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Prevalence and Antimicrobial Susceptibility Patterns of Escherichia Coli, Salmonella, and Shigella Species Among Fewer Than Five Years Age Diarrheal Children Attending Mettu Karl Comprehensive Specialized Hospital, Southwest Ethiopia June to August, 2023

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Research Article

Keywords: Diarrhea, Escherichia coli, Salmonella, and Shigella, Antimicrobial Susceptibility

Posted Date: February 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8980001/v1

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Additional Declarations: The authors declare no competing interests.

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF *ESCHERICHIA COLI*, *SALMONELLA*, AND *SHIGELLA* SPECIES AMONG FEWER THAN FIVE YEARS AGE DIARRHEAL CHILDREN ATTENDING METTU KARL COMPREHENSIVE SPECIALIZED HOSPITAL, SOUTHWEST ETHIOPIA JUNE TO AUGUST, 2023.

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Abstract

Background: Diarrheal diseases are a common disease worldwide associated with high morbidity and mortality rates in children under the age of five especially in developing countries. Enteric bacterial are most common causative agents of diarrhea and become a challenges to prevent and treat diarrhea due to emergency of antimicrobial resistant.

Objective: The aims of this study was to isolate and determine prevalence and antimicrobial susceptibility patterns of *Escherichia coli*, *Salmonella*, and *Shigella* species among under five years age diarrheal children attending Mettu Karl Comprehensive Specialized Hospital, Southwest Ethiopia June to August, 2023

Methods: A cross-sectional study was conducted from June to August 2023 on 382 at Mettu Karl Comprehensive Specialized Hospital, Southwest Ethiopia. A structured questionnaire was used to collect data from children under five who had diarrhea. Data collection was done using convenience sample technique. Each collected sample was inoculated onto Selenite F broth and MacConkey agar. Antimicrobial susceptibility testing was performed using the Kirby Bauer disk method on Muller Hinton agar. Socio-demographic, clinical, and laboratory results data were entered in EpiData v4.6.0.6 and analyzed using SPSS version 25. Descriptive summaries, frequency, tables, and graphs were used to describe the study variables.

Results: From the 382 children screened, bacteria were isolated from 44 (11.52%). This included; 19(5.0%) *Escherichia coli*, 14(3.66%) *Salmonella* species, and 11 (2.9%) *Shigella*. The highest resistance among bacteria was observed against Ampicillin (89.5%), cotrimoxazole (73.7%), and cefazolin (57.1%). Almost all of *E.coli, Salmonella,* and *Shigella* were susceptible to imipenem, cefepime, and cefotaxime. Of the total 44 isolates, 20(45.5%) showed multidrug resistance.

Conclusion and Recommendation: This study indicates that *E. coli, Salmonella*, and *Shigella* were isolated pathogenic bacteria. The identified bacteria were susceptible to Imipenem, cefepime, and cefotaxime. The highest prevalence of antimicrobial resistance was observed for ampicillin followed by cotrimoxazole and cefazolin. Also, isolated bacteria showed resistance to ceftriaxone, ceftazidime, azithromycin, and ciprofloxacin and multiple antimicrobial resistances were high among those bacteria. It is better to use potent antimicrobials like Imipenem, Cefepime, and Cefotaxime for the treatment of bacterial-caused diarrhea.

Key Words: - Diarrhea, *Escherichia coli*, *Salmonella*, and *Shigella,* Antimicrobial Susceptibility.

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Introduction

Background

Diarrhea is a condition of having three or more loose or liquid bowel movement per day. Human diarrheal diseases have been recognized since the beginning of civilization and remain one of the most prevalent public health problems of today. The most significant modes of transmission is believed to be fecal–oral spread by ingestion of contaminated food or water which is frequently facilitated easily by unsanitary and hygienic situations(1). Diarrheal diseases are caused by a lack of access to good health care, a lack of appropriate diagnostics method, poor health facilities, unsanitary water sources and/or a lack of effective treatment strategies (2).

