

# Isolation of *Escherichia coli* and Its Associated Risk Factor from Diarrheic Children in Wolaita Sodo Town, Southern Ethiopia

Amanuel Wolde<sup>1</sup>

Yosef Deneke<sup>2</sup>

Tesfaye Sisay<sup>3</sup>

Mesfin Mathewos<sup>4</sup>

Haben Fesseha<sup>4</sup>

<sup>1</sup>College of Agriculture, Department of Veterinary Science, Jinka University, Jinka, Ethiopia; <sup>2</sup>School of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia; <sup>3</sup>Institute of Biotechnology, Addis Ababa University, Addis Ababa, Ethiopia; <sup>4</sup>School of Veterinary Medicine, Wolaita Sodo University, Wolaita Sodo, Ethiopia

**Introduction:** *Escherichia coli* are among the major causes of mortality and morbidity in under-five children in developing nations including Ethiopia.

**Methods:** A non-analytical observational study design followed by a purposive sampling technique was conducted from October 2017 to June 2018, to isolate *Escherichia coli* and determine its associated risk factors from diarrheic children that were admitted to Christian hospital, Wolaita Sodo town. *E. coli* was confirmed using standard culture and biochemical analyses of the bacterium. In addition, a semi-structured questionnaire was provided to evaluate the potential risk factors that contribute to diarrhea in children.

**Results:** The overall isolation rate of *E. coli* in diarrheic children was 61.8% (68/110) (95% CI: 52.1–70.9%). Factors such as age, contact with either animals or manure, negligence to handwashing before a meal with soap, and exclusive breastfeeding at six months ( $p < 0.05$ ) has significant contribution to the prevalence of the *E. coli* in diarrheic children. The odds of being infected were highest in children whose caretakers had a habit of the negligence of handwashing before the meal (AOR = 6; 95% CI 30.8–49.8%;  $p = 0.01$ ).

**Conclusion:** Improving the hygienic practices amongst parents of children reduces its *Escherichia coli* occurrence. Furthermore, awareness of the importance of exclusive breast-feeding to parents of children should be maximized.

**Keywords:** children, diarrhea, *Escherichia coli*, risk factors, Wolaita Sodo

## Introduction

In developing countries, children, particularly under the age of five years, are susceptible to diarrhea, which is one of the major causes of mortality and morbidity<sup>1,2</sup> and this also results in the annual death of nearly 2.6 million people worldwide, mostly from African children.<sup>3</sup> Diarrhea is a common disease in children in most developing nations including Ethiopia. This is mainly related to consumption of contaminated foods, such as eating undercooked meat, unpasteurized milk, close relationship with reservoir animals, lack of handwashing with soap by the caregiver, lack of proper breast-feeding for children until the first six months and poor health infrastructure have been mainly related with bacterial pathogens that result in diarrheal disease.<sup>4–6</sup>

Infections (sepsis, pneumonia, tetanus, and diarrhea) and preterm delivery are responsible for 4 million neonatal deaths, according to a global review.<sup>7</sup> Early beginning of breast-feeding (or human milk feeding) and exclusive nursing can avoid or decrease the negative consequences of both.<sup>4,5</sup>

Correspondence: Mesfin Mathewos  
Email Aboseme23@wsu.edu.et

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The most genetically adaptable bacteria, *E. coli*, is the source of numerous plasmids and phage-mediated genes. Despite the fact that most of its members are non-pathogens that are found in the normal microflora of people and animals' intestinal tracts, some subsets of this bacterial species have acquired genes that allow them to cause intestinal or extraintestinal illness.<sup>8,9</sup>

Diarrhea syndromes can be caused by single or many etiologic agents, such as bacterial, viral, or parasite diseases.<sup>10</sup> Among the causal agents are the following: Diarrheagenic *E. coli* (DEC) is the most frequent of the numerous enteropathogenic organisms, especially in poor countries and in diarrhea-related mortality in children under the age of five.<sup>11</sup> The total isolation rate for *E. coli* from children under five years of age was 14% and 15.3% as reported by Adugna et al<sup>12</sup> and Getaneh et al<sup>13</sup> from a hospital-based cross-sectional study at Bahir-Dar and Eastern Ethiopia, respectively. Other reports in Hawassa by Mulatu et al,<sup>14</sup> in Bahir Dar by Yemane et al,<sup>15</sup> in Jimma by Beyene et al,<sup>16</sup> and in Ambo by Wagi<sup>17</sup> indicated *Campylobacter*, *Shigella*, and *Salmonella* species, respectively.

