

Causative agents for diarrhoea in under 5 children in a tertiary care hospital

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Abstract

Background: Infectious diarrhoea is a major public health problem in developing countries like Bangladesh. Bacteria and parasites are important etiological agents for diarrhoea in infants and children.

Objectives: To isolate and identify the causative organisms of diarrhoea from diarrhoeal stools 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 upto 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 enteric bacteria, diarrhoeal stools were inoculated on MacConkey agar media and for *Campylobacter* spp. diarrhoeal stools were inoculated 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. Oocysts of *Cryptosporidium* were identified by Modified Ziehl Neelsen staining and other parasites were identified by wet mount preparation.

Results: Out of 217 diarrhoeal stools bacteria were isolated from 97 (44.7%) patients. Among 97 cases, *Escherichia coli* were detected in 51(52.58%) patients; *Campylobacter* spp. were detected in 23(23.71%) patients; *Shigella* spp. were detected in 15(15.46%) patients and *Salmonella* spp. were detected in 8(8.25%) patients. Microscopic finding of diarrhoeal stools were detected by pus cells with or without RBCs in 90(70.31%) samples. Cysts of *Giardia intestinalis* were found in 9(4.14%) samples, cysts of *Entamoeba histolytica* in 2(0.9%) samples, eggs of *Triturus trichiura* in 2(0.9%) samples and oocysts of *Cryptosporidium* were found in 5(2.30%) samples.

Conclusion: *Escherichia coli* was the most common bacteria and *Giardia intestinalis* was predominant parasite causing diarrhoea in under 5 children.

Key words: Diarrhoea, Bacteria, Parasites.

Introduction

Diarrhoea is a major public health problem in developing countries. Globally 1.87 million children die annually within 5 years of age from diarrhoea, accounting for 19% of all childhood deaths.¹ Diarrhoea remains the second leading cause of death under 5 years and the highest mortality occur below 1 year of age.²

Improvement of sanitation, nutrition, education and easy access of ORS have substantially lowered the lethality of severe diarrhoea from 4.6 million in 1982 to 2.5 million in 2003.³ In developed countries an average 3.2 episodes of

diarrhea per child per year has been reported, but in developing countries the number can be as high as 12 episodes per child per year.⁴ Among the developing countries, 78% child mortality occurred in Africa and Southeast Asia regions.¹ In 2007, yearly estimated child death was 50,800 due to diarrhoea and Bangladesh was in 7th position.⁵

The contribution of different diarrhoeal pathogens may differ substantially between regions depending on local meteorological, geographical and socioeconomic conditions.⁶ A wide range of

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bacteria, virus and parasites causes infectious diarrhoea.⁴ In Bangladesh, common bacterial causes of diarrhoea in children under 5 years of age are *Escherichia coli*-29% (ETEC-16.8% & EPEC-12.2%), *Campylobacter jejuni* (17.4%) followed by *Aeromonas* spp. (12.2%), *Shigella* (9.2%) and *Vibrio cholerae* (9%).⁷

Among the bacterial causes, *Escherichia coli* is most important agent for childhood diarrhoea in developing countries. In Bangladesh, 41-46% acute diarrhoea is caused by *Escherichia coli*.⁸ Worldwide *Campylobacter* spp. is one of a major cause of acute bacterial gastroenteritis among infants and children.⁹ In Bangladesh isolation rate of *Campylobacter* within 5 years of age is 11% to 17.4%.¹⁰ Shigellosis is endemic in Bangladesh and it causes 20% of death related to diarrhoea among children.⁵ The intestinal protozoan parasite *Giardia intestinalis* (*Giardia lamblia*) has recently emerged as an important cause of diarrhoea in humans.¹¹ *Cryptosporidium* spp. is a leading cause of diarrhoea, particularly persistent diarrhoea, in children in developing Countries.¹² This study was designed to find out diarrhoeagenic bacteria and parasites from children suffering from diarrhoea under 5 years of age.

Materials 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 to June, 2016 in Microbiological laboratory of Sir Salimullah Medical College, Dhaka.

During this period, 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. Cases were 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 who completed antibiotic treatment within last 15 days were excluded.

Stools were examined microscopically in saline and iodine preparation for detection of pus cells and parasites. Modified Ziehl Neelsen staining were done for detection of oocysts of *Cryptosporidium*. 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 mg/liter and Vancomycin 10 mg/liter) and Non-selective Blood agar media with membrane filter (0.45 mm). A cellulose triacetate membrane with 0.45mm pore and 47 mm diameter were placed on surface of Blood agar plate before inoculation. A Pasteur pipette were used to place 8 to 10 drops of stool suspension on the surface of the membrane. The filter paper were removed after 30-60 minutes. 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. 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.

Results

During the study period a total of 217 stool samples were collected from under 5 diarrhoeal children. Among 217 patients, majority of the children (43.32%) were below 12 months of age group followed by 13-24 months of age group (23.50%) (Figure 1).