According to World Health Organization (2013), diarrhea could be classified into four types, based on clinical symptoms to less than five years children. They are watery diarrhea which defined as a sudden onset of frequent, watery, loose stools without visible blood, lasting less than two weeks. Dysentery is a diarrhea containing blood and mucus in feces. Persistent diarrhea is defined as diarrhea episodes of presumed infectious etiology that have an unusually for longer than 14 days, but less than 1 month. Chronic diarrhea is recurrent or long-lasting due to mainly noninfectious causes. Each of them reflecting different pathogenesis (3). Watery stools, frequent bowel movements, vomiting, nausea, fever, gas, dry mouth, blood in the stools, decreased urine production, loss of appetite, and dehydration are some of the clinical signs of diarrhea (4).

Both infectious and non-infectious can causes diarrhea (5). Bacterial pathogens, viruses, and parasites can all lead to infectious diarrhea(6). Bacterial infections are most common causative agents of diarrhea (7). Global estimates show that among all diarrheal causes, the prevalence of specific types of bacterial diarrhea is 10% to 25% for *E. coli*, 10% for *Shigella*, 3% for *Salmonella*, and 3 to 6% for *Campylobacter* (8).

Children in developing nations experience multiple episodes of diarrheal disease due to early exposure to a broad spectrum of enteric bacterial infections. The most common bacterial infections vary with the child's age and time, showing both seasonal as well as geographical variation. Every child's experience is unique, but the most frequently identified bacterial in young children are *Salmonella* strains, entero-hemorrhagic *E. coli, Shigella* spp., *Clostridium difficile*, *Campylobacter jejuni,* and *Yersinia enterocolitica*. Other less common causes of diarrhea but clinically significant bacterial infections include *Aeromonas hydrophila*,

Campylobacter upsaliensis, Vibrio spp (7,9). Parasites that causes diarrhea include *Giardia lamblia, Entamoeba histolytica, Cyclospora cayetanensis, Cryptosporidium, Ascaris lumbricoides, Trichuris trichiura,* and *Hymnolepis nana.*and viruses such as Adenovirus , Norwalk Virus and Rotavirus which cause diarrhea in children under five (10).

The emergence of multidrug-resistant bacteria has significantly impeded the use of antimicrobials to treat infectious diarrhea (11). There have been reports of high rates of resistance to widely used and readily available antimicrobials such as ampicillin, tetracycline, trimethoprim-sulfamethoxazole (cotrimoxazole), and chloramphenicol (5,12). In addition, the development of antimicrobial resistance (AMR) and multidrug resistance (MDR) has changed the antimicrobial susceptibility pattern of *Salmonella* spp., *Shigella* spp., and *E. coli* gradually (13). When necessary, antimicrobials should only be used to treat diarrhea caused by infectious agents such as bacteria after culture and susceptibility testing have been performed (14). However, antimicrobial susceptibility tests (ASTs) are not usually performed before antimicrobials are prescribed; therefore, empirical treatment is usually considered. Because of this, the prevalence of antimicrobial-resistant bacteria has increased (15). This may be partially explained by the scarcity of ASTs and the increased turnaround time for test results (12). So the future of treatment by antimicrobial is being challenged by infections from resistant organisms, which are affecting treatment costs, treatment outcomes, disease duration and disease spread (16). Therefore, this study was aimed to fill the gap that stated above by assessing the prevalence and antimicrobial susceptibility patterns of *Escherichia coli*, *Salmonella*, and *Shigella* species among fewer than five years age diarrheal children attending Mettu Karl Comprehensive Specialized Hospital, Southwest Ethiopia June to August, 2023.

Materials and Methods

Study Area, Study Design and Period

Hospital-based, cross-sectional study was carried out to determine prevalence and antimicrobial susceptibility patterns of *Escherichia coli*, *Salmonella*, and *Shigella* species among fewer than five years age diarrheal children attending Mettu Karl Comprehensive Specialized Hospital (MKCSH) from June to August 2023. MKCSH Hospital is a specialty hospital founded in 1964 and named after Dr. Karlheinz Boom, the founder and chairman of Mesnchem für Mesnchem (MFM), a German-born Non-Governmental Organization (NGO), who renovated and expanded the hospital in February 2001.

The hospital provides services to a catchment area of more than 2 million people come from Ilubabor Zone, Gambella Region, Southwestern Population Region of Ethiopia and South Sudan Country. Based on the Central Statistical Agency (CSA) projection for 2022, the town has a total population of 59,706, of which 29,569 are male and 30,137 female.

