



Bacterial and Parasitic Etiologies and Antibiotic Susceptibility Pattern among Children with Diarrheal Disease at Adama Hospital Medical College, Adama, Ethiopia

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ABSTRACT

Background: Diarrheal disease is a major health problem worldwide, especially in developing country like Ethiopia. The contribution of the various pathogens of diarrhea may differ substantially between regions depending on local meteorological, geographic and socio-economic conditions.

Objective: The aim of this study was to investigate bacterial and parasitic etiologies and antibiotic susceptibility pattern among children under the age of 15 years at Adama hospital medical college.

Materials and methods: A cross-sectional study was conducted at Adama hospital medical college. A total of 301 were recruited. A consecutive sampling technique was employed. Stool samples were collected with a sterile clean and leak-proof plastic container from children who came with diarrheal disease. SPSS software version 21 was used for the analysis. Multivariable logistic regression was used to identify factors associated with parasites and bacterial isolates. Adjusted odds ratio at 95% confidence interval and p-value was used to measure the strength of association. P-value less than 0.05 were considered as significant association.

Results: A total of 301 cases with diarrheal disease were included in this study. Parasites and bacterial isolates were detected in 44 (14.6%) and 34 (11.2%) cases respectively. The majority of bacterial enteropathogens isolates were *Shigella* spp 17 (5.6%) followed by enterohemorrhagic *E.coli* O157:H7 13 (4.3%) and *Salmonella* spp 4 (1.3%). *G. lamblia* was detected in 19 (6.3%) cases. *E.coli* and *Shigella* were found to be resistant for trimethoprim by 92.3% and 87.5% respectively. All bacterial isolates were sensitive to ciprofloxacin, ceftriaxone, azitromicine, cefapim and cephalosin.

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The most common associated factors for prevalent of bacterial and parasite isolates were age, gender, number of household members and number of rooms in house.

Conclusion: The isolated bacterial causative agents of diarrhea among children under 15 years age were *Shigella* spp 17 (5.6%), *E.coli* 0157 11 (4.3) and *Salmonella* spp 4 (1.3%) and were resistant for one or more drugs typically commonly prescribed drugs such as cotrimoxazol, augmentin and ampicillin.

Keywords: Diarrhea; Enteric pathogens; Antibiotics resistant; Adama

INTRODUCTION

Globally 530,000 children under 5 years old are dying per year due to diarrheal disease. It is a common cause of death in developing countries and the second most common cause of infant deaths worldwide. Infectious diarrhea, particularly due to enteric bacteria pathogen and parasite is a major health problem worldwide.

Diarrhea is a significant health problem worldwide, especially in the developing world where adequate sanitation facilities are lacking. Acute diarrheal disease is a major public health problem throughout the world, with over two million deaths occurring each year and affecting mostly children under 15 years of age in developing countries [1].

The main etiologies of the diarrhea are related to a wide range of bacteria such as *Campylobacter jejuni*, *Escherichia coli*, *Salmonella* spp, *Shigella* spp, *Vibrio cholerae*, *Yersinia enterocolitica* and *Aeromonas* spp. Enteroparasites (*Giardia* spp., *Cryptosporidium* spp. and *Entamoeba histolytica*) and viruses (Adenovirus, Norwalk virus and Rotavirus. Food intolerance, reactions to medicines such as antibiotics and antacids containing magnesium may also contribute to diarrhea. The main cause of death from acute diarrhea is dehydration, which results from loss of fluid and electrolyte in stool.

The frequency and severity of diarrhea is aggravated by lack of access to sufficient clean water and sanitary disposal of human waste, inadequate feeding practices, hand washing, poor housing conditions and lack of access to adequate and affordable health care.

Africa and South Asia are home to more than 80% of child deaths due to diarrhea. Just 15 countries account for almost three quarters of all deaths from diarrhea among children under five years of age annually; India, Nigeria, democratic republic of the Congo, Afghanistan, Ethiopia, Pakistan, Bangladesh, China, Kenya, Niger, Burkina Faso, untied republic of Tanzania, Mali and Angola were ranked by WHO in 2009 from first to fifteen respectively [2].

According to the Ethiopian demographic health survey of 2011, diarrheal disease was the first leading cause for 31% of child illness among children of under 15 years old, followed by acute respiratory infection (7%). During periods of draught or famine, childhood diarrhea becomes the leading cause of death across all ages in Ethiopia.

