

ORIGINAL ARTICLE**Enteropathogens in Under-Five Children with Diarrhea in Health Facilities of Debre Berhan Town, North Shoa, Ethiopia****Tizazu Zenebe Zelelie^{1*}, Demissew Shenkute Gebreyes², Alemnesh Tesema Tilahun³, Hillary A. Craddock⁴, Nigus Zegeye Gishen¹****OPEN ACCESS**

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ABSTRACT

BACKGROUND: Diarrheal disease is a major cause of mortality and morbidity for under-five children in Ethiopia. The purpose of this study was to investigate the behavioral and socioeconomic risk factors, etiology, and drug susceptibility of bacteria isolated from under-five children with acute diarrhea who were treated at Debre Berhan Referral Hospital or Health Center in Ethiopia.

METHODS: A health facility based cross-sectional study design was used to investigate enteropathogens from 163 under-five children with acute diarrhea. After obtaining written consent from parents or guardians, data were collected using a standardized questionnaire. Freshly passed stool samples were collected for microbiological tests for bacteria and parasites. The chi-square test was used for assessing the relationships of variables.

RESULTS: Enteropathogens were detected among 55.8% (91/163) participants. There was a 46%(75/163) bacterial culture positivity rate and a 9.8%(16/163) prevalence of parasites. The isolated enteropathogens were *Escherchia coli*, *Klebsiella specie*, *Proteus species*, *Salmonella species*, *Shigella species*, *Enterobacter species*, *Giardia lamblia*, *Enteameba histolytica*, *Ascaris lumbricoides*, *Trichuris trichiura* and *Hymnoleps nana*. Level of antimicrobial resistance of bacterial isolates ranged from 0 to 87.2%. Poor hand washing and poor cleaning of feeding utensils showed significant association with the presence of enteropathogens.

CONCLUSION: Bacterial enteropathogens with drug resistance were observed in this study. Continuous health education and promotion about diarrheal disease for mothers/caretakers and regular surveillance of entropathogenes are recommended to reduce under-five mortality. **KEYWORDS:** Under-five children, Diarrhea, enteropathogen, drug resistance, Ethiopia

INTRODUCTION

Infectious diarrheal diseases are of great concern, as they are responsible for three million deaths annually as well as considerable morbidity (1). In 2015, diarrhea was the 9th leading cause of death globally, at about 1.3 million deaths per year, but the fourth leading cause in children of under-five years, with roughly 499,000 deaths (2). The highest mortality rate was in sub-Saharan Africa and

Asia (2), and if this trend continues, it is estimated that 4.4 million children under the age of five will die from infectious diseases by 2030 (3). The World Health Organization (WHO) reported that Africa and South-East Asia account for 78% of all diarrheal deaths among children in the developing world. Sub-Saharan Africa, which includes Ethiopia, has the highest rates of child mortality due to diarrhea (4,5). Poor environmental conditions, socioeconomic status and behavioral factors are all strongly associated with the risk of diarrheal disease transmission (6).

Although diarrhea is caused by a variety of infectious agents, bacterial enteropathogens are among the leading causes in developed and developing countries. Bacterial pathogens, including *Escherchia coli*, *Shigella species*, *Salmonella species*, *Proteus species*, *Yersinia species*, *Vibrio cholera* and *Campylobacter species* are some of the common infectious agents which cause enteric disease in under-five children (7-16). Intestinal parasitic infections are also common worldwide. It is estimated that 3.5 billion people around the world, the majority of which are children, are affected as a result of these infections. These infections cause diarrhea in 30-80% of patients, and the most common etiologic agents include *Giardia lamblia*, *Entamoeba histolytica*, *Cyclospora cayetanensis*, *Cryptosporidium*, *Ascaris lumbricoides*, *Trichuris trichiura*, *Blastocystis hominis* and *Hymnolepis nana* (8, 9,11,18,19,20).

One significant challenge regarding the treatment of diarrheal diseases is antimicrobial resistance due to indiscriminate use of antimicrobials (15). Many studies have shown that enteropathogens can develop such resistance. For example, resistance of *E. coli*, *Salmonella species*, *Campylobacter species* and *Shigella species* isolates to ampicillin and trimethoprim-sulfamethazole has been observed (16). Many of these studies also show that resistance patterns can vary and change over time (10,11,15,16, 21-26).