Sample Size Determination

The sample size was calculated based on a single population proportion formula by taking the prevalence of enteric bacteria in under-five children with diarrhea from a previous study done at Debre Berhan town of 46% (53) at a 95% confidence interval and 5% margin of error.

Where: n = sample size

Zα/2 = 95% level of confidence (1.96).

P = diarrhea prevalence from previous study = 46%.

q = 1-p.

d = margin of error (0.05)

$$n = \frac{(Z\frac{\alpha}{2})^2\, P(1-P)}{d^2} \qquad n = \frac{(1.96)^2 x 0.46 x 0.54}{(0.05)^2} = \qquad 382$$

Thus, the totals of **382** children with diarrhea were included in the study

Data and sample collection

Two days of training was provided by the principal investigator for two BSc. Nurse, two BSc. Laboratory technologists and one supervisors on data collection tools and collection techniques, how to approach the interviews, and maintaining the privacy and confidentiality of respondents prior to the actual data collection period. The data was collected using a structured questionnaire. The questionnaire was translated into the local language Afan Orormo and translated back into English to check its consistency. Prior to data collection, 5% of the sample was pre-tested on Bedele general hospital that is not included in the actual sample. All information on the questionnaire was filled in by the data collector. After the data collection, the questionnaires were cleaned daily by the supervisors and principal investigator and checked for completeness.

Children's socio-demographic variables include age and gender, as well as guardian/parent variables such as education level, family size, and residence. Hygiene and sanitation-related variables like source of drinking water, feeding practice of children, utilization of latrines, hand washing of parents/gardians, clinical data including duration of diarrhea, fever, vomiting, bloody diarrhea, mucoid diarrhea, watery diarrhea, the immunization status of the child, and breastfeeding were collected.

Fresh stool specimens were collected from each study participants by trained BSc. Nurses for bacteriological analysis. The specimens were coded with patients ID and information and transported using Cary-Blair transport media to MKCSH bacteriology laboratory at 2-8^{0}C.

Identification of Bacterial Isolates

Isolation and characterization of parasites were examined by preparing a slide using normal saline 0.85% to observe the ova, cysts, and trophozoite stage of the parasites present in the stool at Mettu Karl Coprehensive Specialized Hospital laboratory before inoculated on culture medaia. For bacteriological isolation, each stool sample directly cultured onto Selenite F broth (SFB) and MacConkey agar media. Approximately 1 g of each sample inoculated into 10 ml of SFB. The tubes and plates were incubated for 24 hours at 37^{0}C. From enrichment Selenite F broth subculture onto Xylose-Lysine Deoxycholate (XLD) agar then the plates incubated at 37°C for 18 to 24 hrs. The suspected bacteria were identified by colony morphology and biochemical characteristics. On MacConkey agar suspected enteric bacteria appeared as transparent or colorless colonies. *Salmonella spp.* appeared on XLD agar as red colonies with black center owing to H_2S production. *Shigella spp*. colonies identified on XLD agar the

colonies appeared as transparent red. On MacConkey agar suspected enteric bacteria appeared as transparent or colorless colonies. *E.coli* on MacConkey agar showed transparent colorless colonies. Unfortunately, there was no growth of bacteria on alkaline peptone water. The colonies of each representative isolate were then characterized using standard bacteriological methods. Please see laboratory work flow below (Figure 1).

