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# Prevalence and clinico-demographic characteristics associated with bacterial diarrhea among HIV positive and negative children aged below five years at Moi Teaching and Referral Hospital, Kenya

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**Abstract:** Diarrhea is the second leading cause of morbidity and mortality worldwide. It accounts for over 2 million deaths in children aged below 5 years, majority being from Sub-Saharan Africa. Diarrhea is ranked third as a cause of death and the third leading cause of pediatric admissions in Kenya. The objective of this study was to determine the prevalence and clinico-demographic characteristics associated with diarrhea in relation to patient HIV status. This was a cross-sectional comparative study of 216 HIV seropositive and seronegative children aged below 5 years admitted with diarrhea at Moi Teaching and Referral Hospital in Western Kenya. Clinico-demographic characteristics associated with diarrhea were obtained using questionnaires. Stool samples were collected and analyzed using standard microbiological methods to determine their bacterial etiology. The mean (standard deviation) 24.11(15.61) vs. 11.77(7.88), median and inter quartile range 22(10, 36) vs. 10 (6, 16) was higher among HIV positive than negative cases with no statistically significant differences with regard to gender. The prevalence of diarrhea was 17.1% with 64.4% (139) of our cases being male and 35.6% (77) female. The prevalence of bacterial and non bacterial diarrhea was 8.5% (118) and 7.1% (98) respectively. The main bacterial enteropathogens isolated among HIV positive and negative cases were *Escherichia coli 88.9*% (38 vs. 67), *Shigella* 5.1 %( 1, 5), *Salmonella* 4.2% (0, 5) *and* other enteric species 1.7% (0, 2). EAEC (17.7%) was the main diarrheagenic *E coli* (DEC) followed by EHEC (5.1%), EPEC (3.4%), EIEC (2.5%) and ETEC (1.7%). Other were *S.typhimurium* 4.2% (0, 5), S.typhi *3.4*%) (0, 4), S. *dyenteriae and S. flexneri* 2.5% (0, 3) each respectively. HIV positive individuals recorded more mixed infections (72% vs. 28%) than HIV negative cases. Some of illnesses recorded in HIV positive cases included tuberculosis 4(100%) and meningitis 8(100%) whereas pneumonia 21(71.4 % vs. 28.6), oral thrush 13 (76.9% vs. 23.1%), malaria 19 (57.9% vs. 42.1%) and protein energy malnutrition 13 (61.5% vs. 38.5 %) were recorded in both HIV positive and negative cases respectively. Education level of parent/guardian, socioeconomic status, housing, water, sanitation and seasonal variations were significantly associated with diarrhea. The study depicts a positive correlation between clinico-demographic characteristics and HIV status on prevalence and etiology of diarrhea. We recommend improved hygienic practices, definitive diagnosis of diarrhea etiology and patient HIV status for effective management of childhood diarrhea.

**Keywords:** Prevalence, Socio-Demographic, Bacterial diarrhea, HIV Status

## 1. Introduction

Diarrheal disease is a major public health problem worldwide, with over 2 million deaths each year in children aged below five years [1]. The substantial degree of morbidity and mortality due to diarrheal disease

particularly in sub-Saharan Africa is compounded by HIV/AIDS epidemic [2]. It is believed that bacterial infections in AIDS patients manifest differently from immune-competent hosts [3]. HIV prevalence in Kenya currently stands at 7.4 per cent with an estimated 70,000 - 100,000 infants exposed to HIV every year [4]. Currently, one third of total pediatric admissions in Kenya and 16% of all deaths among pediatric inpatients are diarrhea related [5]. Diarrhea is often accompanied by abdominal pain and may lead to severe dehydration, a serious and potentially life-threatening condition [4]. The causative agents of diarrhea include a wide variety of bacteria, viruses, and parasites [6]. Bacterial organisms such as *Campylobacter* spp., *Salmonella* sp, *Shigella* sp. and various pathogenic *Escherichia coli* are some of the common causes of gastrointestinal disease in children worldwide [7] although their association with HIV infections is controversial.