In Ethiopia trends of percentage of children under the age five who had diarrhea in 2 weeks showed reduction, like for example from 24% in

2000 to 12% in 2016 (27). However, diarrhea is still one of the major contributors to death of children under the age five years (28,29) with recently reported 22% prevalence of diarrhea in this age group in the country (29). Overall, Ethiopia has the fifth highest burden of pneumonia and diarrhea in the world (30). There has been substantial prior research on the prevalence and determinant factors of diarrhea, with several occurring in Ethiopia (6,31-35). These studies show that the frequency and severity of diarrhea could be aggravated by lack of sanitary disposal of human waste, inadequate feeding practices and hand -washing, poor housing conditions, and lack of access to adequate and affordable health care (31). In the country, only 57% and 4% of rural households have access to improved drinking water sources and improved toilet facility respectively (27).

Studies have been carried out in various parts of Ethiopia to investigate the influence of various behavioral factors on diarrheal illness. However, this study is novel in that it investigated behavioral and socioeconomic factors, etiological agents and antibiotic resistance among under-five children with acute diarrhea in health facilities of Debre Berhan town, Ethiopia.

MATERIALS AND METHODS

Study design: A health facility based, cross-sectional study design was utilized. The study was conducted at the Debre Berhan Referral Hospital and Health Center in Debre Berhan, Ethiopia, between November 2015 and August 2016. Debre Berhan is a town in Central Ethiopia. Participants were recruited as they came to the Pediatric Outpatient Department for treatment of acute diarrhea. The sample size (N) was determined using the single population proportion

formula:
$$N = \frac{(Z_{\alpha/2})^2 P(1-P)}{d^2}$$
, and based on assumptions of $Z_{\alpha/2} = 1.96$ at the 95 % level of confidence, taking higher margin of error, $d=7.6\%$ (because of resource limitation), prevalence of enteropathogens in under-five children (P) = 0.635 (36) and 5% non response

rate. Ultimately, 163 children of under-five years of age were enrolled in the study. Data on general socio-demographic characteristics and acute diarrhea exposure factors were collected by a structured questionnaire, and fecal samples were collected for analysis from each patient. Additional clinical data were extracted from patient records.

Sample Processing

Stool sample collection and transportation:

Stool samples were collected by trained nurses from children with acute diarrhea. These samples were collected in a sterile basin in the toilet. Each stool sample for bacterial isolation was immersed into a test tube containing Cary Blair medium (Oxoid). For parasite isolation, stool samples were collected in two additional sample cups, one with and one without 10% formol-saline. The samples were stored in an ice chest with ice packs and transported to the Microbiology Laboratory within two hours of collection. In most cases, the samples were processed immediately after arrival, and otherwise, they were stored at -20°C until processing.

Isolation and characterization of bacteria:

Stool samples were first plated onto supportive enrichment media (Blood agar and Selenite broth, both from Oxoid). Then the bacterial colonies were sub-cultured into a slightly selective and differential media (MacConkey agar and Eosin–methylene blue agar (EMB, from Oxoid). Finally the colonies were sub-cultured onto a moderately selective media, Xylose Lysine Deoxycholate (XLD, from Oxoid), and Salmonella-Shigella (20). All were incubated at 37°C for 24 hours.

All the suspected isolates were tested via Gram staining method and examined biochemically using oxidase test, lysine decarboxylase test, urease test, citrate test, hydrogen sulfide gas production, fermentation test, and motility test to identify the significant characteristic of bacteria according to the standard methods (19,37). Based on culture results and microscopic and biochemical characteristics, bacterial isolates were identified to the genus level.

Antimicrobial susceptibility patterns were determined by disk diffusion, which was done according to the guideline of Clinical and Laboratory Standards Institute (CLSI) (38). The antibiotics tested against bacteria were the commonly prescribed antibiotics. These were Ampicillin (AM, $10\mu\text{g}$), Amoxicillin (AMX, $10\mu\text{g}$), Cephalothin (CF, $30\mu\text{g}$), Chloramphenicol (C, $30\mu\text{g}$), Ciprofloxacin (CIP, $5\mu\text{g}$), Gentamycin (GM, $10\mu\text{g}$), Nalidixic acid (NA, $30\mu\text{g}$), Ceftriaxone (CRO), Tetracycline (TE) and Trimethoprim-Sulphamethazole (SX). According to the size of the zone of inhibition, the organisms were classified as sensitive, intermediately sensitive, or resistant to each antibiotic using CLSI interpretation guideline (38).