Biochemical Tests

Biochemical media were used for the identification of bacterial isolates. The identification were done based on the pattern of biochemical reactions using a standard bacterial identification system. All the suspected isolates were examined biochemically using Nutrient Broth, Simmons Citrate Agar, Klingler Iron Agar (KIA), Sulfide-Indole-Motility (SIM), Urea Broth Base, and Kovac's Reagent to identify bacteria. Among the tests used to identify enteric bacteria are: pigment production, motility, urease, citrate, hydrogen sulfide utilization, oxidase, indole, lysine, and sugar fermentation. Bacterial isolates were categorized based on culture outcomes and biochemical tests. Please see laboratory work flow below (Figure 1).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility patterns were determined by the Kirby Bauer disk method. Three to five pure colonies from agar plate isolates were suspended in the normal saline solution and mixed well to adjust at 0.5 McFarland standards. The 0.5 McFarland turbidity standard was prepared by mixing 0.5 mL barium chloride dehydrate (1.175%) with 99.5mL sulfuric acid (1%). A sterile cotton swab is used to evenly spread bacterial inoculum on a sterile petri dish of Mueller Hinton Agar. 12 disk on 150mm plate and 6 on 100mm plate with up to 24mm apart each other. Each disc must be pressed down to ensure complete contact with the agar surface. The plates were incubating for 16-18hrs at 37^{o}C, which was done according to the guideline. Commonly prescribed Antimicrobials like ampicillin (10µg), amoxicillin-clavulanic acid (20/10µg), cefazolin (30µg), cefotaxime (30µg), ceftazidime (30µg), cefepime (30µg), imipenem (10µg), ciprofloxacin (5µg), co-trimoxazoale (trimethoprim-sulfamethoxazole,(1.25/23.75µg), gentamicin (15µg), azithromycin (15µg) were used to test against bacteria. According to the size of the zone of inhibition, the organisms were classified as susceptible, intermediate, or resistant to each Antimicrobial using the CLSI 31th edition, 2021 interpretation guideline (35). Please see laboratory work flow below (Figure 1).

The figure below indicate laboratory workflow of bacterial identification, Biochemical Tests and Antimicrobial Susceptibility Testing

