufacturer's instruction. The color pattern observed in panel were compared to the shade card and results were entered in the Remel RapID ONE software for the identification.

Antibiogram of all three types of bacteria (*E. coli*, *S. Typhi*, *S. dysenteriae*) isolated from stool samples was performed by Kirby-Bauer disc diffusion method. Following antibiotics were tested for antimicrobial sensitivity; amikacin (AK) 30µg, amoxycillin (AMP) 25µg, ciprofloxacin (CIP) 30µg, cefotaxime (CTX) 30µg, doxycycline (DO) 30µg, D (DA) 2µg, enrofloxacin (ENR) 30µg, sulphamethoxazole/trimethoprim (SXT) 25µg, piperacillin/tazobactam (TZP) 110µg, and ofloxacin (OFX) 5µg. Bacterial lawn was prepared for each isolate with standardized 0.5 McFarland overnight fresh bacterial suspension on Muller Hinton agar plates. All antibiotic discs were placed at equal distance. After 24 hrs incubation at 37°C, zones of inhibition around antibiotics discs were measured and compared to Clinical

Laboratory Standards Institute (CLSI) manual [12].

Results

Results of Prevalence for bacterial isolates in stool sample

Isolates were first characterized on the basis of morphology and microscopy. Presumptive isolates were further confirmed by Remel RapID ONE kit. Based on the results of morphological, microscopic and biochemical tests, three major types of bacterial isolates were identified in stool samples of children with gastroenteritis. These includes *E. coli*, *S. Typhi* and *S. dysenteriae*. Among 200 children's stool samples collected for bacterial isolation, 156 stool samples were positive for *E. coli*, 110 were positive for *S. dysenteriae* and 73 stool samples were positive for *S. Typhi*. Highest rate of prevalence was found for *E. coli*, while *S. Typhi* was least prevalent (78%), followed by *S. dysenteriae* (55%) and *S. Typhi* (36%) as shown in (Fig. 1).

Cultural, Microscopic and biochemical characteristics of all bacterial isolates recovered from the stool samples

On MacConkey agar media, *E.coli* gave lactose fermenting bright pink colored colonies. *S. Typhi* gave smooth, transparent colonies (2-4mm) with the black center,

while *S. dysenteriae* colonies appeared as smooth, circular and colorless on SS agar media. Under microscope, *E. coli* appeared as Gram negative pink rods, while both *S. Typhi* and *S. dysenteriae* appeared as Gram negative straight rods. Remel RapID ONE kit, used for biochemical tests confirmed all the positive isolates as *E. coli*, *S. Typhi* and *S. dysenteriae*.

Results of antibiotic sensitivity test for *E. coli*, *S. dysenteriae* and *S. Typhi* isolates

In (Fig. 2) showed results of antibiotic sensitivity and resistance of *E. coli*, *S. dysenteriae*, and *S. Typhi* isolates to various antibiotics used in the test. All bacterial isolates showed differential pattern in sensitivity and resistance towards various antibiotics. It was found that *E. coli* isolates were most sensitive (80%) to enrofloxacin followed by amikacin (75%) and least sensitive for ciprofloxacin (10%) followed by tazobactam (30%) (Fig. 3). *S. Typhi* isolates were also found most sensitive for enrofloxacin (80%) and resistant to cefotaxime (90%) (Fig. 4). All the isolates of *S. dysenteriae* showed resistance to amoxycillin (95%) followed by doxycycline (90%), sulphamethoxazole/trimethoprim (90%) and cefotaxime (80%) (Fig. 5).

Figure 1. Prevalence of different bacterial isolates in stool samples. Highest prevalence in stool samples of children with gastroenteritis was of *E. coli*

Figure 2. Representing the antibiotic sensitivity pattern (sensitive, intermediate sensitive, resistant) of *E. coli*, *S. dysenteriae* and *S. Typhi* isolated from gastroenteritis infected child's stool sample

Figure 3. Antibiotic sensitivity of isolated *Escherichia coli* isolated from child's stool sample. ENR was found highly sensitive followed by AK and OFX

Figure 4. Antibiotic sensitivity/resistance pattern of *S. Typhi* towards 9 different antibiotics. Intermediate resistance was shown to CIP, while highest resistance was observed against both DO and CTX