Isolation and characterization of parasites:

Microscopic examination of stool was done by preparing a slide using Normal Saline and Lugol's Iodine to observe the ova, cysts and trophozoite stage of the parasites present in the stool. Samples were examined under a magnification of 40x to detect ova and cysts of parasites, and parasites were identified to the species level (37).

Quality control: The researchers strictly adhered to Standard operating procedures (SOPs) during sample processing. Sterility tests were done for prepared culture media. Control organisms (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853) were used in each relevant procedure.

Data management and analysis: Data were entered and analyzed using SPSS version 20 software. The magnitude (prevalence) of each enteropathogen was summarized. Variables with a p-value of ≤ 0.05 were considered a statistically significant.

Ethics approval: Ethical clearance was obtained from the Ethics Review Committee of the Research and Community Service of Debre Berhan University. Official letter was given to the health facilities to create a smooth environment. Written informed consent was obtained from each child's parent or guardian. The results were communicated to the attending physician for providing treatment for children with diarrhea.

RESULTS

Socio-demographic factors: A total of 163 under-five children with acute diarrhea were included in the study. About 92(56.4%) of the children came from Debre Berhan, and the rest (43.6%) were from outside the town. About two-thirds (68.1%) of the guardians or parents were

females, and the majority (96.9%) of the guardians or parents were married. Socio-demographic data of the guardians or parents are presented in Table 1. The mean age of the study participants was 1.6 years (SD \pm 1.032). Further socio-demographic and clinical data are presented in Table 2.

Table 1: Socio-demographic data of the guardians or/parents in two Debre Berhan Health Facilities from November 2015 to August 2016.

Variable for the guardian/parents	Response	Frequency	Percent (%)
Marital status	Currently married	158	96.9
	Currently single	5	3.1
Religion	Orthodox	117	71.8
	Muslim	26	16.0
	Protestant	20	12.3
Ethnicity	Amhara	98	60.1
	Oromo	28	17.2
	Tigre	37	22.7
Educational status	Illiterate	21	12.9
	Primary school	19	11.7
	Secondary school	22	13.5
	College or university	101	62.0
Work status	Unemployed	26	16.0
	Daily worker	17	10.4
	Monthly salary	72	44.2
	Others ¹	48	29.4

Table 2: Socio-demographic and clinical data of under-five children who presented to two Debre Berhan, Ethiopia Health Facilities from November 2015 to August 2016.

Variables for the child		Frequency	Percentage
Sex	Male	99	60.7%
	Female	64	39.3%
Age in months	0-12	75	46.0%
	13-24	52	31.9%
	25-60	36	22.1%
Fever	No	78	47.9%
	Yes	85	52.1%
Stool appearance	Bloody	18	11.0%
	Mucoid	82	50.3%
	Watery	63	38.7%
Treatment in the last two weeks	No	103	63.2%
	Yes	60	36.8%

¹ Other includes farmer, merchant, students

Associated variables related to child diarrhea were assessed, and it was found that only hand-washing practices and poor cleaning of utensils

used in feeding the child were statistically significant for the presence of enteropathogens in stool samples (Table 3).

Table 3: Results of chi-squared test comparing socioeconomic and demographic factors to diarrhea outcomes in under-five children who presented to two Debre Berhan, Health Facilities from November 2015 to August 2016

Variables	Enteropathogen (n=91), n(%)	P-value	Bacterial isolates	P-value	Parasites	P-value
Hand washing practice						
Poor	58(77.3%)	P=0.000	43(57.3%)	P=0.006	15(20.0%)	P=0.000
Good	33(37.5%)		32(36.4%)		1(1.1%)	
Defecation site						
Traditional pit latrine	63(62.4%)	P=0.099	51(50.5%)	P=0.338	12(11.9%)	P=0.488
Ventilated pit latrine	17(44.7%)		15(39.5%)		2(5.3%)	
Open field	11(45.8%)		9 (37.5%)		2(8.3%)	
Latrine cleaning frequency						
Every day	12(52.2%)	P=0.188	10(43.5%)	P=0.294	2(8.7%)	P=0.904
1-2 times per week	27(47.4%)		22(38.6%)		5(8.8%)	
Not cleaned	52(62.7%)		43(51.8%)		9(10.8%)	
Contact with domestic animals						
No	39(54.2%)	P=0.412	34(47.2%)	P=0.457	5(6.9%)	P=0.204
Yes	52(57.1%)		41(45.1%)		11(12.1%)	
Cleaning of utensil for child feeding						
Poor	59(83.1%)	P=0.000	44 (62.0%)	P=0.000	15(21.1%)	P=0.000
Good	32(34.8%)		31(33.7%)		1(1.1%)	

