

Antibiotic sensitivity pattern of diarrhoeal pathogens in under five children

MK Pervin¹, ST Jhora², S Paul³, A Naher⁴

Abstract

Background: Different bacterial enteropathogens are responsible for diarrhoea in children less than 5 years of age in Bangladesh. This study aimed to determine the antimicrobial sensitivity pattern of diarrhoeal pathogens that is necessary to know for the specific management of acute bacterial diarrhoea.

Objectives: To find out the bacterial enteropathogens causing diarrhoea and their antimicrobial sensitivity pattern in under 5 children.

Methodology: This was an observational cross sectional study carried out at Microbiology Department of Sir Salimullah Medical College, Dhaka. A total of 217 diarrhoeal patients 0-5 years age, attending Pediatric unit of Sir Salimullah Medical College and Mitford Hospital, Dhaka and Dhaka Shishu Hospital, Dhaka during the period of July, 2015 to June, 2016, were enrolled in the study. For isolation of the common enteric bacteria, diarrhoeal stools were cultured on MacConkey agar media and for *Campylobacter* spp, diarrhoeal stools were cultured on selective Modified Campy BAP media and nonselective Blood agar media with membrane filter. Positive culture was confirmed by Gram stain morphology and standard biochemical tests. Antimicrobial susceptibility test were used to identify the bacterial sensitivity pattern in the isolates.

Results: Out of 217 diarrhoeal stools, bacteria was isolated from 97 (44.70%) patients by culture. Among 97 culture positive cases *Escherichia coli* (51-52.58%), *Campylobacter* spp. (23-23.71%), *Shigella* spp. (15-15.46%) and *Salmonella* spp. (8-8.25%) were isolated. Mecillinam is the most sensitive drug for *Escherichia coli*, *Campylobacter* spp., *Shigella* spp. and *Salmonella* spp. Among the other antimicrobials, *Campylobacter* spp. were sensitive to azithromycin, erythromycin and nitrofurantoin. *Escherichia coli* were sensitive to nitrofurantoin, gentamicin and azithromycin. *Shigella* spp. were sensitive to ceftriaxone, ciprofloxacin and nitrofurantoin and *Salmonella* spp. were sensitive to ceftriaxone, cotrimoxazole and gentamicin.

Conclusion: Mecillinam and nitrofurantoin were highly effective against bacteria causing diarrhoea in children.

Key words: Diarrhoea, Bacteria, Culture, Sensitivity.

Introduction

Acute infectious diarrhoea is one of the leading causes of morbidity and mortality among infants and children in developing countries of the world. Diarrhoea remains the second leading cause of death and causes 1.3 million deaths every year in children under 5 years of age.¹

Diarrhoea defined as excessive and frequent evacuation of watery faeces usually indicating gastrointestinal disease or disorder of 3-7 days

duration.² In developing countries nearly 1.4 billion episodes of diarrhoea occur annually in children below 5 years, of which 123.6 million episodes required outpatient medical care and 9 million needed hospitalizations.³ In Bangladesh one in fifteen children die before their fifth birth day and among them diarrhoea causes 7% of death.⁴

Aetiology of diarrhoea is variable according to geographic and climate conditions, host factors

1. Mst Khashruba Pervin M Phil, Lecturer, Department of Com. Med, Khulna Medical College (Email: drsurovimicrobiology@gmail.com)

2. Sanya Tahmina Jhora M Phil, Professor and Ex Head, Department of Microbiology, Sir Salimullah Medical College, Dhaka

3. Shikha Paul M Phil, Professor and Head, Department of Microbiology, Sir Salimullah Medical College, Dhaka

4. Akhtarun Naher M Phil, Professor and Ex Head, Dept. of Microbiology, Sir Salimullah Medical College, Dhaka

and socioeconomic situations. A wide range of bacteria, virus and parasites causes infectious diarrhoea.⁵ Among the bacterial causes, *Escherichia coli* is most common pathogen for childhood diarrhoea in developing countries and now being an emerging antimicrobial resistant enteropathogen in developed countries.⁶ In Bangladesh, about 41-46% acute diarrhoea is caused by *Escherichia coli*.⁷ Worldwide *Campylobacter* spp. is one of a major cause of acute bacterial gastroenteritis among infants and children.⁸ In Bangladesh isolation rate of *Campylobacter* within 5 years of age is 11% to 17.4%.⁹ Shigellosis is endemic in Bangladesh and it is estimated that 2,00,000 to 3,00,000 cases of shigellosis are found per year.¹⁰ For earlier diagnosis and specific treatment for diarrhoea, it is very important to know the exact pathogen and its antimicrobial sensitivity pattern. But currently antibiotic resistance has been increasing and is a major threat to public health. The study was designed to find out bacteria and its antimicrobial sensitivity pattern from children suffering from diarrhoea under 5 years of age.