Furthermore, the incidence and other epidemiological characteristics of these infections as causative agents of diarrhea differ from one region to the next, as well as across and among nations within the same geographical area.<sup>18</sup> In

the southern parts of the country, notably in the city of Wolaita Sodo, there is a scarcity of knowledge on the causes of diarrhea in children and the risk factors connected with it. As a result, the goal of this study was to estimate the prevalence of *E. coli* and its associated risk factors among under-five children who were hospitalized at Wolaita Sodo Christian Hospital in Southern Ethiopia.

## Materials and Methods

### Study Area

The current research work was carried out in Wolaita Sodo town at Sodo Christian Hospital from October 2017 to June 2018. Wolaita Sodo is a town in the SNNPR situated 383 kilometers from Addis Ababa. The region is bordered on the north by Damot gale woreda, on the south by Humbo woreda, on the east by Damot Wilde woreda, and on the west by Damot Sore woreda (Figure 1). It has an annual rainfall of 100–1200 mm and a temperature of 25–35°C. Its height ranges from 1650 to 2980 meters above sea level. The area is Woina-Dega (mid-altitude) at an elevation below 1600 feet, and the livestock population in the Wolaita Sodo zone includes 128,919 cattle, 29,191 sheep, 4606 equines, and 55,278 poultry.<sup>19</sup> According to the results of the May 2007 housing and population census, Wolaita Sodo town has a population

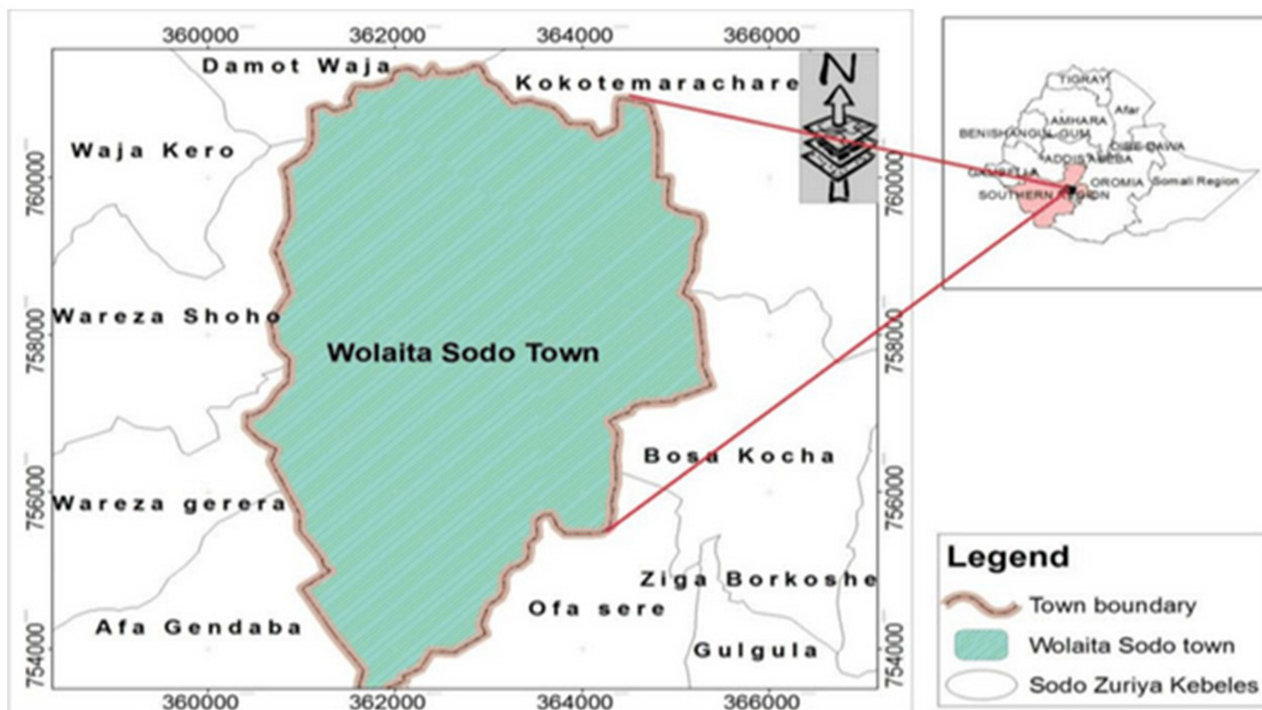


Figure 1 Map of the study area.