Studies conducted in Ethiopia, documented many socioeconomic factors such as overcrowding and low maternal education, poor sanitation, contaminated water, failure to

continue breast feeding until one year of age, using infant bottles which are difficult to clean, storing food at room temperature, failure to wash hands, failure to dispose of faces hygienically and inadequate food hygiene were associated with a high incidence of under fifteen diarrheal disease [3].

The evolution of antibiotic resistance in bacteria is a topic of major medical importance. Antimicrobial resistance in enteric pathogens is of great importance in the developing world where the rate of diarrheal diseases is highest. The resistance of enteropathogenic bacteria to commonly prescribed antibiotics is increasing both in developing as well as in developed countries. Resistance has emerged even to newer, more potent antimicrobial agents.

This study aimed to assess bacterial and parasitic etiologies and antibiotic susceptibility pattern of isolated bacteria among pediatric patients with diarrheal disease at Adama hospital medical college [4].

MATERIALS AND METHODS

A cross-sectional study was conducted at Adama hospital medical college among children with diarrheal disease. The sample size for the study was determined using a single population proportion formula by the following assumptions; 95% of confidence interval, 5% of margin of error and prevalence of diarrheal disease under the age of five years is 0.147. The total sample was 301. A consecutive sampling technique was employed [5]. Stool samples were collected with a sterile clean and leak-proof plastic container from children who came with diarrheal disease. The specimen were transferred to cary blair medium and examined for parasitological examination. A wet mount was prepared and examined for each sample and observed under light microscope for cyst, ova and trophozoites of various parasites. The specimen was also observed for inflammatory cells, WBC and RBC associated with diarrhea at AHMC. The samples were cultured on differential and selective media for bacterial cultivation in order to isolate bacterial enteropathogens. All specimens were placed transported to examined at Adama public health research and referral laboratory center where they were inoculated into *Salmonella-Shigella* medium (SS agar), Xylose-Lysine-Deoxycholate (XLD) agar/Hektoen Enteric (HE) agar, Sorbitol Macconkey medium (10 ug), thiosulfate citrate bile salt sucrose agar TCBS agar.

The culture plates were incubated aerobically at 37°C for 18-24 hours. The significant growth colonies were examined morphologically for size, shape and ability to ferment lactose and sorbitol. Further identification of enteric bacterial

pathogens was done by subculture of single colony to multiply which were used for biochemical test and antimicrobial susceptibility test [6]. Control strains were *E. coli* ATCC25922, were performed parallel to test to assure the isolation. The isolated pathogens were identified using conventional biochemical tests. The isolates were grouped into Lactose Fermenting (LF) and Non-Lactose Fermenting (NLF) colonies which were then characterized based on the following standard biochemical tests by indole test, urease test, manitol broth, hydrogen sulphide production and gas production test (using triple sugar iron agar), citrate utilization test, motility test, carbohydrate fermentation test, malonate test-, Lysine Decarboxylase Test (LDC). oxidase test was used to differentiate *Shigella* which is oxidase negative from pseudomonas, oxidase positive after subculture pinkish colonies from XLD medium [7].

Pure colony of isolated bacterial organism was mixed with normal saline and measured at 0.5 Mcfarland standards for susceptibility testing. Susceptibility was determined by using Mueller Hinton agar. The following antibiotics were used to screen for the susceptibility of the isolates; ciprofloxacin-CIP (5 µg), augmentin-AUG (30 µg), gentamicin-GN (10 µg), chloramphenicol-C (30 µg) and trimethoprim-sulphamethoxazole-SXT (30 µg), Ampicillin-AM (10 µg), naldilic acid, cefraxom- FEP (30 µg), azithromycin AZT (30 µg), augmentin cefatazidin and after incubation, the diameter of each inhibition zone was measured with a pair of calipers and recorded in mm. The results then interpreted according to CLSI guidelines antimicrobial susceptibility breaking points 2016 recorded as Sensitive (s), Intermediate (I) or Resistance(R).

Data was entered to epi-info software version 7.1 and transferred to SPSS software version 21. Descriptive summaries using frequencies and proportions were used to present the study results. Multivariable logistic regression was used to identify factors associated with parasites and bacterial isolates. Adjusted odds ratio at 95% confidence interval and p-value was used to measure the strength of association. P-value less than 0.05 were considered as significant association.

RESULTS

Socio demographic characteristics

A total of 301 children were recruited in this study. Most of them (87%) were between 0-5 years old and 12% of them were 10-15 years old. The mean age was 3.41 years. Nearly half of children’s parents (49%), had monthly income less than 1500 Ethiopian Birr (ETB). Half of parents (51.2%) said that they live in a single room house. Most of them (86.4%) use pit latrine.