Material and methods

This was an observational cross sectional study. The study protocol was approved by Ethical Review Committee of Sir Salimullah Medical College and Mitford Hospital, Dhaka. The study was conducted from July, 2015 - June, 2016 in Microbiological laboratory of Sir Salimullah Medical College, Dhaka.

During this period a total of 217 stool samples were collected from acute diarrhoeal children upto 5 years of age in Pediatric unit of Sir Salimullah Medical College and Mitford Hospital and Dhaka Shishu Hospital, Dhaka. Stools were collected in a clean dry, wide mouth and leak proof container and brought to the Microbiological laboratory, of Sir Salimullah Medical College, Dhaka within 2 hours. The selected cases were the children upto 5 years of age passing watery or loose stools three or more times in 24 hours with/without mucus and or blood, vomiting, fever abdominal pain. Children on antibiotic or completed antibiotic treatment within last 15 days were excluded.

Stools were examined microscopically in wet film preparation for detection of pus cells. Then all samples were plated on MacConkey agar media and incubated at 37°C for 24 hours in aerobic condition for isolation of enteric bacteria. For isolation of *Campylobacter* spp. stool samples were inoculated in Selective Modified Campy BAP media (Brucella agar base with 10% defibrinated

sheep blood and 3 antimicrobials Polymixin B 2.5 IU/ml, Amphotericin B 2.0mg/liter and Vancomycin 10 mg/liter) and Non selective Blood agar media with membrane filter (0.45 µm). A cellulose triacetate membrane with 0.45 µm pore and 47 nm diameter were placed on surface of Blood agar plate before inoculation. After inoculation media were incubated in candle jar at 42°C for 48 hours. Suspected colonies were identified by colony character, Gram stain and standard biochemical methods. Susceptibility to antimicrobial agents was done by Kirby Bauer modified disk diffusion technique using Mueller Hinton agar with 5% sheep blood was used for *Campylobacter* spp. Mueller Hinton agar was used for other enteric bacteria.

The data of the patients were collected on a structured questionnaire. All data were compiled and analyzed using Microsoft excels (2007) and presented in table to describe it in percentage. As the values were small, comparison between them could not be done.

Results

During the study period from July, 2015 to June, 2016 a total of 217 stool samples were collected from under 5 years children with diarrhoeal disease.

Table-1 shows isolation of bacteria from diarrhoeal stools. Of the 217 samples, bacteria were isolated in 97(44.70%) samples. Among the culture positive 97 samples, *Escherichia coli* were isolated in 51(52.58%) sample, *Campylobacter* spp. were isolated in 23(23.71%) samples, *Shigella* spp. were isolated in 15(15.46%) samples and *Salmonella* spp. were isolated in 8(8.25%) samples. No bacteria were isolated in 120(55.30%) samples.

Table I

Isolation of bacteria from diarrheal stools by culture (n=217)

Name of isolated bacteria	No. of cases (%)
Culture positive cases	97(44.70)
<i>Escherichia coli</i>	51(52.58)
<i>Campylobacter</i> spp.	23 (23.71)
<i>Shigella</i> spp.	15(15.46)
<i>Salmonella</i> spp.	08(8.25)
Culture negative cases	120(55.30)
Total	217(100)

Table II shows sensitivity pattern *Escherichia coli* to different antimicrobials by disc diffusion method. Among these tested antimicrobials, E

coli were highly susceptible (100%) to mecillinam followed by nitrofurantoin (84.32%), gentamicin (88.24%) and cotrimoxazole (76.47%). Moderately sensitive to azithromycin (50.98%), less sensitive to ceftriaxone (41.18%) and ciprofloxacin (39.22%). E coli were highly resistant to ampicillin (96.08%), erythromycin (90.2%) and nalidixic acid (80.39%).