of 102,922, with 54,315 men and 48,617 females, and a 5.3% annual population growth rate. This makes Wolaita Sodo the second most populous city in the South Region after Hawassa. Mixed farming, which includes the development of grain crops, cash crops, especially coffee, and animal production, is the most important economic activity.<sup>20</sup>

## Study Population

All under-five children with diarrhea who visited Sodo Christian hospital in Wolaita Sodo town were included in the study, as described by Black et al.<sup>19</sup>

## Inclusion and Exclusion Criteria

### Inclusion Criteria

Diarrheic children under the age of five who attended Sodo Christian Hospital with diarrhea and whose caregivers were willing to participate in the study were included in the study's sample population.

### Exclusion Criteria

Diarrheic children over the age of five who had been on antibiotic medication for at least two weeks and whose caregivers refused to collect a sample were excluded from the research.

## Study Design and Sampling Methodology

### Study Type, Type of Sampling, and Source of Samples

A non-analytical observational study starting from October 2017 to June 2018 was conducted at the Sodo Christian Hospital found in Wolaita Sodo town. The Sodo Christian hospital was chosen purposefully, based on the availability of clinical cases (diarrheic youngsters) and the willingness of the children's parents. The pediatrician provided information about the children's health. Children with normal stool consistency and/or indications of dehydration, sunken eye, diarrhea, and weakness were categorized as healthy, whereas ill children with abnormal stool consistency and/or signs of dehydration, sunken eye, diarrhea, and weakness were classified as diarrhea. The kind, features, and color of diarrhea were also documented.

### Questionnaire Survey

The childhood information was assessed by using a questionnaire survey. A semi-structured questionnaire was administered to parents or caretakers of the children to assess the general family life standard, ways of handling the children, and hygienic practices of the family during admission to the hospital. The questionnaires were designed in accordance with

the study's aims and were prepared in a straightforward and easy-to-understand manner in order to obtain reliable information from the children's parents. The questionnaire covered all family practices that may have an influence on the correct management of the children's related risk factors for diarrhea. General health care, breastfeeding, hand cleaning, and illness prevention and control are among them.

### Sample Size Determination

The selection of participants from Sodo Christian hospital and stool samples from children was based on a non-probability purposive sampling technique. During sampling, the availability of clinical cases (diarrheic children) and the willingness of the parents of the children were the main factors considered to determine the total number of stool samples. Accordingly, 110 stool samples were collected from children who had diarrhea.

### Sample Collection Procedures

The sample collection procedure was applied by medical laboratory technicians having the stool laboratory request of the pediatricians for diarrheic children under five years of age who visit Sodo Christian hospital. The stool samples were collected by researchers and were collected from diapers of the children. The collected stool samples were labeled properly and aseptically transported to the Wolaita Sodo regional veterinary laboratory in an icebox containing ice packs and then immediately processed for bacterial isolation.

## Isolation and Identification of *Escherichia coli*

*E. coli* was isolated and identified based on the standard procedures described by Quinn et al<sup>21</sup> and the techniques recommended by the ISO.<sup>22</sup> After immediately arriving at the laboratory and/or overnight storage in the refrigerator at 40°C and thawing at room temperature, the samples were manually homogenized by using a vortex mixer for about 40 seconds. When there is a small quantity of samples in a sterile flask, 25 g of fecal samples were mixed into 225 mL of sterile buffered peptone water (Himedia, India) in a 1:9 ratio. The pre-enriched samples were homogenized in the flask for two minutes before being incubated aerobically for 24 hours at 37°C. All the study's media were prepared according to the manufacturer's instructions.

The sample dilution of pre-enriched broth was inoculated aseptically onto sterile MacConkey agar (Himedia, India) and incubated at 37°C for 24 hours. The presence of growth on MacConkey agar was employed as the major criterion for

moving on with *E. coli* isolation and identification. Suspected *E. coli* colonies were subcultured on Eosin methylene blue (EMB) agar medium (Himedia, India) to confirm their identity as *E. coli*. The green metallic sheen appearance of *E. coli* colonies on EMB was used to identify their features. For biochemical testing, all isolated colonies were maintained on a nutrient agar slant (Oxoid, England). Gram staining was used to evaluate the morphology and purity of all isolates<sup>23</sup> and they were subjected to the standard biochemical tests: Indole test, Methyl red (MR), Voges Proskauer (VP) test, Citrate test, and triple sugar iron. The isolates that exhibited an IMViC pattern (++- -) were presumed to be *E. coli* isolates.<sup>21</sup> All the presumed *E. coli* isolates were subcultured on nutrient agar slant (Oxoid, England) for short-term preservation or maintenance.