Some of the factors leading to increased risk of diarrhea include failure to breast-feed exclusively for the first 4-6 months of life. Diarrhea has been noted to be far greater in non-breastfed than exclusively breastfed infants [8]. Host susceptibility to infection is determined by the child's age, presence of protective maternal factors (transplacental antibodies), nutritional and immunological status, prior exposure and acquired immunity and genetic susceptibility [9]. Diarrhea as an opportunistic infection may result from immunological impairment due to current illness or immunodeficiency in persons with HIV/AIDS [10]. In rare cases, overuse of antibiotics leads to overgrowth of commensal *Clostridium difficile* which releases a toxin that causes diarrhea. Moreover, some of the antiretroviral medications in HIV patients, particularly protease inhibitors, cause diarrhea as a side-effect [9]. Children with diarrhea are at risk of dehydration and therefore early and appropriate fluid replacement is a main intervention to prevent death [11].

Although diarrheagenic organisms have been studied in different parts of the African continent, most research has targeted specific organisms and their role in the production of diarrhea with little consideration of the presence of other agents and their role in the production of inflammation which might be a considerable part of the pathogenesis of the organisms [12]. Previous studies in Kenya have documented the prevalence of bacterial agents associated with diarrhea [13-17]. However, there is limited data on clinico-demographic characteristics associated with diarrhea versus HIV status. This study aimed at bridging the gap with a view to improving diarrhea management protocols in the region.

# 2. Materials and Methods

### *2.1. Study Site*

The study was conducted at Moi Teaching and Referral Hospital (MTRH) in Eldoret, Uasin-Gishu County located about 320 km North West of Nairobi between latitude 0° 31' 54" N and longitude 35° 15' 58" E. This is the second Referral Hospital in Kenya serving the Western Kenya region including the North and South Rift Valley, Nyanza, Western Province, parts of Eastern Uganda and Southern Sudan. The USAID–Academic Model for Prevention and Treatment of HIV/AIDS (AMPATH) Partnership's clinics and laboratories located within the hospital provide free diagnosis, monitoring and treatment for HIV/AIDS patients. Kenya is a developing country located in sub-Saharan Africa, bordered by Tanzania, Somalia, Uganda, Sudan, and Ethiopia. 63% of households have access to improved drinking water sources, with large discrepancies among urban and rural residents while 75% of households do not have access to an improved sanitation facility predisposing to gastrointestinal infections [5].

### *2.2. Study Population and Research Design*

A cross sectional prospective comparative study involving 236 children aged below five years admitted with diarrhea out of a total population of 1382 inpatient children was undertaken between July 2011 and April 2012. Diarrhea was defined as the passage of loose or watery stools at least three times a day or increase in volume, fluidity or frequency of bowel movement relative to the usual pattern for a particular person [11].

### *2.3. Determination of HIV Status*

HIV status was routinely determined on admission using HIV screening rapid methods, Determine **(**Abbott, Tokyo, Japan) and Uni-Gold_ (Trinity Biotech, Ireland) at USAID-Academic Model for Prevention and Treatment of HIV/AIDS (AMPATH) laboratories located within the hospital. HIV status is categorized as positive, negative or sero-exposed cases. HIV status is defined as positive for children aged above 18 months and confirmed to be HIV antibody positive using rapid Anti-HIV–1/2 antibody ELISA, Determine **(**Abbott, Tokyo, Japan) and Uni-Gold (Trinity Biotech, Ireland), based on the criteria laid down by the Center of Disease Control and Prevention, Atlanta [18]. A HIV antibody positive case aged 0-18 months, born to a HIV positive mother was considered HIV sero-exposed while an antibody positive child aged above 18 months was HIV positive if confirmed positive by Western blot assay. Individuals who were neither HIV infected nor sero-exposed were considered HIV negative.

### *2.4. Isolation and Identification of Bacterial Enteropathogens*

Demographic data was obtained using questionnaires administered to parents/guardians who consented to the study. Stool samples were collected in leak proof containers and sent to the Microbiology laboratories at MTRH for processing to detect bacterial enteropathogens using the gold standard conventional microbiological methods [19]. The stool was examined macroscopically for consistency, color and atypical components such as mucous and blood.

A loopful of stool sample was inoculated aseptically on deoxycholate citrate Agar (DCA) and MacConkey *agar* and incubated aerobically at 37°C for 18–24 hours. The plates were observed for growth of lactose and non-lactose fermenting colonies and further identified by biochemical tests [20] including indole, methyl red, voges proskaeur, citrate utilization (IMViC) and triple sugar iron (TSI) tests. Further, serological tests were performed by slide agglutination with polyvalent and monovalent sera (Welcome Diagnostics) to detect virulent strains of *E. coli, Salmonella* and *Shigella* through their O and H antigens [19].