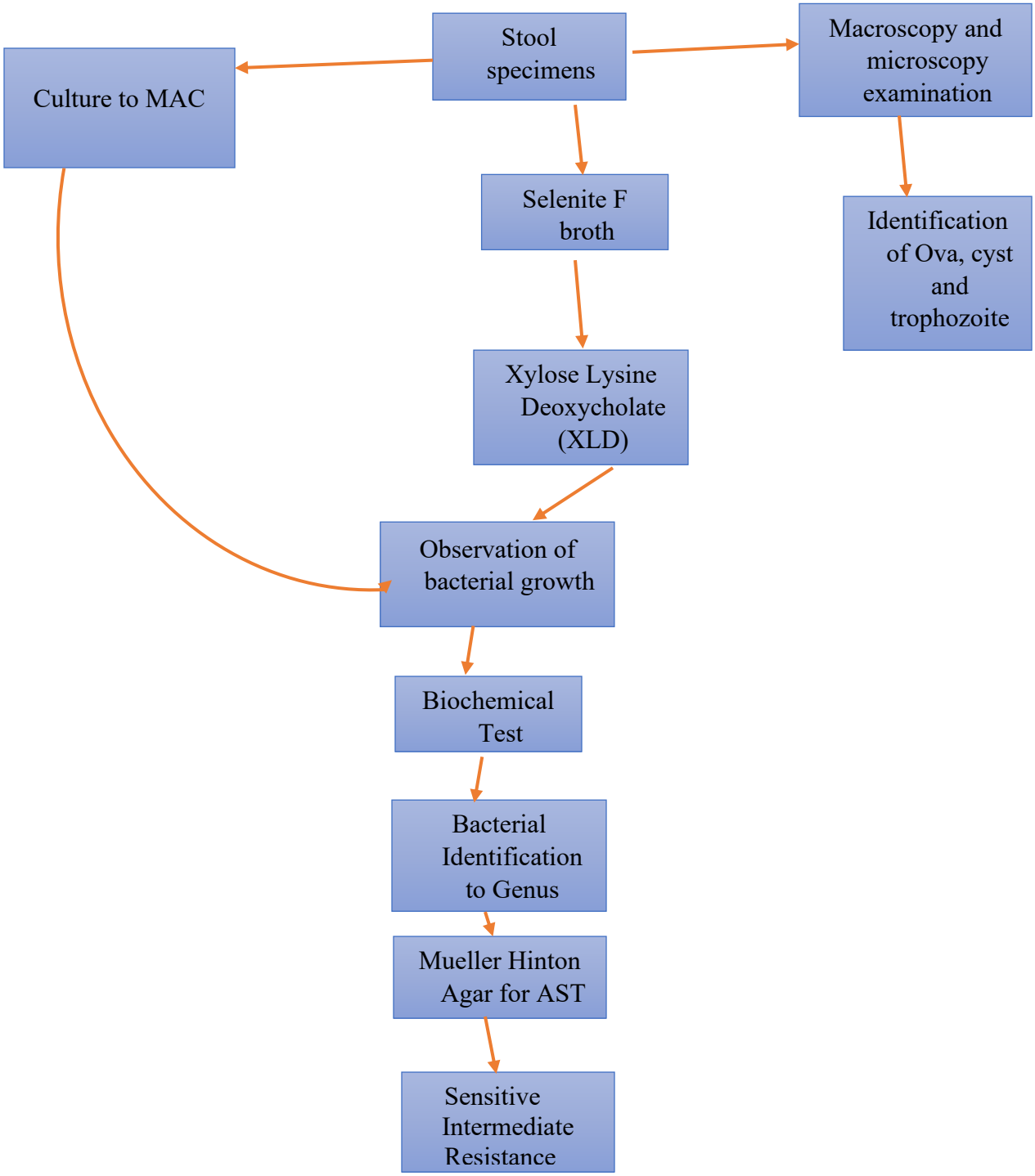


Figure 1. The isolation and identification of *Escherichia coli, Salmonella,* and *Shigella* from under five children at Mettu Karl Comprehensive Specialized hospital, Southwest Oromia, Ethiopia, 2023.

Data Quality Assurance

The data quality control measure of the study was maintained throughout the whole process of data collection and laboratory work. For laboratory analysis, the pre-analytical, analytical, and post-analytical stage of quality assurance and standard operating procedures (SOPs) of testing laboratory was followed.

Stool samples were collected by trained healthcare personnel using sterile stool containers that are labeled with participant identification numbers and transported to the Microbiology Laboratory at the MKCSH immediately. The collected samples were registered in the logbook to avoid re-sampling. Before using culture medium, the expiration date was checked. Two plates of prepared culture media from the test batch were taken and placed into the incubator set at 37°C for 24 hours. After required incubation, the plates were checked for any growth. If there is any growth, contamination has occurred in the prepared batch and the entire batch is not used.

The sample was analyzed at MKCSH. The test was performed by a well-trained and experienced laboratory technologist. The collected samples were tested once for bacterial culture and for intestinal parasites. A known strain of the American Type Culture Collection (ATCC) of *Escherichia coli* 25922 and *Pseudomonas aeruginosa* ATCC 27853 control were used as the zone of inhibition for these organisms is known in accordance with the CLSI 31th edition (2021) (35).

The results were recorded with an identification number on the laboratory result form and logbook. The laboratory results were given to the physician immediately for medical treatments, and parents and guardians were informed. All bacterial isolates were stored at refrigerator until the end of this research project for any kind of checkup & future use.

Statistical Analysis and Interpretation

Following data collection, it was reviewed for completeness and consistency. EpiData v4.6.0.6 version software was used for data entry and exported to the statistical package for social science (SPSS) 25version for analysis. Finally, descriptive summaries, frequency, tables, and graphs were used to describe the study variables.

Results

Socio-Demographic Characteristic of the Study Participants

In this study, a total of 382 under-five children with diarrhea disease were included. Out of 382 childrens, 178(46.6%) were male and 204 (53.4%) were female; with male to female ratio of 1:1.2. The age ranges from 4 – 60 months with mean of 32.64 (SD ±14.39) months, while majority of participants (213 (55.8%)) lived in urban areas and (294(77%)) had family size 3-4 persons. It was found that among the respondents, 35(9.2%) parents/guardians were not able to read and write and less than three-fourths of parents or guardians 104(27.2%) had completed college and above. Almost half 170 (44.5%) of the guardians had a monthly income of 1,001-3000 birr (Table 1).

Table 1. Distribution of *Escherichia coli*, *Salmonella*, and *Shigella* among study participants with respect to sociodemographic variables at Mettu Karl Comprehensive Specialized Hospital Southwest Oromia, Ethiopia from June to August, 2023