Table II

Sensitivity pattern of *Escherichia coli* to different antimicrobials (n=51)

Name of antimicrobials	No. of isolates (%)		
	Sensitive(S)	Intermediate(I)	Resistant(R)
Ampicillin	2(3.92)	00	49(96.08)
Erythromycin	5(9.80)	00	46(90.20)
Nalidixic acid	7(13.73)	3(5.88)	41(80.39)
Cotrimoxazole	18(35.29)	00	33(64.71)
Ciprofloxacin	20(39.22)	9(17.64)	22(43.14)
Azithromycin	26(50.98)	00	25(49.02)
Ceftriaxone	21(41.18)	2(3.92)	28(54.90)
Gentamicin	41(80.40)	5(9.80)	5(9.80)
Nitrofurantoin	43(84.32)	4(7.84)	4(7.84)
Mecillinam	51(100)	00	00

Table-III shows sensitivity pattern of *Campylobacter* spp. to different antimicrobials. In this study *Campylobacter* spp. were highly sensitive (100%) to gentamycin and mecillinam followed by azithromycin (95.65%) erythromycin (86.96%) and nitrofurantoin (78-26%). Moderately sensitive to nalidixic acid (62.5%) and ciprofloxacin (73.91%). *campylobacter* spp. were highly resistant to cotrimoxazole (91.3%) followed by ampicillin (82.61%) and ceftriaxone (65.22%).

Table III

Sensitivity pattern of *Campylobacter* spp. to different antimicrobials (n=23)

Name of antimicrobials	No. of isolates (%)		
	Sensitive(S)	Intermediate(I)	Resistant(R)
Ampicillin	4(17.39)	00	19(82.61)
Erythromycin	20(86.96)	00	3(13.04)
Nalidixic acid	15(65.23)	00	8(34.78)
Cotrimoxazole	2(8.70)	00	21(91.30)
Ciprofloxacin	17(73.91)	00	6(26.09)
Azithromycin	22(95.65)	00	1(4.35)
Ceftriaxone	7(30.43)	1(4.35)	15(65.22)
Gentamicin	23(100)	00	00
Nitrofurantoin	18(78.26)	00	5(21.74)
Mecillinam	23(100)	00	00

Table IV shows sensitivity pattern of *Shigella* spp. to different antimicrobials by disc diffusion method. In this study, *Shigella* spp. were highly sensitive (100%) to mecillinam followed by ceftriaxone (86.67%) and ciprofloxacin (80%). Moderate to low rate of sensitivity to nitrofurantoin (73.34%), azithromycin (73.34%) and gentamicin (53.34%); Highly resistant to erythromycin (93.34%), cotrimoxazole (86.67%), ampicillin (80%) and nalidixic acid (60%).

Table IV

Sensitivity pattern of *Shigella* spp. to different antimicrobials (n=15)

Name of antimicrobials	No. of isolates(%)		
	Sensitive (S)	Intermediate (I)	Resistant(R)
Ampicillin	3 (20)	00	12(80)
Erythromycin	1(6.66)	00	14(93.34)
Nalidixic acid	5(33.33)	1(6.67)	9(60)
Cotrimoxazole	2(13.33)	00	13(86.67)
Ciprofloxacin	12(80)	2(13.33)	1(6.67)
Azithromycin	11(73.34)	00	4(26.66)
Ceftriaxone	13(86.67)	00	2(13.33)
Gentamicin	8(53.34)	2(13.33)	4(33.33)
Nitrofurantoin	11(73.34)	1(6.66)	3(20)
Mecillinam	15(100)	00	00

Table V shows sensitivity pattern of *Salmonella* spp. to different antimicrobials by disc diffusion method. In this study, *Salmonella* spp. were

Table V

Sensitivity pattern of *Salmonella* spp. to different antimicrobials (n=8)

Name of antimicrobials	No. of isolates (%)		
	Sensitive(S)	Intermediate(I)	Resistant(R)
Ampicillin	5(62.5)	00	3(37.5)
Erythromycin	00	00	8(100)
Nalidixic acid	00	00	8(100)
Cotrimoxazole	7(87.5)	00	1(12.5)
Ciprofloxacin	4(50)	2(25)	2(25)
Azithromycin	2(25)	00	6(75)
Ceftriaxone	8(100)	00	00
Gentamicin	7(87.5)	00	1(12.5)
Nitrofurantoin	6(75)	1(12.5)	1(12.5)
Mecillinam	8(100)	00	00

highly sensitive (100%) to ceftriaxone and mecillinam, followed by cotrimoxazole (87.5%) and gentamicin (87.5%). Among the other antimicrobials sensitivity pattern were as follows: nitrofurantoin (75%), ampicillin (62.5%), ciprofloxacin (50%) and azithromycin (25%). *Salmonella* spp. were 100% resistant to erythromycin and nalidixic acid.