Hygiene and environmental health characteristics

From the total subjects, half of them live in a single room (51.2%). Most of children (86.7%) were cared by their mothers. Almost all children use tap water, 99.7%. It was found that 94.7% of them didn’t use any method of water treatment. Most of them (86.4%) use pit latrine. Most of them (86.7%) have

clean type of latrine while few of them (8.3%) have very clean type of latrine [8].

Clinical feature of children

It was said that 34.2% children had more than three time frequency of diarrhea per day the other diarrhea and 33.6% had mucoid type of diarrhea. It was reported that nearly half of them (50.5%) had vomiting. Children who had more than three days of diarrhea were 34.2%.

Prevalence of pathogens among children with diarrheal disease

Protozoan parasite: Among total children who had diarrheal disease, 25 (8.3%) had *E. histolotica* parasite. It also found that *G. Lamblia* was detected in 19 (6.3%) children. Whereas 131 (43.5%) of them had no ova or parasite (Figure 1).

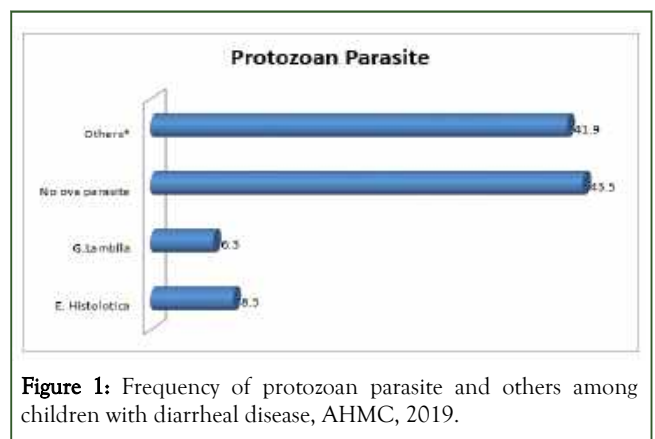


Figure 1: Frequency of protozoan parasite and others among children with diarrheal disease, AHMC, 2019.

Bacterial isolates: From the total isolated specimen, the common bacterial pathogen was *Shigella*, 17 (5.6%). It was detected that *Salmonella* spp and *E.coli* 0157 pathogens were presented by 4 (1.3%) and 13 (4.3%) respectively (Figure 2).

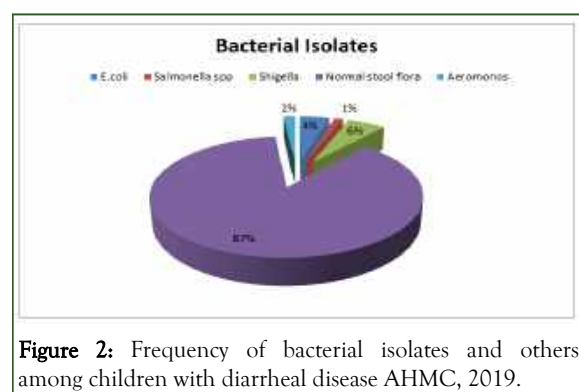


Figure 2: Frequency of bacterial isolates and others among children with diarrheal disease AHMC, 2019.

Distribution of pathogens

Among 301 children, 25 (8.3%) of them were presented with *E. histolotica*. From these, 20 (80%) of them were 0-5 years old. Among children who were detected for *G. lamilla*, 16 (84.2%) of them were 0-5 years old. Similarly, children who were presented

with *Shigella* 15 (88.2) and *E.coli* 12 (92.3) were 0-5 years old. *E.coli* was isolated by 12 (92.3%) from those who don't use water treatment. *E. histolytica* was detected in 20 (80%) male children while 5 (20%) in female children.

The most common associated factors were age, gender, number of household members and number of rooms in house. Among 301 children, *E. histolytica* was seen for 25 (8.3%) children. From these, 20 (7.6%) of them were 0 years to 5 years old. It was found that children who were 0 years to 5 years old were more likely at risk for *E. histolytica* by 4.0 times (COR=4.0, CI 95% (1.0, 16.1), AOR=3.4 CI 95% CI (08, 16.1) p-value 0.048) [9].

Drug sensitivity test for bacterial isolates

Drug sensitivity test was done for *E.coli*, *Salmonella* and *Shigella* bacterial isolates. *E.coli* was found to be resistant for trimethoprim (12/92.3%), augmentin (12/92.3%), azitromycin (1/7.7%) and gentamycin (1/7.7%). *Shigella* was found to be resistant for trimethoprim 14 (87.5%) and ampicillin 14 (87.5%).