## Data Analysis

Discrete data (positive or negative for *E. coli* from diarrheic children of under the age of five) were subject to contingency table analysis, and Pearson's Chi-square test was used to determine the statistical significance of the relationships and/or associations between the dependent and independent variables from discrete data. To examine the relationship between each predictor variable and the result variable, a logistic regression analysis was used. The association was measured using crude odds ratios (CORs) for illness and 95% confidence intervals for covariates. All the significant factors from the bivariate analysis were included in a multivariable analysis. This provided the Adjusted Odds Ratios (AORs) at a 95% confidence interval. Multicollinearity diagnosis among independent variables by contingency coefficient was used to assess the model's suitability. The Hosmer and Lemeshow test was used to determine the model's goodness of fit to the data. Throughout the investigation, a significance level of  $p < 0.05$  was employed. All data from the questionnaire survey and laboratory tests were coded, filtered, and recorded in Microsoft Excel spreadsheet 2007 (Microsoft Corporation) before being analyzed with SPSS version 20.0 software (SPSS INC. Chicago, IL).

## Ethical Considerations

This study received ethical approval from the Wolaita Sodo University of Research Ethics and Review Committee. Before collecting the samples, the parents or guardians of the patients were asked for verbal agreement to collect samples from their children while adhering to stringent sanitary guidelines. All the studies were done in

accordance with the Helsinki Declaration. The best practices for human care were followed, and the patients' parents or guardians were told of the study's objective and that the Wolaita Sodo University of Research Ethics and Review Committee authorized the oral informed consent process.

## Results

### Overall Isolation of *Escherichia coli* from Diarrheic Children

From a total of 110 stool samples from diarrheic children, 68 (61.8%) (95% CI: 52.1–70.9%) were positive for *Escherichia coli*. The occurrence of *E. coli* in child diarrhea showed a significant difference by age ( $p=0.007$ ), contact with either animal or manure ( $p=0.009$ ), and exclusive breast-feeding in six months ( $p=0.036$ ). A higher isolates of *E. coli*, 47 (42.8%), was observed in children of age groups  $>2$  years, 48 (43.7%) in those who had a history of contact with either animals or manure, and 46 (41.8%) in children who did not get only breast-feeding until six months of age. There was no significant difference in the occurrence of *E. coli* by sex (Table 1).

### Univariable and Multivariable Logistic Regression Analysis of Risk Factors Associated with Diarrhea in Children

In the analysis of Risk factors concerning diarrhea in children independent variables like age, contact with either animals or manure, and exclusive breastfeeding at six months in univariable analysis were significantly associated with the occurrence of *E. coli* isolates ( $p < 0.05$ ). On the other hand, sex and negligence in handwashing before the meal was not significantly associated with *E. coli* isolates. To control confounders, the variables with ( $p < 0.25$ ) in the univariable logistic regression analysis were transferred to the multivariable logistic regression analysis. In multivariable logistic regression analysis, the occurrence of *E. coli* isolates in diarrheic children were more likely higher in age groups  $>2$  years (AOR=2.4; 95% CI: 2.4 (33–52.5%);  $p=0.003$ ) and children having a history of contact with either animal or manure (AOR=3.5; 95% CI: 3.5 (34.2–53.4%);  $p=0.001$ ) than other age categories and in those having no history of contact with either animals or manure, respectively.

Similarly, children experiencing a habit of negligence to handwashing before the meal (AOR=6; 95% CI: 6 (30.8–49.8%);  $p=0.01$ ) and those who did not get only

