RESEARCH ARTICLE

# Acute Diarrhea in Children Less than Five Years of Age: Epidemiology of Bacterial Pathogens

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## ABSTRACT

Objectives: Acute diarrhea is among the leading causes of mortality and morbidity worldwide. Bacteria tend to cause more fatal illnesses and complications such as septicemia and persistent diarrhea. This study aimed to determine the causes of acute diarrhea, laboratory and clinical predictors of bacterial causes, and antimicrobial resistance pattern among the isolates among children in Dar es salaam, Tanzania.

Methods: A cross-sectional hospital-based study was conducted in Dar es salaam Hospitals from April 2015 to March 2016 among children below five years of age who presented with acute diarrhea. Demographic characteristics and results from stool specimen analysis, complete blood count, C- reactive protein and antimicrobial resistance results were recorded using a pre-structured clinical research form.

Results: Among 200 children enrolled, viruses were identified in 149 (74.5%) of the cases. Bacterial pathogens were found in 15 (7.5%) cases only. Elevated stool red blood cell count, stool white blood cell count, and fever were highly associated with enteric bacterial pathogens (p<0.001, p=0.002 and p=0.04 respectively). Most of the bacterial isolates were resistant to Cotrimoxazole and erythromycin but highly sensitive to ciprofloxacin and Ceftriaxone.

Conclusion: Fever, elevated stool leukocyte and elevated stool red blood cells are significant predictors of bacterial enteric pathogens in children with acute diarrhea. These parameters may guide clinicians in resource-limited settings in the diagnosis and management of acute diarrhea. Further studies should be conducted to determine local antimicrobial resistance patterns. *J Microbiol Infect Dis 2020; 10(3): 208-214.*

Keywords: *Acute diarrhea, causes, predictors, antimicrobial resistance, children*

## INTRODUCTION

Diarrhea is the second cause of mortality among children under five years of age worldwide preceded by neonatal complications. It is estimated that around 2.5 billion diarrhea cases occur per year worldwide with 50% of these cases in Africa and South Asia. Among these cases, 1.5 million die with 85% of death taking place in Africa [1,2]

The most common causes of diarrhea in children are viruses, bacteria, and other parasites but these vary from one region to another [3-6]. Studies were done in Dar es salaam and Ifakara in Tanzania demonstrated variations in these pathogens depending on age, location (rural or urban) and seasons (rainy or dry) [3,7,8]. The most common pathogens were *Escherichia coli, Cryptosporidium parvum, Shigella spp, Rotavirus,* and *Giardia lamblia.*

Diarrheal diseases are transmitted by fecal-oral route with the major risk factors for transmission being an inadequate supply of safe water, poor sanitation and hygiene [1,2].

Clinical presentation of acute diarrhea is almost the same regardless of the etiology. These features may include acute onset of passing loose stool more than three times in 24 hours, fever, vomiting, abdominal clumps and dehydration. Bloody diarrhea may be present in the case of enteroinvasive organisms [9]. Most of diarrhea cases resolve spontaneously or by supportive rehydration and zinc supplementation [9]. Systemic antibiotics are warranted in case of life-threatening bacterial infections such as enteroinvasive *E. coli, Shigella spp* or cholera [9,10]. If not well treated, bacterial pathogens may lead to disseminated infection such as septicemia, meningitis, pneumonia and subsequently death [11-14]

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World Health Organization (WHO) recommends the use of antibiotics such as ciprofloxacin and ceftriaxone [9] in case of dysentery due to suspected shigellosis but deaths have been reported in other non-dysenteric shigella and other enteric bacterial pathogens which do not present with bloody diarrhea [5,11,14,15].

It is usually difficult to differentiate bacterial diarrhea from other pathogens by using clinical signs and symptoms. Standard confirmatory tests of etiologies are usually not available in resource-poor countries and some of them are expensive to perform. This may lead to delayed diagnosis and inappropriate management of children with bacterial diarrhea leading to complications and death.