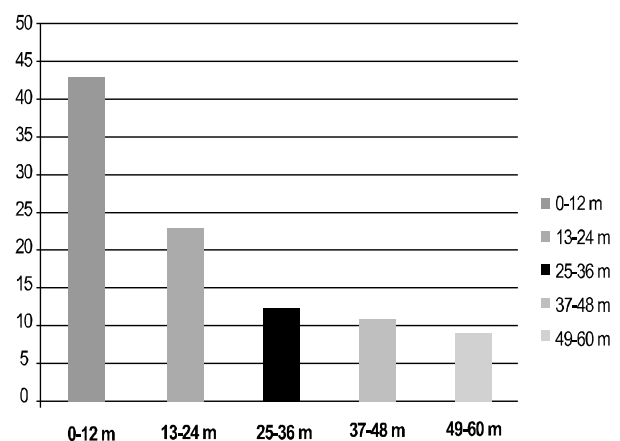


Figure 1. Different age distribution in diarrhoeal patients

Among 217 stool samples, bacteria were isolated in 97 samples (44.7%) which were more predominant than parasites 18(8.29%) (Figure 2).



Figure 2. Distribution of causative agents of diarrhoea.

Of the 217 samples, parasites were present in 18(8.29%) stools and pus cells and/ or RBCs were present in 90(75%) stools. Among 18 parasite positive stools (Table I), cysts of *Giardia intestinalis* in 9(4.15%) samples, cysts of *Entamoeba histolytica* in 2(0.92%) samples and eggs of *Trichuris trichiura* were present in 2(0.92%) samples. Modified Z-N staining detected oocysts of *Cryptosporidium* in 5(2.30%) samples.

Table 1

Microscopic finding of parasites in diarrhoeal stools (n= 217).

Microscopic finding	No. of cases (%)
Finding present	18(8.29)
Wet preparation	
Cysts of <i>Giardia intestinalis</i>	09(4.15)
Cysts of <i>Entamoeba histolytica</i>	02(0.92)
Eggs of <i>Trichuris trichiura</i>	02(0.92)
Modified Z N stain	
Oocysts of <i>cryptosporidium</i>	05(2.30)
Negative finding	199(91.71)
Total	217(100)

Of the 217 samples, bacteria were isolated in 97(44.70%) samples (Table II). Among the positive 97 samples, *Escherichia coli* were isolated in 51(52.58%) samples; *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 II

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

Name of isolated bacteria	No. of cases (%)
Total Detected	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)
Not detected	120(55.30)
Total	217(100)

Discussion

Infectious diarrhoea is one of the common causes of morbidity and mortality among infants and children in developing world. In Bangladesh, ICDDR, B treats more than 1,00,000 diarrhoeal patients in a year and this number is increasing year after year.⁸ Knowledge about different diarrhoeal pathogens is essential for implementation of appropriate public health measure to control the diseases.¹³

The study included children ranging from 0 to 60 months of age. Majority of children were within 0-12 months (43.32%) and 13-24 months (23.5%) of age which has similarity with other study (Figure 1).¹⁴ During 0-12 months the infants have immature immune system because Protective maternal antibody begins to decline after 6 months and formula milk or food starts after 6 months.

In the study, pus cells with or without RBCs were present in 75% diarrhoeal stools. In other study of Bangladesh, 79% pus cells and 33% RBCs were found in diarrhoeal Stools.¹⁵ Among 217 stool samples, cysts of *Giardia intestinalis* was highest in 9(4.15%) samples followed by oocysts of *Cryptosporidium* spp. in 5(2.30%) samples and cysts of *E. histolytica* and eggs of *Trichuris trichiura* in 2(0.92%) diarrhoeal stools. These three parasites previously reported as the most common diarrhoea causing parasites in Bangladesh.¹⁶

Out of 217 stool samples, 97(44.7%) bacteria were isolated in stool samples (Table-II). The aetiology of diarrhoea due to bacteria was varied due to various factors. Use of transport media, actual history of taking antibiotics before sample collection, locality, seasonality and environmental condition might affect the isolation of diarrhoeal pathogens. Among these diarrhoeal pathogens *Escherichia coli* was predominant and isolated in 51(23.5%) samples in this study. Roy reported 50.37% *Escherichia coli* from culture of diarrhoeal Stool.¹⁴ Due to poor sanitation, lack of education about personal hygiene, *Escherichia coli* can be transmitted from patients to patients through feco- oral route.

Campylobacter spp. were isolated in 23(10.6%) samples of diarrhoeal stool in the current study. In Dhaka, Roy reported 13.5% *Campylobacter* found in acute diarrhoeal patients.¹⁷ So the rate of isolation varies from place to place where contaminated water and food of animal origin, especially improperly processed chicken acts as

vehicle for transmission of campylobacteriosis of human.¹⁸

In this study *Shigella* spp. was isolated in 15(6.9%) samples from diarrhoeal stool. In Bangladesh incidence of shigellosis is higher in winter (October - January) and 9.20% *Shigella* was isolated in another study.⁸ But frequency of isolation of *Shigella* spp. 36.8% were higher in other developing countries like Nepal.¹⁹ In our study the percentage of *Salmonella* spp. was 8.25% whereas Nessa reported 16% *Salmonella* spp. which was higher.²⁰

Conclusion

In Bangladesh, *Escherichia coli* was the most common bacteria and *Giardia intestinalis* was common parasite causing diarrhoea in under 5 children. Therefore proper diagnosis and management of diarrhoea requires microscopy and culture facility which should be available at all level.

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