Figure 5. Antibiogram of *S. dysenteriae* isolated from child's stool sample

Discussion

Gastroenteritis is a major cause of infant mortality in developing countries and is caused by many pathogenic microorganisms along with several risk factors. Risk factors included are; age of children, immune status, travelling, exposure to sick person and socioeconomic status (family structure,

sanitation conditions, provision of clean food and water) etc. [13]. The present study was conducted to determine prevalence and antibiotic susceptibility profiling of enteric pathogens isolated from stool samples collected from gastroenteritis cases. The prevalence results showed that out of total 200 stool samples, *E. coli* was found to be

most prevalent (78%) and *S. Typhi* was the least (36%). Similar work was also reported by others [14]. In Korea, a study by Kim *et al.* [15] was conducted to determine prevalence of enteropathogens from stool samples and prevalence results reported were as; *E. coli* (22.0%), *S. Typhi* (13.5%) and *S. dysenteriae* (0.37%). In Nigeria, gastroenteritis suffering patients showed 11.1% prevalence for *E. coli* followed by *S. dysenteriae* (11.4%) and *S. Typhi* (9.2%) [16]. In Tehran, the prevalence of *E. coli* reported was (38.8%), *S. dysenteriae* (45%) and *S. Typhi* (13.8%) [17]. A study conducted by Scallan *et al.* [18] reported contradictory results that *Salmonella* was found to be prevalent (42%), followed by *Shigella* (28%) and *E. coli* O₁₅₇ (3%). In present study, the low prevalence of *S. Typhi* may be due to proper usage of antibiotics and hygienic conditions against this bacterium.

The antibiotic sensitivity pattern results of present study indicated that *E. coli* isolates were the most sensitive (80%) to ENR, and least sensitive to CIP (10%). While on the other hand, the least prevalent *S. Typhi* showed resistance to CTX (90%) and highest sensitivity to ENR (80%). *S. dysenteriae* was highly sensitive against OFX (80%) followed by CIP (75%), AK (70%), TZP (70%), and ENR (70%). It was resistant against AMP (95%), followed by CTX (80%) and SXT (80%). In another study, *Salmonella* isolates were found resistance against sulfisoxazole (55.0 %) followed by ampicillin (46.8 %), streptomycin (44.0 %), tetracycline (29.4 %) and chloramphenicol (16.5 %) [19]. The resistance of *Shigella* to almost all antibiotics was much more obvious than *Salmonella* except ciprofloxacin (CIP). Moderate resistance to tetracycline (45.9 %) and cefotaxime (29.7 %) was also observed; however, resistance to chloramphenicol and ciprofloxacin was relatively low (16.2 and 2.7 %, respectively) [20]. In Beijing, *Salmonella* and *Shigella* showed resistance to

ciprofloxacin isolated from diarrheal children 9.3% and 29.7%, respectively [21]. *Salmonella* and *Shigella* isolates showed resistance rates to CIP 9.2% and 2.7% at Shanghai and Anhui in China [22]. In Azad Kashmir, 48.6% *E. coli* isolates were resistant to carbenicillin (48.6%) followed by 47.8% to ampicillin, 42.1% to cefotaxime 39.6% to co-trimoxazole, 36.2% to streptomycin, 34.0% to amoxicillin, 33.3% to amikacin, 31.2% to tetracycline, 28.5% to erythromycin, 27.9% to nalidixic acid, 25.5% to chloramphenicol, 25.4% to gentamicin, 21.5% to cefotaxime and 1.2 to ciprofloxacin. But all these *E. coli* isolates were sensitive to cefotaxime [23].

Conclusion

Our study emphasizes the need to monitor the prevalence of enteric pathogens in children and to determine antibiotic susceptibility testing for effective treatment. The practice of ineffective antibiotics therapy is going to lead the increase in resistance of pathogens against antibiotics. Since antimicrobial resistance patterns are constantly evolving and it is a present global public health problem, there is the necessity for constant antimicrobial resistance surveillance.

Authors' contributions

Conceived and designed the experiments: F Masood, Performed the experiments: FR Anjum & RM Abdullah, Analyzed the data: S Anam, Contributed materials/ analysis/ tools: S Anam, Wrote the paper: S Anam.

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