DISCUSSION

Among the protozoan infection specifically *E. histolytica* was the most frequently causing diarrhea in children with an overall rate of (8.3%) which was lower with another study in Addis Ababa accounting 25.8%. It is may be due to seasonal variation of data collection time. However, the present result is higher than a study conducted in western Ethiopia; Jimma which was 0.8%. This big difference might be due to geographic variation and variation in age group [10].

The predominant enteric bacterial pathogen isolated in this study was *Shigella* spp. accounts 5.6%. In this study was higher with study done in Jimma, southwest Ethiopia which count 2.3% but lower than in other study in Jimma which account 8.8% from the same study. This finding is also higher compared to other studies including Kenya 4.0%. This difference could be due to sample size and study period.

The other bacterial etiology in this study was *Salmonella* spp. It accounts 1.3% which is similar to study done in Addis by Wagi, et al. (1.3%) in contrast lower than study done in Addis Ababa by Zeleke, et al. (2.8%) and in Bahir Dar town 7.8%. This is may be due to that sample use in Addis Ababa lower than in our study [11].

One major problem of developing countries the emergences of increased antimicrobial resistances not only in developing countries but also it is a global challenge. The causes of Antimicrobial Resistance (AMR) in developing countries are more complex and may be rooted in the practices of health care. All *Shigella*, *Salmonella* and *E.coli* species isolates from this study displayed resistance to one or more drugs including trimethoprim, tetracycline, augmentin, ampicillin and trimethoprim. *Shigella* spp in our study was resistant for trimethoprim (87.3%), ampicillin (84.6%), augmentin (76.4%), tetracycline (72.2%) and cefuroxime (73.3%). Study done in Addis Ababa the antimicrobial resistance pattern of *Shigella* spp. Against trimethoprim-sulphamethoxazole, augmentin, gentamycin and ciprofloxacin were 68.2, 36.4%, 9.0% and 0%

(intermediate 4.5%), respectively. It is in line with our study this is may be due to the same antibiotic usage pattern.

Antibiotic resistance of *Shigella* spp against tetracycline (72.2%) was comparable with a study done in Jimma which count 63.6%, in Harar which count 70.6% but lower than a study done in Gondar university teaching hospital 86% in Hawassa which count 90%. This may be due to those strains moderately susceptible for tetracycline at certain corner of the country. The antibiogram of *E. coli* 0157:H7 in our study showed resistance of 92.3% for trimethoprim-sulphamethoxazole, 92.3% to augmentin, 76.9% to tetracycline and 84.6% to ampicillin. The study showed comparable with the study done in Bahir Dar in which high levels of antimicrobial resistance to ampicillin (86.8%), tetracycline (76%) and cotrimoxazole (76%) was documented [12].

CONCLUSION

Parasitic and bacterial infections are common public health problems among children's under 15 years of age. Those parasite and enteric bacteria were found in association with mucoid diarrhea and bloody diarrhea. The isolated parasite was *E. histolytica* (8.3%) and *G. lamblia* (6.3%). The isolated bacterial causative agents of diarrhea among children under 15 years age were *Shigella* spp 17 (5.6%), *E.coli* 0157 11 (4.3) and *Salmonella* spp 4 (1.3%) and were resistant for one or more drugs typically commonly prescribed drugs such as cotrimoxazol, augmentin and ampicillin.

LIMITATIONS OF THE STUDY

Further confirmation of *Salmonella* spp and *E. coli* 0157:H7 with serology and molecular PCR method were not done and other susceptibility test methods which would show us other strain distribution and antibiotic profile at the site.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical clearance was obtained from institutional ethics review board of Adama hospital medical college. Formal permission letters were obtained from the district health office. An informed written consent was obtained from the parents/guardians of every child before taking the stool samples.

AUTHORS' CONTRIBUTION

Abie Lemma planned, designed the study, supervised data collection, did statistical analysis and wrote the manuscript. Biruk Yeshitila, Dr. Mekonen Teferi and Dr. Tolossa Eticha participated from the conception of research idea and reviewed the proposal and manuscript.

Bedado Dula and Meryma Abdo reviewed and approved the proposal and the manuscript. Shimeles Teshome and Haile Abera participated in data collection and did all laboratory investigations. Roza Teshome did statically analysis and wrote the manuscript. The authors read and approved the final manuscript.

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