Variables		Frequency	Percentage	*Escherichia coli*	*Salmonella* spp	*Shigella* spp
Age categories (in month)	0-6	15	3.9			
	7-12	23	6.0		1	
	13-24	89	23.3	9	5	2
	25-36	101	26.4	3	2	3
	37-48	97	25.4	3	3	2
	49-60	57	14.9	4	3	4
Gender	Male	178	46.6	8	6	6
	Female	204	53.4	11	8	5
Residence	Urban	213	55.8	14	9	7
	Rural	169	44.2	5	5	4
Educational status of parents or guardians	Can't read and write	57	14.9	5	2	5
	Primary school	101	26.4	6	5	3
	Secondary School	119	31.2	5	6	2
	College and above	105	27.5	3	1	1
Family size	1-2	2	0.5			
	3-4	294	77.0	5	5	7
	5-6	86	22.5	14	9	4
Average monthly income	<1,000	16	4.2	5	5	2
	1001-3,000	170	44.5	4	2	3
	3,001-5,000	139	36.4	5	3	2
	>5,000	57	14.9	4	4	2

Clinical Characteristics of Respondents

The clinical complaints of the diarrheal children indicated that, out of 382 diarrheal children, 128(33.5%) had fever and 80(20.9%) had vomiting. With regard to consistency of stools, 243(63.6%) had watery diarrhea. Almost all (372(97.4%) of children were vaccinated. The

study also revealed that the duration of diarrhea ranged from 1-6 days with a mean of 2.030 days with 265 (69.4%) had three days of duration. One hundred ninty five (51%) of chidren had stool frequency greater than 5 per days and 192(50.2%) children were breastfeeding for 1-2 years (Table 2).

Table 2. Distribution of *Escherichia coli*, *Salmonella*, and *Shigella* among diarrheal children with respect to clinical variables at Mettu Karl Comprehensive Specialized Hospital Southwest Oromia, Ethiopia from June to August, 2023

Variables		Frequency	Percentage	*Escherichia coli*	*Salmonella* spp	*Shigella* spp
Fever	Yes	128	33.5	5	10	9
	No	254	66.5	14	4	2
Duration of diarrhea	1 day	54	14.1	3	0	1
	2 days	64	16.8	4	3	3
	3 days	162	42.4	6	5	2
	4 days	102	26.7	6	6	5
Stool Frequency (per day)	1-2 per day	8	2.1	1	2	0
	3-4 per day	177	46.3	8	5	4
	>5 per day	197	51.6	10	7	7
Type of diarrhea	Watery diarrhea	243	63.6	6	5	2
	Mucoid diarrhea	116	30.4	8	2	3
	Bloody diarrhea	23	6.0	5	7	6
Vomiting	Yes	80	20.9	3	8	3
	No	302	79.1	16	6	8
Dehydration	No	380	99.5	19	14	11
	Mild	2	0.5	0	0	0
Weaning Age	1 year breast feeding	66	17.3		1	
	1-2 years breast feed	222	58.1	15	10	9
	>2 breast feed	94	24.6	4	3	2
Immunization	He/she take an age appropriate vaccine	45	11.7	0	1	0
	Complete vaccine	327	85.6	18	13	9
	Incomplete vaccine	10	2.6	1	0	2

This study indicated that around half of the respondents 169 (44.9%) used pipe water as sources of drinking. Almost all parents/guardians used traditional pit latrines (340(89%)) but only 28(7.3%) had hand washing facilities near their latrines. The study also revealed that the majority of parents/guardians (327(88.2%)) washed their hands before and after cooking food and more than half of parents/guardians (255(66.8%)) used bottles for feeding their children. (Table 3).

Table 3. Distribution of *Escherichia coli*, *Salmonella*, and *Shigella* among diarrheal children with respect to behavioral characteristics at Mettu Karl Comprehensive Specialized Hospital Southwest Oromia, Ethiopia from June to August, 2023

Variables		Frequency	Percentage	*Escherichia coli*	*Salmonella* spp	*Shigella* spp
Source of drinking water	Pipe water	228	59.7	5	3	2
	Spring/protected	96	25.1	5	2	0
	Well water	23	6.0	2	4	3
	River water	35	9.2	7	5	6
Utilization of latrine	Yes	365	95.5	16	12	7
	No	17	4.5	3	2	4
Type of latrine	Traditional pit latrine	340	89.0	17	14	10
	Ventilated improved latrine	25	6.5	2	0	1
Hand washing Facility near the latrine	Yes	28	7.3	1	2	0
	No	337	88.2	18	12	11
At what time did you wash your hand	Before and After cooking food	327	85.6	6	7	3
	Before feeding child	227	59.4	6	5	4
	After Toilet	222	58.1	7	2	4
Material used for feed child	Bottle	255	66.8	14	10	4
	Cup with spoon	118	30.9	5	3	7
	Glass Tube	9	2.4	0	1	0