Enteropathogens in under-five children:

Enteropathogens were detected from 91(55.8%) of the 163 stool samples from children and infants. There was 46%(75/163) culture positivity and 9.8% (16/163) prevalence of parasites. The most predominant bacterial isolate was *Escherchia coli* (62.7%), followed by *Klebsiella specie* (14.7%), *Proteus species* (9.7%), *Salmonella species* (6.7%), *Shigella species* (4%) and *Enterobacter species* (2.7%) (Table 4). The isolated parasites were *Giardia lamblia*, *Enteameba histolytica*, *Ascaris lumbricoides*, *Trichuris trichiura* and *Hymnoleps nana*. The prevalence varied based on the clinical presentation of diarrhea. In bloody diarrhea cases, *Giardia* and *Shigella* were the most common. In samples with watery diarrhea, *E. coli* was the most common, and in mucoid diarrhea cases, *Klebsiella* and *Proteus* were the most common. Overall enteropathogens prevalence is indicated in Table 4.

Antimicrobial susceptibility test results:

Overall, antimicrobial resistance of the isolates ranged from 0 to 87.2%. High resistance was seen to ampicillin, tetracycline, nalidixic acid and trimethoprim-Sulphamethazole. With the exception of two *E. coli* isolates, all samples were susceptible to Ciprofloxacin. *E. coli* showed more resistance to antibiotics; however, there was a wide range of resistance. For example, 4.3% of the samples were resistant to Ciprofloxacin, while 87.2% of the samples were resistant to Trimethoprim-sulphamethazole. As seen in Table 3, the other pathogens also exhibited a wide range of resistance. *Salmonella species* ranged from 0-80% resistance, 0-66.7% in *Shigella species*, 0-81.8% in *Klebsiella species*, 0-57.1% in *Proteus species* and 0-50% in *Enterobacter species*.

The majority of the bacterial isolates were resistant to more than three antibiotics. Overall, thirteen, two and one distinct antibiogram (resistance pattern) were observed in *Escherchia*

coli, *Salmonella species*, and *Klebsiella species* respectively (Table 5). The resistance pattern of *Escherichia coli* varied from 3-6 drugs. Forty of the total *E. coli* isolates were multi-drug resistant (resistant to 3 or more drugs). Two of the

Salmonella species and five *Klebsiella species* isolates were multi-drug resistant. *Shigella species*, *Proteus species* and *Enterobacter species* did not show antibiogram patterns.

Table 4: Prevalence of pathogens in stool from under-five children who presented to two Debre Berhan, Health Facilities from November 2015 to August 2016.

Pathogen	Samples testing positive (n=91)	Samples testing positive with bloody diarrhea (n = 18)	Samples testing positive with watery diarrhea (n = 63)	Samples testing positive with mucoid stool appearance (n = 82)
<i>Eschechia coli</i>	47(51.6%)	7(38.9%)	31(49.2%)	9(11.0%)
<i>Salmonella spp</i>	5(5.5%)	1(5.5%)	1(1.6%)	2(2.4%)
<i>Shigellaspp</i>	3(3.3%)	3(16.6%)	0	0
<i>Klebsiellaspp</i>	11(12.1%)	0	8(12.7%)	3(3.6%)
<i>Proteus spp.</i>	7(7.7%)	0	4(6.3%)	3(3.6%)
<i>Enterobacter spp.</i>	2(2.2%)	0	2(3.2%)	0
<i>Giardia lamblia</i>	5(5.5%)	3(16.6%)	2(3.2%)	0
<i>Enteamebahistolytica</i>	4(4.4%)	0	2(3.2%)	2(2.4%)
<i>Ascarislumbricoides</i>	3(3.3%)	0	0	3(3.6%)
<i>Trichuristrichiura</i>	2(2.2%)	0	0	2(2.4%)
<i>Hymnoleps nana</i>	2(2.2%)	0	0	2(2.4%)

Table 5: Antibiotic resistance of bacterial isolates from stool samples from November 2015 to August 2016.