### *2.5. Ethical Approval*

Ethical review and approval was obtained from the Institutional Research and Ethics Committee of Moi University/Moi Teaching and Referral Hospital (FAN: IREC 000711).

### *2.6. Data Analysis*

Data was analyzed using STATA 10 and descriptive statistics including mean, median, standard deviation (SD) and inter quartile range (IQR) were used for categorical variables while frequency listings were applied for discrete variables. To obtain independence among proportions, we applied Chi square ($X^2$) test for associations between categorical variables and Kruskal-wallis non-parametric test to compare means between groups. The adopted significance level for statistical inference was 0.05.

## 3. Results

### *3.1. Prevalence of Diarrhea*

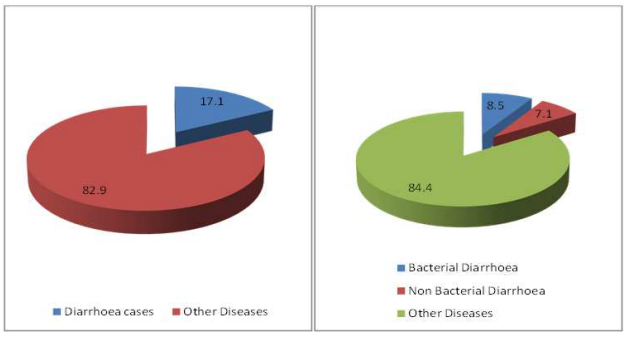


***Figure 1.*** *Prevalence of Diarrhea*

Prevalence of diarrhea among children aged below five years admitted at Moi Teaching and Referral Hospital was 17.1%. 46.2% (109) of our cases were HIV positive and 45.3% (107) HIV negative while 8.5% (20) were HIV sero-exposed cases (Figure 1). Prevalence of bacterial diarrhea (BD) was 8.5% (118) while non-bacterial diarrhea (NBD) was 7.1% (98). 34.7% (41) and 65.3% (77) of bacterial diarrhea cases were from HIV positive and negative cases respectively. NBD occurred at considerably higher frequency among HIV positive 68 (69.4%) than HIV negative cases 30 (30.6%). However, there was no difference in the frequency of diarrhea episodes per day in both categories.

### *3.2. Clinico-Demographic Characteristics of Diarrhea Cases*

The mean age and standard deviation 24.1(15.6) vs. 11.8(7.9), median (IQR) 22(10, 36) vs. 10(6, 16) was significantly higher among HIV positive compared to HIV negative children respectively (p = 0.001), (Table 1). There was a tendency for higher frequency of diarrhea in males (68.8% vs. 59.8%) than females (31.2% vs. 40.2%) although this was not statistically significant, in both categories (p = 0.164). The minimum age was (3 vs. 4) months while maximum age was (60 vs. 25) months respectively in HIV positive and negative cases. More than 50% of the households resided in mud-walled to semi-permanent dwellings commensurate to their monthly household income below 10,000 Kenya shillings. It was evident that most parents/guardians of diarrhea cases had either no education (54.9% vs. 45.1%) or primary level (56.6% vs. 43.4%) and used well/borehole water for drinking and domestic use (54% vs. 46%). Although these factors were associated with diarrhea, there was no statistical difference between HIV positive / and HIV negative cases.

Kruskal-wallis test for association between duration of diarrhea and patient HIV status revealed significant differences (p = 0.001), with higher mean duration and standard deviation 8.95(6.77) vs. 4.23(2.89) median and IQR 7(4, 14) vs. 3(3, 5) in HIV positive than negative cases. Patients either experienced diarrhea only or accompanied by vomiting and / or fever with varying frequencies but with no significant differences with regard to HIV status, (p= 0.079) There was evidence of multiple infections among HIV positive cases who presented with other illnesses including tuberculosis 4(100% vs. 0) and meningitis 8(100% vs. 0) whereas pneumonia 21(71.4 % vs. 28.6), oral thrush 13 (76.9% vs. 23.1%), malaria 19 (57.9% vs. 42.1%) and protein energy malnutrition (PEM) were recorded in both groups. This was statistically significant, (p = 0.000).

