



## Investigation of Diarrheal Stool from Hospitalized Patient and Compare Their Soci-economical Condition, Dhaka, Bangladesh

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### Authors' contributions

*This work was carried out in collaboration between all authors. Author KF designed the study, wrote the protocol and wrote of the manuscript. Author KM managed the literature searches and analyses of the study performed analysis. Author TA managed the experimental process. Author SD revised the paper. All authors read and approved the final manuscript.*

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### ABSTRACT

**Aims:** This study was possible to identify factors associated with the severity of different pathogens and duration of hospitalization in diarrheal patients from stool depending and find the difference in the socio-economical background of their families.

**Study Design:** Samples were collected from 60 hospitalized patients in Upazila Health Complex; Munshigonj, Bangladesh who were infected by diarrhoea from 2013. The study population included infants, young children and adults

**Methods:** Stool samples were collected from the patient's in clear, transparent, wide mouthed bottles. Information was also obtained from each patient regarding age, sex, feeding patterns, and maternal education. The study was conducted in Centre for Excellence Laboratory (CEL) Department of Microbiology, Primeasia University, Dhaka, Bangladesh.

**Results:** A total of 60 samples were collected of which 50% was positive isolates. Among these isolates *Salmonella* was detected in 40% and *E. coli* in 30% of the positives. The antimicrobial susceptibility testing showed that the *Salmonella* sp. were highly sensitive (90%) to Ceftriaxone,

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moderately sensitive to Ciprofloxacin; *Salmonella* and *E. coli* strains showed 100% resistance towards Cotrimoxazole, Ampicillin, Tetracycline and Nalidixic acid.

**Conclusion:** To addresses this issue, antibiotic therapy should be taken into consideration along with the pathogen. In addition, the incidence of pathogens in human can be traced primarily to faulty weaning practices and poor personal hygiene.

**Keywords:** Diarrhoea; antimicrobial susceptibility; socio economical condition.

## 1. INTRODUCTION

Worldwide, around 1.1 billion people have no access to clean water sources, and 2.4 billion have no basic sanitation. Each year, there are approximately four billion cases of diarrhoea worldwide, and infectious diarrhoea is widespread throughout the developing world [1]. Poor quality of water, sanitation, and hygiene account for some 1.7 million deaths a year, mainly due to infectious diarrhoea. Nine out of ten of such deaths occur in children, with a significant majority of deaths in developing countries [2]. Human diarrhoea is the third leading cause of infectious deaths worldwide and is responsible for ~1.8 million deaths in children under age five each year [3]. Bacteria, protozoa and viruses have all been implicated as causal agents. Chief among the known etiological agents are rotaviruses, noroviruses, astroviruses, and adenoviruses [4]. However, it is estimated that on average up to 40% of diarrhoea cases are of unknown etiology, suggesting that unrecognized infectious agents, including viruses, remain to be discovered [5–9]. Diarrhoea is a second leading cause of illness and death among children in developing countries, where an estimated 1.3 thousand million episodes and 4 million deaths occur each year in under-fives [10]. Worldwide, these children experience an average of 3.3 episodes each year in under-fives.

About 80% of deaths due to diarrhoea occur in the first two years of life [11]. In Bangladesh 20% to 30% of all deaths are due to diarrheal disease. In rural Bangladesh [12] cases of severe diarrheal illness occurs population per year. In untreated cases the mortality is very high which from 20% to 60%. According to WHO, Diarrhoea is the passage of 3 or more loose or liquid stools per day, or more frequently than is normal for the individual. It is usually a symptom of gastrointestinal infection caused by microorganisms. Infection is spread through contaminated food or drinking-water, or from person to person as a result of poor hygiene [13].

Diarrhoea is usually defined in epidemiological studies as the passage of three or more loose or watery stools in a 24-hour period, a loose or looser or that would take the shape of a container. However, mothers may use a variety of terms to describe diarrhoea, depending, for example upon whether the stool is loose, watery, bloody or mucous, or there is vomiting [13]. It is important to be familiar with these terms when asking whether a child has diarrhoea. Exclusively breast-fed infants normally pass several soft, semi-liquid stools each day, for them, it is practical to define diarrhoea as an increase in stool frequency or liquidity that is considered abnormal by the mother [12]. Three clinical syndromes of diarrhoea have been defined, each reflecting a different pathogenesis and requiring different approaches to treatment. Acute watery diarrhoea, this term refers to diarrhoea that begins acutely, lasts less than 14 days (most episodes last less than seven days), and involves the passage of frequent loose or watery stools without visible blood. Vomiting may occur and fever may be present. Acute watery diarrhoea causes dehydration; when food intake is reduced, it also contributes to under nutrition. When death occurs, it is usually by acute dehydration. The most important causes of acute watery diarrhoea in young children in developing countries are rotavirus, enterotoxaemia *Escherichia coli*, *Shigella*, *Campylobacter jejuni*, and *cryptosporidium*. In some areas, *Vibrio cholerae* 01, *Salmonella* and enter pathogenic *E. coli* are also important causes [14]. Dysentery; the term dysentery refers to diarrhoea with visible blood in the faeces. Important effects of dysentery include anorexia, rapid weight loss, and damage to the intestinal mucosa by the invasive bacteria. A number of other complications may also occur. The most important cause of acute dysentery is *Shigella*; other causes are *Campylobacter jejuni* and, infrequently, enter invasive *E. coli* or *Salmonella*, *Entamoeba histolytica* can cause serious dysentery in young adults but is rarely a cause of dysentery in young children [15]. Persistent diarrhoea, this term refers to diarrhoea that begins acutely but is of unusually long duration (at least 14 days). The episode may