This study aimed to determine the causes, clinical and routine laboratory test which may predict bacterial etiology in children with acute diarrhea and antimicrobial sensitivity pattern among the bacterial isolates. If these children are identified early and specific confirmatory tests done, it may reduce the diagnostic costs of testing every child with diarrhea and may also guide clinicians on the appropriate management of children with suspected enteric bacterial pathogens.

## METHODS

### Study site and design

A cross-sectional hospital-based study was conducted at Temeke, Amana and Mwananyamala Hospitals in Dar es Salaam from April 2015 to March 2016. Children below five years of age with acute diarrhea attending inpatient and outpatient services were enrolled in the study. Those who were on antibiotics, or had a known history of food intolerance, or persistent diarrhea for more than 14 days were not included.

### Ethical issues

Ethical clearance was obtained from the Ethical Review Committee (ERC) of the Hubert Kairuki University followed by permissions form the respective municipal authorities and hospitals.

After the informed consent process, social demographic information and clinical evaluation of the participants and study specimen collection were done. Data were recorded on a pretested clinical research form.

### Study sample collection, transport, and analysis

Blood and stool samples were collected and according to the current protocols [16] and then transported the central research laboratory at Hubert Kairuki Memorial University for analysis. Complete blood count and differentials were performed (Beckman-Coulter, Model Act 10) as well as C-Reactive protein using standard technique (SYNCHRON CX®, Beckman-Coulter, 2008).

Stool microscopy analysis using wet preparations was performed to detect stool red and white blood cells, protozoa cysts, and helminths ova. Bacterial pathogens were identified in stool samples as described in the Clinical and Laboratory Standard Institute Guidelines as described in other similar studies [3,10]. The disc diffusion method was used to determine the antimicrobial sensitivity of bacterial isolates (CLSI,2006). Antibiotics tested were co-trimoxazole, Erythromycin, ciprofloxacin, Gentamicin, and Ceftriaxone. Rapid immunochromatographic tests for the detection of viral antigens were used to detect rotavirus, adenovirus, norovirus, and astrovirus (Ridaquick, Generic Assays, 2014).

### Data analysis

Data were analyzed using Statistical Package for Social Sciences for Windows (SPSS Version 20). Proportions and categorical variables were compared using the Chi-square test. Binary logistic regression analysis was used to determine the clinical and laboratory predictors of bacterial causes of diarrhea. A p-value of less than 0.05 was considered statistically significant.

### RESULTS

A total of 200 participants were recruited in the study. Males were 125 (62.5%) while females were 75 (37.5%). Most of the participants (92.5%) presented with diarrhea for less than 5 days. On presentation, 134 (66%) children had no dehydration, 63 (31%) had some dehydration while only 3 (1.5%) had severe dehydration. Other baseline characteristics are shown in Table 1.

Viruses comprised the major causes of diarrhea. Viral causes were identified in 149 (74.5%) of the study participants. Rotavirus was responsible for 84 cases

(42%) followed by adenovirus (29%) and norovirus (3.5%). Astrovirus was not detected (Figure 1).

Table 1. Baseline characteristics of participants

| Variable | No. of Cases (%) |
|---|---|
| **Hydration status** | |
| Normal | 134 (66.0) |
| Some dehydration | 63 (31.0) |
| Severe dehydration | 3 (1.5) |
| Total | 200 (100) |
| **Gender** | |
| Male | 125 (62.5) |
| Female | 75 (37.5) |
| Total | 200 (100) |
| **Age** | |
| less than 2 years | 168 (84) |
| more than 2 years | 32 (16) |
| Total | 200 (100) |
| **Body temperature** | |
| No fever (<37.5 $^0$C) | 177 (88.5) |
| With fever | 23 (11.5) |
| Total | 200 (100) |
| **Bodyweight** | |
| below 10kg | 133 (66.5) |
| between 10-20kg | 66 (33) |
| above 20kg | 1 (0.5) |
| Total | 200 (100) |
| **Duration of Diarrhea** | |
| less than 5 days | 185 (92.5) |
| between 5-10 days | 15 (7.50) |
| more than 10 days | 0 (0) |
| Total | 200 (100) |

Bacteria were isolated in 15 stool samples only (7.5%). Pathogens isolated were *Escherichia coli* [7(46.7%)], Salmonella typhii [3(20%)], Shigella dysenteriae [3(20%)]. Vibrio cholerae was isolated in 2 cases (13.3%) as shown in figure 2. Other pathogens were Amoeba histolytica 10 (5%) and intestinal worms 10(5%).