***Table 1.*** *Demographic and Clinical characteristics of Diarrhea cases*

| Variable | HIV Positive N= 109 | HIV Negative N=107 | p-value |
|---|---|---|---|
| Age of child (months) | | | |
| Mean (std) | 24.11(15.61) | 11.77(7.88) | |
| Median (IQR) | 22(10, 36) | 10(6,16) | 0.0001[2] |
| Minimum | 3 | 4 | |

| Variable | HIV Positive N= 109 | HIV Negative N=107 | p-value |
|---|---|---|---|
| Maximum | 60 | 25 | |
| Sex | | | |
| Female | 34(44.2) | 43(55.8) | 0.164[1] |
| Male | 75(54) | 64(46) | |
| Monthly household Income in Kenya Shillings | | | |
| Below 10,000 | 65(54.6) | 54(45.3) | |
| 10,000-19,000 | 30(44.8) | 37(55.2) | |
| 20,000-29,000 | 12(50) | 12(50) | 0.683[1] |
| 30,000-40,000 | 1(25) | 3(75) | |
| Above 40,000 | 1(50) | 1(50) | |
| Type of residential house | | | |
| Mud-walled | 39(50.6) | 38(49.4) | |
| Semi-permanent | 30 (50.9) | 29(49.1) | 0.921[1] |
| Stone/brick walled | 40 (50) | 40(50) | |
| Education level of mother/guardian | | | |
| None | 36 (49.3) | 37 (50.7) | |
| Primary | 47 (56.6) | 36 (43.4) | 0.276[1] |
| Secondary | 16 (40) | 24 (60) | |
| Post secondary | 10 (50) | 10 (50) | |
| Types of Drinking Water | | | |
| Piped treated water | 32 (43.8) | 41 (56.2) | |
| River | 10 (52.6) | 9 (47.3) | 0.318[1] |
| Well/borehole | 67 (54) | 57(46) | |
| Duration of diarrhea | n=109 | n=107 | |
| Mean (std) | 8.95 (6.77) | 4.23(2.89) | 0.0001[2] |
| Median (IQR) | 7(4,14) | 3(3,5) | |
| Symptoms | | | |
| Diarrhea only | 11(17.5) | 24(27) | |
| Diarrhea and vomiting | 24(38.1) | 30 (33.7) | 0.079[1] |
| Diarrhea and vomiting and fever | 28 (44.4) | 35(39.3) | |
| Diarrhea and other illness | | | |
| Meningitis | 8 (100) | 0 | |
| Pneumonia | 5 (71.4) | 2 (28.6) | |
| Protein Energy Malnutrition | 8(61.5) | 5 (38.5) | 0.000[1] |
| Malaria | 11(57.9) | 8 (42.1) | |
| Oral thrush | 10 (76.9) | 3 (23.1) | |
| TB | 4 (100) | 0 | |
| Diarrhea episodes per day | | | |
| Five times or more | 76(52.8) | 68(47.2) | |
| Four times | 27(48.2) | 29(51.8) | 0.677[1] |
| Three times or less | 6(40) | 10(60) | |
| Stool culture Result | | | |
| Positive bacterial cultures | 41(34.7) | 77(65.2) | 0.000[1] |
| Negative Bacterial cultures | 68(69.4) | 30 (30.6) | |

[1]Chi square test; [2]Kruskall-wallis test

### *3.3. Bacterial Enteropathogens Associated with Diarrhea*

Bacteriological analysis of the 118 isolates among HIV positive and negative cases (Table 2), identified the main etiological agents as; diarrheagenic *E coli* 88.9% (38 vs. 67), *Shigella species* 5.1% (1 vs. 5) *Salmonella species* 4.2% (0 vs. 5) and other enteric bacteria 1.7% (0, 2) respectively (Table 1). There was a significant difference in isolation rates of the specific enteropathogens in relation to HIV status with the three species recording higher frequencies in HIV negative than HIV positive cases ($p< 0.05$). Serotypic analysis of isolates among HIV positive and negative cases identified the following *E. coli* strains; *Enterohaemorrhagic E. coli* (EHEC) 2 (0 vs. 100%), *Enterotoxigenic E. coli* (ETEC) 2 (0 vs. 100%)), *Enteroaggregative Enterotoxigenic E. coli* (EAEC) 21 (71.4 vs. 28.6), *Enteroinvasive E. coli* (EIEC) 2 (66.7% vs. 33.3%),) and *Enteropathogenic E. coli* (EPEC) 6 (33.3% vs. 66.7%). Strains detected in *Salmonella* and *Shigella* species were *S.typhimurium* 1(0 vs. 100%), *S.typhi* 4 (0 versus 100%), S. *dyenteriae* 3 (0 vs. 100%) and *S. flexneri* 3 (33.3% vs. 66.7%), respectively in both categories.