Isolation of Enteric Bacterial

From the total 382 stool specimens, isolated enteric bacteria were *Escherichia coli, Salmonella* and *Shigella* species. The positive rate of enteric bacteria identified from this research was 44(11.52%). There is no other species of bacteria is isolated in this study from a total of 382 stool samples processed. From the positive stool sample 43(97.7%) had single infection of bacterial pathogen whereas 1(2.3%) had protozoan parasite and bacterial infection.

Out of 44(11.52%) isolated bacteria, *E.coli* was detected in 19(5.0%), *Salmonella* species was detected in 14(3.7%) of study participants and *Shigella* species was detected in 11(2.9%) of target children. About half of detected *E.coli* and *Salmonella* cases were in the age group between 13 and 24 months whereas *Shigella* species were detected in almost all age groups

equally. In this study, protozoan parasites were identified of which 6 (1.6%) *Giardia lamblia* and 2(0.5%) *Entamoeba histolytica/dispar* (Figure 2).

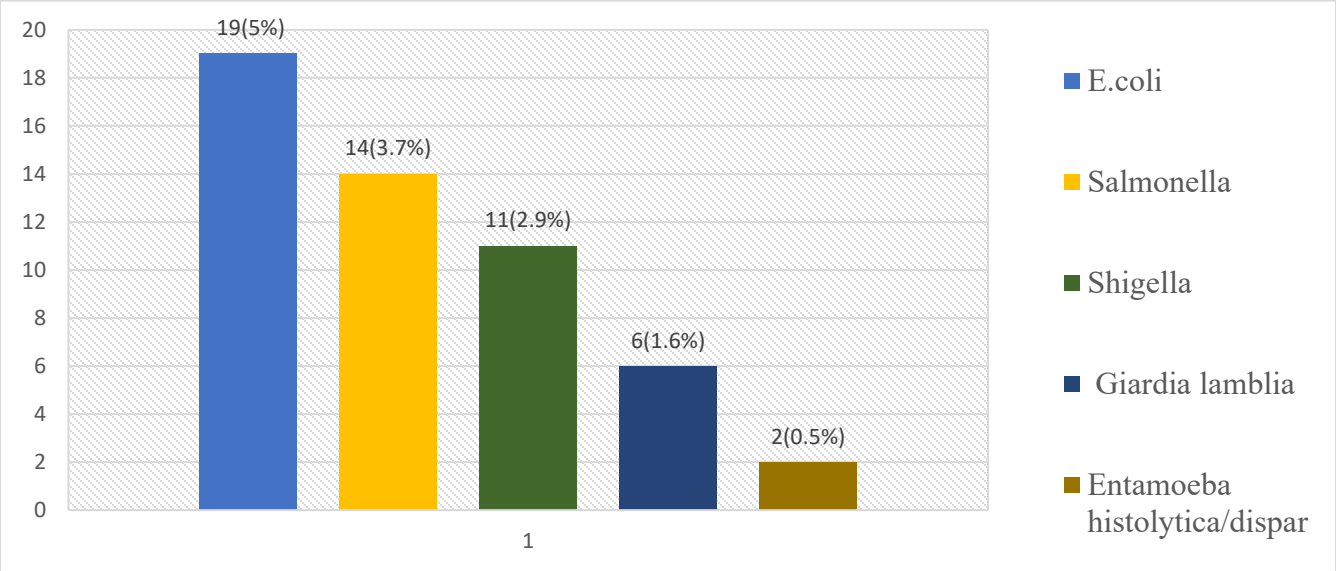


Figure 2. Prevalence of entero-pathogens isolated from under-five children with a compliant of diarrhea at Mettu Karl Comprehensive Specialized Hospital Southwest Oromia, Ethiopia from June to August, 2023