There were no specific findings in 16 (8%) of the stool samples. Most of the bacterial pathogen isolates were resistant to Cotrimoxazole and highly sensitive to Ciprofloxacin and Ceftriaxone (Table 2).

The presence of red blood cells in stool was found to be highly associated with bacterial causes of diarrhea [OR =50.7, CI=8.7-296.7 (P<0.001)]. Other factors found to be associated with enteric bacterial pathogens were elevated white blood cells in stool [OR =42.9, CI=1.8-1026(P=0.02)] and presence of fever with body temperature >37.5 $^0$C [OR=7, CI=1.1-47(P=0.04)]. Age, elevated total white blood count, and C-reactive proteins were not significantly associated with bacterial causes of diarrhea (Table 3).

## DISCUSSION

Enteric pathogens were found in 186 (92%) of stool specimens from children with acute diarrhea who participated in our study. This is higher compared with other studies that were done in Dar es Salaam and Ifakara whereby enteric pathogens were found only in 67.1% and 71.8% of the stool samples collected respectively [3,7]. High yield in our study could be because other studies did not investigate other enteric pathogens than bacteria and viruses. In our study we also excluded children with persistent diarrhea, and who had a previous history of antibiotic use during the index illness.

We found that viruses were the most common pathogens (rotavirus, adenovirus, and norovirus) compared to other pathogens. This slightly varies from other studies in Tanzania whereby bacterial pathogens were commonly isolated. A study done by Moyo et al in Dar es salaam found the bacterial causes to be 33.2% with *Escherichia spp* leading followed by *Shigella spp* [3]. Another study done by Vargas and others in Ifakara demonstrated that bacteria were the most common pathogens with *Escherichia spp* leading (35.7%), followed by rotavirus (23%) [7]. Our findings are similar to these studies by showing that *Escherichia spp* are the most common bacterial pathogens in stool samples of children with diarrhea [4,7,17]. This is also consistent with other findings in different parts of the world showing the major three causes of diarrhea to be rotavirus, *Escherichia spp*, and *Shigella spp* [18-22].

Table 2: Antimicrobial sensitivity pattern among the enteric bacterial pathogens isolated in stool of children with acute diarrhea.

| Antimicrobial agent | *Escherichia spp* (n=7) | *Shigella spp* (n=3) | *Salmonella spp* (n=3) | *Cholera spp* (n=2) |
|---|---|---|---|---|
| Cotrimoxazole | 0% | 33% | 0% | 0% |
| Erythromycin | 45% | 0% | 0% | 0% |
| Ciprofloxacin | 91% | 100% | 100% | 100% |
| Gentamicin | 45.5% | 0% | 0% | 50% |
| Ceftriaxone | 100% | 100% | 100% | 100% |

Table 3. Clinical and laboratory features associated with bacterial causes of acute diarrhea.