begin either as watery diarrhoea or as dysentery. Marked weight loss is frequent. Diarrheal stool volume may also be great, with a risk of dehydration. There is no single microbial cause for persistent diarrhoea; enteroadherent *E. coli* and cryptosporidium may play a greater role than other agents. Persistent diarrhoea should not be confused with chronic diarrhoea, which refers to recurrent or long-lasting diarrhoea due to non-infectious causes, such as sensitivity to gluten or inherited metabolic disorders [14]. Diarrheal disease also represents an economic burden for the developing countries. In many nations more than a third of the hospital beds for children are occupied by patients with diarrhoea. These patients are often treated with expensive intravenous fluids and ineffective drugs. Although diarrhoeal disease is usually less harmful to adults than to children, it can also affect a country's economy by reducing the health of its workforce [16]. Simple and effective treatment measures are available that can markedly reduce diarrhoea deaths, make hospitalization unnecessary in most cases, and prevent the adverse effect of diarrhoea on nutritional status. Practical preventive measures can also be taken that substantially reduce the incidence and severity of diarrheal episodes. There are two principal mechanisms by which watery diarrhoea occur: (i) secretion, and (ii) osmotic imbalance. Intestinal infections can cause diarrhoea by both mechanisms, secretory diarrhoea being more common, and both may occur in a single individual. [17] Secretory diarrhea: Diarrhoea is caused by the abnormal secretion of fluid (water and salts) into the small bowel. This occurs when the absorption of sodium by the villi is impaired while the secretion of chloride in the crypts continues or is increased. Net fluid secretion results and leads to the loss of water and salts from the body as watery stools; this causes dehydration. In infectious diarrhoea, these changes may result from the action on the bowel mucosa of bacterial toxins, such as those of *Escherichia coli* and *Vibrio cholera* 01, or viruses, such as rotavirus; other mechanisms may also be important. Osmotic diarrhoea: The small bowel mucosa is a porous epithelium; water and salts move across it rapidly to maintain osmotic balance between the bowel contents and the blood [18]. This study focused to identify the risk factors that are infected patients the duration of hospitalized patients with diarrhea including their nutritional status, type and morbidity of disease and diarrhea, to isolate and identify the causative agent of diarrheal stool in those who had during treatment drug resistance pattern of the isolates.

## 2. MATERIALS AND METHODS

### 2.1 Isolation of Samples

All culture were inoculated and incubated under aseptic condition. A compound microscope was used to observe the microscopic characteristics of the bacteria. 10 g of solid sample were weighed aseptically into a sterile jar and 90 ml of distilled water was added, it was homogenized with sterile blender at 3000 rpm for 5-10 minutes. 1 ml of homogenate was transferred to a test tube containing 9 ml of sterile distilled water to make  $10^{-1}$  dilution and shaken with vortex mixer. A serial dilution up to  $10^{-8}$  was also made in the same procedure. The bacterial count was performed by standard method.

### 2.2 Growth of Microorganisms

The microbiological condition safety and hygiene were assayed using the methods recommended by ICNSF. Total viable bacterial count was carried out by the spread plate technique. The media used for viable bacterial count plate count agar (PCA) count; Mannitol salt agar, *Salmonella-Shigella* (SS) agar medium was used. Homogenate sample (0.1 ml) from each dilution was taken on to each sterile petridish. The sample was homogeneously distributed on the plate using a glass spreader in backward and forward movement while rotating the plate. Then the plates were incubated at 37°C for 24 hours. Typical or suspected colonies from plate were selected and streaked on nutrient agar plates. MacConkey agar media was used for counting of total coli form bacteria. Then the prepared dishes were incubated at 37°C for 24 hrs Potato dextrose agar (PDA) was used for fungal species count at 25°C for 5 days.