***Table 2.*** *Bacterial Enteropathogens associated with Diarrhea*

| Bacterial Species | Pathotype | HIV Positive | HIV Negative | |
|---|---|---|---|---|
| *Escherichia coli* | EHEC | 0 | 2 (100) | |
| | ETEC | 1(33.3) | 2 (66.7) | 0.002 |
| | EAEC | 15 (71.4) | 6 (28.6) | |
| | EIEC | 2 (66.7) | 1 (33.3) | |
| | EPEC | 2 (33.3) | 4 (66.7) | |
| | Other DEC | 19 (26.7) | 52(73.2) | |
| Sub total | | 39(36.2) | 67(63.8) | |
| *Salmonella* | *S.typhimurium* | 0 | 1(100) | 0.001 |
| | *S.typhi* | 0 | 4(100) | |
| Sub total | | 0 | 5(100) | |
| *Shigella species* | *S. dyenteriae* | 0 | 3(100) | 0.002 |
| | *S. flexneri* | 1(33.3) | 2(66.7) | |
| Sub total | | 1(16.7) | 5(83.3) | |
| Other Bacteria | | 0 | 2(100) | |

Total: 41(34.7), 77(65.3)

### *3.4. Seasonal Variations versus Frequency of Diarrhea*

Seasonal variations had a significant effect on frequency of diarrhea admissions during the study period. BD cases recorded were notably higher during the dry season between November and February with a peak in December while non bacterial diarrhea was associated with the rainy season with a peak in May (Figure 3). An in-depth analysis revealed that BD episodes among HIV positive patients were lower (46% vs. 54%) in HIV negative cases during the dry months but rose sharply (73.3% vs. 26.7%) during the wet months.

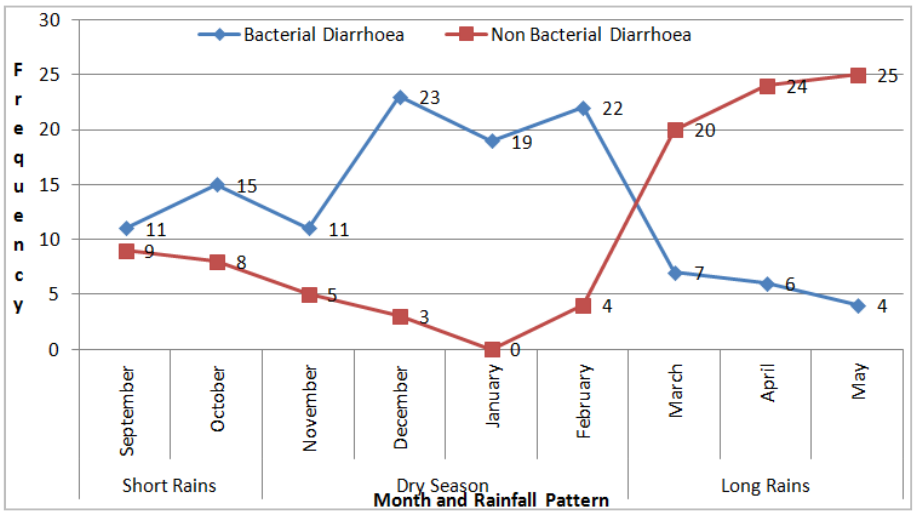


***Figure 3.*** *Frequency of Diarrhea Admissions versus Seasonal Variations*

## 4. Discussion

Acute and persistent diarrhea in infants and children aged below five contributes to substantial mortality and morbidity in Kenya and other developing countries. In HIV infected children, the resultant gastro enteric complications from infections can contribute to nutritional deficiency with consequent aggravation of the immunologic conditions [21]. Diarrhea in such patients is typically difficult to treat because the specific etiology is infrequently determined. The prevalence of diarrhea in this study was established at 17.1%. This was within the range of other recent studies in Kenya which established a prevalence of 17.7% (16) and 16.7% in 0-36 cohort in Busia, Kenya [15]. According to the Kenya Demographic Health Survey, diarrhea prevalence in Kenya stands at 17% (5). Although there was a high number of male than female admissions during the period, there was no significant difference in gender distribution among HIV positive and negative cases. This concurred with earlier findings within the same region which established no gender differences among HIV positive and negative children admitted with diarrhea [22].