**Table 1** Overall Occurrence of *E. coli* with Different Risk Factors in Diarrheic Children

Risk Factors	Category	No. Examined	No. Positive	Category Proportion (%)	Samples Proportion (%)	( $\chi^2$ )	df	P-value
Age	≤2 years	49	21	42.8	19	10.2	2	0.007
	>2 years	61	47	77	42.8			
Sex	Male	62	38	61.3	34.5	0.13	1	0.96
	Female	48	30	62.5	27.3			
CAM	Yes	73	48	65.7	43.7	11.3	1	0.009
	No	37	20	54	18.1			
NHWM	Yes	63	44	69.8	40	14.2	1	0.21
	No	47	24	51	21.8			
EBMF6M	Yes	39	22	56.4	20	13.5	1	0.036
	No	71	46	64.8	41.8			

breastfed in six months of age (AOR=5.6; 95% CI: 5.6 (32.5–51.6%);  $p=0.02$ ) were more likely susceptible to *E. coli* infection than those who wash their hands and those who get only breastfed in six months of age, respectively. There was no collinearity among the variables, and there was an insignificant difference between the observed and predicted values using ( $\chi^2= 1.24$ ;  $p=0.64$ ), which suited the data well (Table 2).

## Description of Socio-Demographic Situation Pattern Based on Questionnaire Survey Findings

One hundred ten diarrheic children with ages below five years admitted to Sodo Christian hospital were considered. A semi-structured questionnaire comprised contacts of manure or domestic animals in the house, breast milk feeding, treatment history of the child, and hand-washing practices were administered to caretakers/parents/based on their consent. Fifty-two

(47.3%) out of 110 diarrheic children were females and 58 (52.7%) were females-to-male ratio of 1.2:1. The age level of fewer than five years of which the highest number of participants 54 (49%) is found in age groups above two years. Of all parents of the sampled children, 102 (92.7%) used the toilet for defecation and the remainder were not in which children were susceptible to diarrheal infection because they did not pay attention to hygiene. Fifty-three (48.3%) of the parents reared domestic animals in the home and 46 (41.8%) used animal products as a supplement feed source.

## Discussion

Diarrhea is a multifactorial disease since it is caused by interactions between the patient, its environment, diet, and infectious pathogens.<sup>24</sup> As a result of this study's focus on *E. coli*, other microbiological, parasite, or management variables as a possible cause of infant diarrhea may be ruled out. The overall isolation rate of *E. coli* isolates was

**Table 2** Risk Factors Associated with *Escherichia coli* Isolates in Diarrheic Children by Univariable and Multivariable Logistic Regression

Risk Factors	Category	No Examined	Samples Positive (%)	Univariable		Multivariable	
				P-value	COR (95% CI)	P-value	AOR (95% CI)
Age	≤2 years	49	21 (19)	0.007	2.9 (34.2–53.8%)	0.003	2.4 (33–52.5%)
	>2 years	61	47 (42.8)				
CAM	Yes	73	48 (43.7)	0.009	3.8 (33.6–55.3%)	0.001	3.5 (34.2–53.4%)
	No	37	20 (18.1)				
NHWM	Yes	63	44 (40)	0.21	4 (32.0–52.4%)	0.01	6 (30.8–49.8%)
	No	47	24 (21.8)				
EBMF6M	Yes	39	22 (20)	0.036	6.8 (31.5–49%)	0.02	5.6 (32.5–51.6%)
	No	71	46 (41.8)				



68 (61.8%) out of 110 stool samples collected from diarrheic children. This result is higher when compared with the results of Adugna et al,<sup>12</sup> Nguyen et al,<sup>25</sup> Mandomando et al,<sup>26</sup> Ansari et al<sup>27</sup> who reported 48.3% in Bahir Dar, 2.3% in Nepal, 22.6% in Mozambique, and 31% in Austria, respectively. However, it was found to be lower when compared to the findings of Shah et al<sup>28</sup> 86.5% in Kenya and Ugwu et al<sup>29</sup> 88% (23/26) in Nigeria. The difference in isolation rate might be attributed to different sociodemographic characteristics, sample size variation, and the season when the studies were carried out.<sup>30</sup>

In the present study, *E. coli* was isolated from diarrheic children of all age groups. Its occurrence was differed statistically by age ( $p < 0.05$ ). The odds of being diarrheic due to *E. coli* was more likely to occur in children above the age of two years. This finding agrees with a study conducted by Moyo et al<sup>31</sup> in Tanzania and by Ansari et al<sup>27</sup> in Nepal. This might be because maternal antibodies protect babies under the age of two years from severe diarrhea to some extent at first, and they appear to have developed sufficient immunity between the ages of 12 and 16 months. It can also be explained according to Sherchand,<sup>32</sup> as children in this age range, are more interested in outdoor activities, careless about feeding themselves, and lack understanding about healthcare and personal cleanliness, all of which put them at risk for serious health problems as they get older. Therefore, this relatively higher chance of contact with either animals or their manure among higher children age groups than lower age groups could be a possible reason. There was a significant association ( $p < 0.05$ ) between the presence of *E. coli* in diarrheic children and a history of contact with animals or manure. This might be due to activities of small children in the vicinity of livestock and manure has a high probability of carrying *E. coli* because of the habit of hand contact with the mouth, eyes, and nose after touching the livestock and manure without wearing protective clothing and due to the negligence of hand-washing with soap that could be a vehicle for the occurrence of *E. coli* in children under five years of age.