Discussion

Diarrhoeal diseases still remains as a second leading cause of child death causing 1 in every 5 death in developing world. In Southeast Asia, child mortality rate is high (31.3%) due to diarrhoea.¹¹ In this study, out of 217 stool samples, enteric bacteria were isolated in 44.7% samples. Among these diarrhoeal pathogens *Escherichia coli* was the most predominant (52.58%) bacteria in this study. Like Bangladesh *Escherichia coli* is the most common aetiology of diarrhoea in other developing countries like India (44.2%) and Sudan (53.8%).^{12,13}

Campylobacter spp. were isolated as second prevalent diarrhoeal pathogen (10.6%) from diarrhoeal stool in this study. In the present study, *Shigella* spp. was isolated (15.46%) from diarrhoeal stool. In Bangladesh incidence of shigellosis is higher in winter (October-January) and (9.20%) *Shigella* was isolated in another study.⁶ Frequency of isolation of *Shigella* spp. (36.8%) were higher in other developing countries like Nepal.¹⁴ In present study the percentage of *Salmonella* spp. was 8.25%. Nessa reported 16% *Salmonella* spp. which was higher than present study.¹⁵

Current study showed that *E. coli* were susceptible to mecillinam, nitrofurantoin and gentamicin above 80%. Resistance of ampicillin and cotrimoxazole were reported in previous study which agreed with the present study.¹⁶ Sensitivity to azithromycin, ciprofloxacin and ceftriaxone were low. The high incidence of antibiotic resistance isolates of *E. coli* may be due to indiscriminate use of antibiotics.¹⁷

Most of the *Campylobacter* infections do not require antimicrobial treatment since they are clinically mild and self limiting in nature. Fewer than 50% need antimicrobial treatment. In the present study, *Campylobacter* was 100% sensitive to gentamicin, 95.65% to azithromycin. 86.96% to erythromycin, 73.91% to ciprofloxacin. Marked resistance to ciprofloxacin in both developed and developing countries as a result of inappropriate and indiscriminate, use of quinolones. Macrolides are the drug of choice for *Campylobacter* enteritis

and intravenous aminoglycosides for systemic manifestations.¹⁸ So, a specific sensitivity pattern guideline is needed for the treatment of enteric and systemic campylobacteriosis.

Among 15 *Shigella* spp. all isolates were sensitive to mecillinam followed by ceftriaxone, ciprofloxacin and azithromycin that showed similarity with Rahman.¹⁹ An increasing resistance had been reported in developing countries including Bangladesh to ampicillin, cotrimoxazole and nalidixic acid.²⁰

Out of 8 *Salmonella* spp. all (100%) were sensitive to ceftriaxone and mecillinam. Nessa reported that *Salmonella* spp. were highly sensitive to ciprofloxacin (100%) and less in cotrimoxazole (40%) but that did not match with the present study.²¹ In the present study, ciprofloxacin were 50% and cotrimoxazole were 87.5% sensitive to *Salmonella* spp. 75%-100% isolates were resistant to azithromycin, erythromycin and nalidixic acid. So, the overall sensitivity pattern of antibiotics varies from country to country according to prescription of local physician and also on quality of discs and methods used.

Conclusion

Mecillinam and nitrofurantoin were highly effective antibiotic against diarrhoea in children. As the antibiotic sensitivity pattern to bacterial pathogens is changing, so stool culture should be done before treatment. Sensitivity pattern of diarrhoeal pathogens specify treatment and shortens sufferings of child.

References

1. Black RE, Cousens S, Johnson HL. For the Child Health Epidemiology Reference Group of WHO and UNICEF. Global, regional and national cause of child mortality in 2008: a systematic analysis. *Lancet* 2010; 375: 1969-87
2. Thapar N, Sanderson IR. Diarrhoea in children : an interference between developing and developed countries *Lancet* 2004; 363: 641-53
3. Kosek M, Bern C, Guerrant R. L The Global Burden of Diarrhoeal Disease, as Estimated from Studies Published between 1992 and 2000. *Bulletin of the World Health Organization*. 2003; 81: 197-204
4. Bangladesh demographic and health survey (BDHS), 2007; Available at: <http://w.w.w.measuredhs.com/pubs>
5. WGO. World Gastroenterology Organization Practice Guideline: Acute diarrhea Available at <http://www.omge.org/global guideline,htm.2008>.