We recorded a higher prevalence of bacterial than non bacterial diarrhea (8.5 vs. 7.1%) with a higher proportion among HIV negative than positive cases (65.3% vs. 34.7%). Although we did not further analyze the culture negative stools, some studies have implicated parasites and viruses as etiological agents of diarrhea in HIV patients [23-24]. A study among HIV infected children Kenya implicated rotavirus as the main etiological agent [25] while another identified *Cryptosporidium parvum* as an opportunistic pathogen associated with diarrhea in HIV patients [26]. Other studies in Tanzania [27-28] and India [29], have established a positive correlation between *C. parvum, I. belli* and *E. histolytica* parasitic infections and HIV. The absence of a regular detection of particular bacterial species in HIV-seropositive children, may also be attributed to a fluctuation in the gastrointestinal micro biota or pathogenic agent's due to multidrug chemoprophylaxis (sulfamethoxazole-trimethoprim) and /or treatment to which this group is constantly exposed [30].

Seasonal variations had a significant influence on frequency of diarrhea admissions during the study period

with bacterial diarrhea being predominant during drought and non-bacterial diarrhea during the rains. The dry season in Kenya coincides with the availability of cheap fruits in the region. Children playing outdoors are likely to soil their hands with dirt and contaminate fruits and other foods they consume increasing the likelihood of diarrhea. High frequency of non bacterial diarrhea during wet season may be attributed to rotavirus or parasitic agents. This concurred with findings of a related study in North Eastern Kenya [31]. Temperate regions have also been noted to exhibit seasonal variations with bacterial diarrhea occurring more frequently during the summer and viral diarrhea due to rotavirus with a peak during winter [32].

There was an inverse relationship between age and frequency of diarrhea with most cases recorded below two years of age but extending up to five years in HIV positive cases. Our findings concur with previous studies which established that most diarrheal episodes occur during the first two years of life with incidence being highest in the age group 6-11 months, when weaning often occurs [33]. This pattern has been noted to reflect combined effects of declining levels of maternally-acquired antibodies, the lack of active immunity in the infant, the introduction of food that may be contaminated with fecal bacteria and direct contact with dirt and other infectious particles as the infant starts to crawl [34]. The decline in diarrhoea cases beyond two years relates to acquisition of protective immunity [35]. Our findings however, did not agree with related studies in Kenya [16-17] probably because patient HIV status had not been considered.

Diarrheagenic *E coli* species (88.9%), *Shigella species* 5.1%, *Salmonella species* 4.2% were identified as the main etiological agents with significantly higher rates of isolation among HIV positive (56.7%) than negative cases (32.2%). EAEC (17.7%) was the main diarrheagenic E coli (DEC) strain followed by EHEC (5.1%), EPEC (3.4%), EIEC (2.5%) and ETEC (1.7%). Detection of DEC, *Salmonella* and *Shigella* in diarrheic stool samples has also been demonstrated in other studies in Kenya [36-37] and elsewhere in other developing countries (38-39). Our findings agreed with other studies [21, 23, 41-42] which recorded a higher infection rate with EAEC among HIV positive patients.

# 5. Conclusion

The prevalence of diarrhea in children a below five years Western Kenya is 17.1% while the prevalence bacterial and non-bacterial diarrhea are 8.5% and 7.1% respectively. *E coli, Shigella* and *Salmonella species* were the main bacterial etiological agents with diarrheagenic *E coli,* EAEC being the most common with higher frequencies among HIV negative than positive cases. The study depicts a positive correlation between age, clinico-demographic and HIV status on prevalence and etiology of diarrhea. We recommend improved hygienic practices, definitive diagnosis of diarrhea etiology and HIV status for effective management of childhood diarrhea.

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