| Variable | Number Bacteria isolated | No. of bacteria isolated | AOR | 95% confidence interval | P Value |
|---|---|---|---|---|---|
| Age(years) | | | | | |
| ≤2 Years | 7 (4.2%) | 160 (95.8%) | 1.835 | .218-15.411 | .576 |
| >2 years | 8 (25%) | 24 (75%) | | | |
| Body Temperature(C°) | | | | | |
| No fever | 7 (46.7%) | 169 (91.8%) | 7.104 | 1.071-47.108 | .042* |
| With fever(>37.5°C) | 8 (53.3%) | 15 (8.2%) | | | |
| Total white blood cell count | | | | | |
| Normal(<12,000/µl) | 7 (46.7%) | 123 (68%) | .848 | .151-4.776 | .852 |
| Significantly elevated | 8 (53.3%) | 56 (32%) | | | |
| Stool red blood cells(/HPF) | | | | | |
| Normal(<5rbc/hpf) | 4 (26.7%) | 176 (92.2%) | 50.712 | 8.667-296.730 | .000* |
| Significantly elevated | 11 (73.3%) | 7 (3.8) | | | |
| Stool Leucocytes (HPF) | | | | | |
| Normal (<10wbc/hpf) | 4 (26.7%) | 182 (99.5%) | 42.952 | 1.797-1026.383 | .020* |
| Significantly elevated | 11 (73.3%) | 1 (0.5%) | | | |
| C-Reactive protein (CRP) | | | | | |
| Normal | 9 (64.4%) | 165 (90.2%) | 3.066 | 0.415-22.677 | .272 |
| Significantly elevated (>6 gm/L) | 5 (35.7%) | 18 (9.8%) | | | |

AOR=Adjusted Odd Ratio

We found that fever was among the significant predictors of bacterial diarrhea among the study participants. These findings are consistent with other studies done elsewhere [21-25]. This could be because most of the bacterial pathogens cause more severe inflammation with more severe systemic response compared to viruses and other less virulent pathogens.

Another significant predictor was the presence of blood or red blood cells in stool analysis. This is consistent to most of other research finding [22-27]. This is the result of the destruction of the intestinal lining through different mechanisms. Some bacteria such as *Shigella spp* cause damage by directly invading the intestinal mucosa (entero-invasive)  while others such as  Shiga like toxin-producing *E. coli* cause damage by producing toxins that destroy the integrity of the luminal wall leading to either macro or micro bleeding [28].The presence of red blood cells in the stool may not be used to predict the presence of other pathogenic bacteria that cause secretory diarrhea by producing enterotoxins that interact with multiple ion and solute transporters across the intestinal epithelium such as cholera and enterotoxigenic *E. coli* [29].

Elevated stool leukocyte was significantly associated with the presence of bacterial pathogens. Most of the other researchers have also demonstrated this whereby elevated stool leukocyte above 10/high power field was highly predictive of bacterial diarrhea [26,30-32]. Other studies have further demonstrated that invasive bacterial pathogens such as *Shigella spp* have a more inflammatory response with elevated stool leukocyte compared to non-invasive organisms such as *Cholera spp*.

Abdominal pain was not among the predictors of bacterial pathogens in our study. This is contrary to the general belief and findings from other studies that have shown abdominal cramps to be among the indicators of bacterial enteric pathogens [25,33]. Because the majority of our study participants were children below 2 years of age, probably it was difficult for parents to tell if they were crying because of abdominal pain or something else.

In our study, we could not find any relationship between elevated serum C-Reactive Protein (CRP) and the presence of bacterial pathogens. Other studies have demonstrated moderate sensitivity and specificity of CRP in distinguishing bacterial from other causes of diarrhea in children [30,34,35] but compared to our study, in these studies, the CRP cut off point values were set much higher to give significant results.

Most of the bacterial isolates were resistant to commonly used antibiotics such as erythromycin and co-trimoxazole but sensitive to ciprofloxacin and ceftriaxone. Although the number of bacterial cases was not enough to generalize the results, it conforms with the current trend of microbial resistance to the commonly used antibiotics, which has been demonstrated by other studies in Tanzania [3,17]. This could be due to the irrational use of antibiotics during the management of infections whereby these antibiotics are prescribed even in the absence of convincing evidence for the presence of bacterial infections.

In conclusion, fever, elevated stool leukocyte, and elevated stool red blood cells are significant predictors of bacterial enteric pathogens in children with acute diarrhea. These parameters may be used by clinicians in a resource-limited setting, either to provide supportive treatment for diarrhea or perform further confirmatory tests such as blood culture do identify the etiology and provide appropriate treatment. Further studies should be conducted to determine local antimicrobial resistance patterns, and establish functional antimicrobial stewardship bodies.

## ACKNOWLEDGMENTS

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