6. Akinjogunl OJ, Eghafona NO, Ekoi OH. Diarrhoeagenic Escherichia Coli (DEC): prevalence among in and ambulatory patients and susceptibility to antimicrobial chemotherapeutic agents. *J Bacterial Res* 2009; 1: 34-38
7. Nessa K, Ahmed D, Islam J, Kabir FML and Hossain MA. Usefulness of a Multiplex PCR for Detection of diarrhoeagenic Escherichia coli in a diagnostic microbiology laboratory setting Bangladesh. *J. Med Microbiol* 2007; 1: 38-42
8. Friedman CR, Hoekstra RM, Samuel M, Marcus R, Bender J et al. Risk Factors for Sporadic Campylobacter Infection in the United States: A Case Control Study in FoodNet Sites. *Clin Infect Dis* 2004; 38: 285-96
9. Talukder KA, Aslam M, Islam Z, Azmi IJ, Datta DK, Hossain S et al. Prevalence of Virulence Genes and Cytotoxic Distending Toxin Production in Campylobacter jejuni Isolates from Diarrheal Patients in Bangladesh. *Journal of Clinical Microbiology* 2008; 46: 1485-88
10. National Encyclopedia of Bangladesh 2010. Available at <http://www.banglapedia.org/httdocs/HT/-0336.HTM> accessed on 4.11.2015
11. Walker CL, Aryee MJ, Boschi Pinto C., Black RE Estimating diarrhea mortality among young children in low and middle income countries. *PLoS One* 2012; 7: C29151
12. Rauther VK, Pathana M, Jayara A, Yadav N. Clinical study of acute childhood diarrhea by bacterial enteropathogens. *J Clin Diagn Res.* 2014; 8: 1-5.
13. Saseed A, Abd h and Sandstrom G. Microbial aetiology of acute diarrhea under five years of age in Khartoum Sudan. *J Med Microbiology.* 2015; 64: 432-437
14. Lama C and Sherchan JB. Enteropathogens associated diarrhea in hospitalized patients of Children's Hospital, Kathmandu, J Nepal Heal Res Coun 2006; 5: 50-57
15. Nessa MK, Khan MSR and Alam. Isolation, identification and characterization of Salmonella serovars from diarrhoeic stool samples of human. *Bangl. J. Vet. Med.* 2011; 9: 85-93.
16. Aslani MM, Ahrabi SS, Alikhani YM. Molecular detection and antimicrobial resistance of diarrheagenic, Escherichia coli strains isolated from diarrhoeal cases, Saudi Med J 2008; 29: 388-392
17. Chakraborty S, Deokule JS, Garg P, Bhattacharya SK, Nandy RK, Nair GB et al. Concitant infection of enterotoxigenic E. coli in an outbreak of cholera caused by Vibrio cholerae 01 and 0139 in Ahmedabad, India. *J Clin Microbiol* 2001; 39: 3241-49
18. Gaudreau, C., and H. Gilbert. Antimicrobial resistance of clinical strains of Campylobacter jejuni subsp. jejuni isolated from human in 1998 to 2001 in Montreal, Canada. *Antimicrob. Agents and Chemotherapy* 2003, 2027-29
19. Rahman M, Shoma S, Rashid H, Arifeen S et al. Increasing spectrum in antimicrobial resistance of Shigella isolates in Bangladesh: Resistance to azithromycin and ceftriaxone and decreased susceptibility to ciprofloxacin. *J health popul nutr.* 2007; 25: 158-67
20. Fulla N, Prado V, Duran C, Lagos R, Levine MM. Surveillance for antimicrobial resistance profiles among Shigella species isolated from a semirural community in the northern administrative area of Santiago, Chile *Am J Trop Med.* 2005; 72: 851-54
21. Nessa MK, Khan MSR and Alam Isolation, identification and characterization of Salmonella serovars from diarrhoeic stool samples of human. *Bangl J Vet Med* 2011: 9: 85-93