### 2.3 Biochemical Test

Pure bacterial colony was picked up by a sterile needle and immersed in 2-3% hydrogen peroxide ( $H_2O_2$ ) solution in a test tube. The production of bubbles inside the test tube was taken as positive for catalase production. This test was performed to assess the mode of sugar utilization by stabbing the butt and streaking the slant of KIA media. After incubation at 37°C for 18-24 h result were recorded for change in colour of butt and slant,  $H_2S$  or other gas production. One suspected isolated colony was touched with a sterile wire and stabbed into agar very carefully down the tube, without touching the bottom.

The tube was incubated at 37°C for 18 to 24 hours

### 3. RESULTS

60 samples diarrheal stool inoculated in Primeasia University laboratory during the study period were studied for the various bacterial pathogens. Among these 50 positive samples was identified. The highest number of pathogen was *Salmonella* (40%), *E. coli* and lowest *Staphylococcus aureus* (10%) and *Shigella*. The stool samples of culturally positive patients were collected and analyzed by standard

microbiological procedure. Among these 50 positive samples, 20 samples were associated with *Salmonella* (40%), the causative agents, that was found to be most prevalent (40%), followed by *E. coli* (30%) (Table 1).

Socio-economical conditions of the diarrheal patients among 50% patients are poor. All the isolates were found to be multidrug resistant. Most of the *E. coli* was found to be resistant against amoxicillin, tetracycline, cotrimoxazole. All the isolates were found to be resistant against amoxicillin, tetracycline, cotrimoxazole, azithromycin, ceftriaxone, ciprofloxacin.

**Table 1. Determination of pathogen from diarrhea samples**

Total number of positive patient studied (50 Patients)	Different isolated pathogen			
	<i>Salmonella</i>	<i>E. coli</i>	<i>Shigella</i>	<i>Staphylococcus aureus</i>
50	20(40%)	15(30%)	5(10%)	10(20%)

**Table 2. Percentage of total positive hospitalized diarrheal patients from the total suspected patients according to sex, age and socio-economic condition**

	Number (%)
<b>Sex</b>	
Female	20(40%)
Male	30(60%)
<b>Age group</b>	
0-5	25(50%)
15-20	10(20%)
25-30	10(20%)
35-45	5(10%)
<b>Socioeconomic condition</b>	
Poor	30(60%)
Medium	20(40%)
<b>Nutritional status</b>	
Malnourished	20(40%)
Normal	30(60%)

**Table 3. Antimicrobial resistance pattern of bacterial pathogens isolated from diarrheal patients**

Antimicrobial agent	<i>Salmonella</i> (n= 20)	<i>E. coli</i> (n=15)	<i>Staphylococcus aureus</i> (n=10)
Nalidixic acid	0	0	0
Ciprofloxacin	10	5	0
Erythromycin	0	5	5
Tetracycline	0	0	0
Ampicillin	0	5	0
Cotrimoxazole	0	0	0
Azithromycin	0	0	0
Ceftriaxone	10	2	5

#### 4. DISCUSSION

Diarrhoea is one of the most important causes of death in children and is still a considerable public health in developing countries [18]. A large proportion of diarrhoea episodes end up in hospitalization. There are few studies to investigate the impact of diarrhoea on hospitalization, however it is suggested that among causative pathogens, rotavirus alone causes approximately 111 million episodes of gastroenteritis require home care, million consultations, 2 million hospitalization and 325,000 to 870,000 death in children <5 years of age [19]. Despite many studies related to diarrhea, there is a little mention of factors increasing risk of hospitalization in diarrhea in developing countries. This study revealed that having a history of consultation, a history of previous hospitalization and the presence of blood in the stools were independent factors when comparing hospitalized patients.

#### 5. CONCLUSION

This study has shown that multi drug resistance is associated with diarrhea in Munshigonj, Bangladesh. To addresses this issue, this is in agreement with studies denoting that parent's education, especially illiteracy, is a very important factor in the management of diarrhea [18,19]. In conclusion, the risk factors for hospitalization due to acute diarrhea were the presence of bloody or watery stools, lack of ORS use, having non-semi liquid stools, use of unsafe (spring/river) water, vomiting during the past day or week prior to consultation, hospitalization during the previous year, the presence of rotavirus, *Salmonella* or *E. coli* in the stool, shorter duration of breast feeding and illiteracy of mother. Antibiotic therapy should take into along with the susceptibility pattern of the pathogen. In addition, the incidence of pathogens in human can be traced primarily to faulty weaning practices and poor personal hygiene.

#### CONSENT

All authors declare that written informed consent was obtained from the for publication of this paper.

#### ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the

appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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