Name of isolates	Number of strains (%) resistance to antibiotics									
	AMX	AM	C	CF	CIP	GM	CRO	TE	NA	SX
<i>Eschechia coli</i>	5 (10.6)	29 (61.7)	20 (42.6)	8(17)	2(4.3)	10 (21.3)	4(8.5)	38 (80.8)	7 (14.9)	41(87.2)
<i>Salmonella species</i>	2(40)	0(0)	0(0)	0(0)	0(0)	1(20)	0(0)	2(40)	4(80)	2(40)
<i>Shigella species</i>	0(0)	1 (33.3)	1 (33.3)	0(0)	0(0)	0	0(0)	2 (66.7)	0(0)	1(33.3)
<i>Klebsiella species</i>	4(36.4)	3 (27.3)	2 (18.2)	2(18.2)	0(0)	4 (36.4)	0(0)	9 (81.8)	1(9.1)	8(72.7)
<i>Proteus species</i>	3(42.9)	2 (28.6)	1 (14.3)	0(0)	0(0)	3 (42.9)	1(14.3)	2 (28.6)	4(57.1)	2(28.6)
<i>Enterobacter species</i>	0(0)	1(50)	0(0)	0(0)	0(0)	1(50)	0(0)	0(0)	1(50)	0(0)

AMX: Amoxicillin; NA: Nalidixicaci; AM: Ampicillin; CF: Cephalothin; TE: Tetracycline; C: Chloramphenicol; CIP: Ciprofloxacin; SXT: Trimethoprim-Sulphamethazole; GM: Gentamicin; CRO: Ceftriaxone

Table 6: Multi-drug resistance antibiogram pattern of bacterial isolates in two Debre Berhan, Health Facilities from November 2015 to August 2016

Isolates	Resistance antibiogram	Number	
<i>Eschechia coli</i>	CIP, NA, SX	2	
	C, TE, SX	10	
	AMX, CRO, NA, SX	2	
	AMX, AM, C, TE	2	
	AM, GM, TE, SX	4	
	C, TE, NA, SX	2	
	AM, C, CRO, TE	2	
	CF, CIP, GM, TE	2	
	AMX, AM, CF, TE, NA	2	
	AMX, AM, CRO, TE, NA	2	
	AM, C, CF, TE, NA	2	
	AM, C, GM, TE, SX	6	
	AM, CIP, GM, TE, NA, SX	2	
	<i>Salmonella species</i>	AMX, TE, NA	2
		AMX, GM, TE, SX	3
<i>Klebsiella species</i>	AM, GM, TE, SX	2	

AMX: Amoxicillin; NA: Nalidixicaci; AM: Ampicillin; CF: Cephalothin; TE: Tetracycline; C: Chloramphenicol; CIP: Ciprofloxacin; SXT: Trimethoprim-Sulphamethazole; GM: Gentamicin; CRO: Ceftriaxone

DISCUSSION

Diarrhea remains one of the major illnesses in under-five children, and enteropathogens play significant roles as etiologic agents. In this study, the burden of bacterial and parasitic agents in children suffering from diarrhea, drug susceptibility pattern of bacterial isolates, and social and behavioral factors were investigated. This study additionally builds and expands on a previous study done in the area (6). However, the previous study did not investigate hand-washing or the washing of utensils used for feeding; so we are unable to compare these findings directly. On the other hand, another study done in Addis Ababa revealed that hand-washing with soap after defecation and before preparing food was associated with acute diarrhea (39). In agreement to this, the present study found that hand-washing is associated with occurrence of enteropathogens.

Since contact with domestic animals was associated with disease in the previous study (6), it was an unexpected finding that was not significant in this study. Agriculture is common practice around Debre Berhan that may result to have contact with domestic animals. And also a 2013 study found that 83% of diarrheatic lambs in the area tested positive for *E. coli* (34). Other

studies also found association between domestic animal contact and diarrheal disease (40). This unexpected finding may be due to the season in which data were collected. For example, children may be in greater contact with animals during different times of the year.

Two new aspects of the present study include testing for specific bacteria and parasites as well as the testing of bacterial isolates for antibiotic resistance. Enteropathogens were detected in 55.8% of the patients in this study.