The odds of diarrheic children who had a history of contact with either animals or manure were more likely to be diarrheic than those who did not have contact. According to a study conducted in Kenya by Edward et al,<sup>33</sup> children having contact with manure were independently associated with the occurrence of *E. coli*

infectious diarrhea; farm residents and manure contacted children were significantly more likely to have diarrhea compared with non-farm resident children. Similarly, according to Radostits et al,<sup>34</sup> a small number of *E. coli* pathogens in feces from cattle have the potential to infect humans, since children are vulnerable to be infected with *E. coli* through direct contact with calves, ingesting fecal contaminated food or water, and indirectly by contact with objects soiled with animal feces.

According to Aiello et al<sup>35</sup> handwashing with soap could protect one out of three young children who suffered from diarrhea, and proper handwashing before meals and after defecation can lower exposure of children to enteric pathogens. The habit of the negligence of washing both hands with soap before feeding was significantly related to *E. coli* occurrence in diarrheic children. The odds of being infected with *E. coli* were observed to be more likely in children whose caretakers did not wash their hands with soap before feeding than those who washed their hands before feeding by soap. This agrees with the results of Sylvester et al<sup>36</sup> in Bolivia and Adugna et al<sup>12</sup> in Bahir Dar, indicating the likelihood of children being diarrheic whose caretakers have the habit of negligence to wash their hands.

In the present study, a significant ( $p < 0.05$ ) correlation was observed between exclusive breastfeeding practice of the mothers within the first six months and the isolation rate of *E. coli* from diarrheic children. Children who did not get exclusive breast milk feeding at six months of age were more likely to be susceptible to diarrhea due to *E. coli* than those children who were only provided with breast milk. This result is inconsistent with the study conducted in Kenya by Kageni,<sup>37</sup> in which the number of diarrheic children was the least when complementary feeding was initiated after six months of age. In addition, the National Institute of Child Health and Development report, exclusive breast milk feeding supplies oligosaccharides and lactoferrin bioactive components for effective diarrhea prevention along with the nutritional components. Therefore, children should only be provided with their mother's breast milk for the first six months without any supplementary food items.<sup>38</sup> In line with Adugna et al,<sup>12</sup> the present study also revealed that the sex of the child has no significant impact on the occurrence of diarrhea due to *E. coli*.

## Conclusion

The study finding of the current investigation revealed that there is a high proportion of *E. coli* isolates from under-

five diarrheic children of the study area. Factors, such as age, breast milk feeding, the habit of washing hands without soap, and contact with manure in children were significantly correlated with the incidence of *E. coli*. Therefore, awareness creation to parents or caretakers of children on the importance of exclusive breastfeeding and personal hygiene is highly recommended.

## Abbreviations

AOR, Adjusted odds ratio; COR, Crude odds ratios; CAM, Contact with either Animals or Manure; CI, Confidence Interval; *df*, Degree of freedom; DEC, Diarrheic *Escherichia coli*; EBMF6M, Exclusive Breast Milk Feeding in Six Month; ISO, International Organization for standardization; NHW, Negligence to Hand Washing; NHWBM, Negligence to Hand Washing Before Meal, ®, Reference point; SNNPR, Southern Nation Nationalities and Peoples Region.

## Data Sharing Statement

The datasets used and analyzed throughout the study will be provided by the corresponding author on reasonable request.

## Ethics Approval and Consent to Participate

This study received ethical approval from the Wolaita Sodo University of Research Ethics and Review Committee. Before collecting the samples, the parents or guardians of the patients were asked for verbal agreement to collect samples from their children while adhering to stringent sanitary guidelines. All the studies were done in accordance with the Helsinki Declaration. The best practices for human care were followed, and the patients' parents or guardians were told of the study's objective and that the Wolaita Sodo University of Research Ethics and Review Committee authorized the oral informed consent process.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design,

execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

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