Specifically, regarding the prevalence of bacteria, studies in other parts of the world show more variation, with some reporting rates similar to those seen here, some reporting lower rates (23,41-44) and some reporting higher rates (45-47). The most similar rates were from Tanzania, 67.1%(23), Mozambique, 42.2%(41), Palestine, 57.9%(42) and Burkina Faso (24). However, it contradicts with another report from Trinidad by Zobida, *et al* (43), which found a prevalence of 17.4%. This may be due to differences in handling of the specimens prior to processing, a true difference in the prevalence, or seasonal variation. For example, our study was conducted in the rainy season in a town surrounded by pastoral land.

Despite the relatively high prevalence of bacterial infection observed in our study, low prevalence (9.8%) of parasitic infections was seen among children with acute diarrhea. This finding agrees with studies done in Trinidad (43), the United Arab Emirates (48), Nigeria (46) and others (11, 49). However, there are other studies that reported a high prevalence of parasitic infections in Ethiopia (33,50) plus in Tanzania (23) and Burkina Faso (45).

The low prevalence of parasitic infection in the present study could again be due to differences in study methodology, types of enteropathogen assayed in the study, true difference of the parasitic infection in the area and seasonal variations. Among parasitic infections, *Girdia lamblia* and *Entameoba histolytica* were relatively higher than other parasites, including helminths. This may be because of their nature for easily transmitting in the community.

In most areas of developing countries, regional knowledge of antibiotic resistance profiles among various bacterial isolates would be highly valuable, as it can help inform antibiotic choices by healthcare professionals. The rate of resistance observed here was consistent with other studies (10,11,21,23,25). Significant resistance rate has been found to Tetracycline and Trimethoprim-sulfamethazole. One significant challenge was that the most common bacterial agent, *E. coli*, also exhibited the most resistance. The observed high multi-drug resistance of *E. coli* is comparable with other studies (10,23). As *E. coli* is more resistant, it may be difficult to treat and requires careful decision at choice of antibiotics. Isolates of *E. coli* that showed low resistance to amoxicillin, cephalothin, ciprofloxacin and ceftriazone were detected. In the present study, multi-drug resistance to *E. coli*, however, would be crucial to choose such treatment with care, lest *E. coli* become widely resistant to these antibiotics as well.

The high resistance of bacterial isolates in this study may be due to misuse of therapeutic doses of commonly available antibiotics and the result of widespread use of antibiotics in

agriculture, as antibiotic resistant *E. coli* have also been found on meat in abattoir studies in Ethiopia (51). The rates of resistance among *Salmonella spp* to Amoxicillin (40%), Tetracycline (40%), Nalidixic acid (80%) and Trimethoprim-sulfamethazole were found to be high which is consistent with other studies (10,16,24) but contradicts with the study done by Frederique (25). The difference may be the study period in that resistant species may be emerging through time. Two multi-drug resistant *Salmonella spp* were found. Similarly, *Shigella spp* showed resistance to Ampicillin (33.3%), Chloroamphenicol (33.3%), Tetracycline (66.7%) and Trimethoprim-sulfamethazole (33.3%), and this is a similar finding with other reports (23,24,25).

Enteropathogens were not detected in 44.2% of the study participants. This could be due to potential enteric pathogens such as viruses like norovirus and bacteria that were not tested for, such as *Campylobacter spp*, and *Yersinia spp*. Therefore, a possible limitation was the fact that the study design assayed for a limited selection of bacteria and parasites. It is possible that viral agents or other bacteria and parasites may also be important in childhood diarrhea in the area, and this question needs further investigation.

Further limitations of the study stem from the cross-sectional design. Other studies in Ethiopia have found that non-symptomatic carriage of intestinal parasites and various bacterial pathogens is common among adults (52). Thus, it is possible that the pathogens found were not the direct cause of the diarrhea. Additionally, lack of a control group makes it difficult to determine how significantly the various factors contributed to illness risk.

In conclusion, it was observed from this study that *E. coli* is predominant amongst other enteropathogens detected in children with diarrhea. It also found relatively low prevalence of *Salmonella species*, *Shigella species*, and parasites. High rate of resistance and many multi-drug resistant bacteria isolates were found. The isolation of enteropathogens was statistically associated with poor hand washing practices and

poor cleaning of utensils used to feed the children. However, a more extensive community-based cohort study is needed in order to explore the etiologic importance of this pathogen in diarrheic children. Education and outreach are critical components to reducing the burden of diarrhea observed in this study. Healthcare providers, veterinarians and farmers could work together to reduce unnecessary use of antibiotics in order to reduce the risk of resistance. It is important to continue surveillance on these microorganisms.

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