Antimicrobial Resistance Pattern

The antimicrobial susceptibility pattern for isolated bacteria to was determined by the disk diffusion method by following the recommendations of the CLSI 31 2021(67). Resistance to Ampicillin was 89.5%, 85.7%, and 72.7% for *E.coli, Salmonella* species, and *Shigella* species respectively. Resistance to co-trimoxazoale was 73.7% for *E.coli*, 72.75% for *Shigella* spps and 54.5% for *Salmonella* spp isolates. Resistance to ceftriaxone was 64.3% for *Salmonella*, 47.4% for *E. coli* spps, and 45.5% for *Shigella*. From 19 isolates of *E.coli* species, most of them have shown sensitivity for imipenem (94.7%), cefepime and cefotaxime (89.5%), and azithromycin (73.7%). From 14 isolates of *Salmonella* species, 100% sensitivity has shown for imipenem and cefepime whereas three-fourths of them were susceptible for azithromycin, cefotaxime, augmentin, cotrimoxazole, ciprofloxacin. From 11 isolates of *Shigella* species, 100% sensitivity has shown for imipenem, 81.8% for azithromycin, and 72.7% for cefotaxime, and ciprofloxacin and more than half shows sensitivity for augmentin, Cefepime, ceftriaxone. Almost all *E.coli, Salmonella,* and *Shigella* were susceptible to imipenem, cefepime, cefotaxime (Table 4).

Table 4. Antimicrobial susceptibility pattern of *Escherichia coli*, *Salmonella*, and *Shigella* identified fewer than five age children at Mettu Karl Comprehensive Specialized Hospital Southwest Oromia, Ethiopia from June to August, 2023

Antimicrobial discs	*E.coli* n=19(5.0%)			*Salmonella* spp *n*=14(3.7%)			*Shigella* spp n=11(2.9%)		
	S	I	R	S	I	R	S	I	R
AMP(10µg)	2(10.5)	0(0.0)	17(89.5)	1(7.1)	1(7.1)	12(85.7)	2(18.2)	1(9.1)	8(72.7)
AUG (210/0µg)	13(68.4)	3(15.8)	3(15.8)	9(64.3)	1(7.1)	4(28.6)	8(72.7)	1(9.1)	3(27.3)
GN (10µg)	15(78.9)	0 (0.0)	4(21.1)	-	-	-	-	-	-
TMP-SXT (1.25/23.75µg)	4(21.1)	1(5.3)	14(73.7)	6 (42.9)	0(0.0)	8(57.1)	2(18.2)	1(9.1)	8(72.7)
IPM(10µg)	18(94.7)	0(0.)	1(5.3)	14(100)	0(0.0)	0(0.0)	11(100)	0(0.0)	0(0.0)
FEP(30 µg)	17(89.5)	0(0.0)	2(10.5)	14(100)	0(0.0)	0(0.0)	9 (81.8)	1(9.1)	1(1.9)
AZT(30 µg)	14(73.7)	1(5.3)	4(21.1)	12(85.7)	0(0.0)	2(14.3)	7(63.6)	0(0.0)	4(36.4)
CRO(30 µg)	7(36.8)	3(15.8)	9(47.4)	3(21.4)	2(14.3)	9(64.3)	6(54.5)	0(0.0)	5(45.5)
CIP(5 µg)	11(57.9)	1(5.3)	7(36.8)	11(78.6)	0(0.0)	3(21.4)	8(72.7)	1(9.1)	2(18.2)
CZ (30µg)	7(36.8)	1(5.3)	11(57.9)	5(35.7)	1(7.1)	8(57.1)	5(45.5)	0(0.0)	6(54.5)
CTX (30µg)	17(89.5)	0(0.0)	2(10.5)	9(64.3)	0(0.0)	5(35.7)	8(72.7)	0(0.0)	3(27.3)
CAZ (30µg)	9(47.4)	2(10.5)	8(42.1)	7(50.0)	5(35.7)	2(14.3)	4(36.4)	4(36.4)	3(27.3)

Key S-Susceptibility I- Intermediate, R-Resistance, TMP- SXT-trimethoprim sulphamethoxazole, AMP-Ampicillin, AUG-Augmentin, IPM-imipenem, CAZ-ceftazidime, CTX-Cefotaxime, CZ-cefazolin, FEP-Cefepime, AZT-Azithromycin, CRO-Ceftriaxone, CIP-Ciprofloxacin.

Multi-Drug Resistant Isolates

From total 44 isolates, 20(45.5%) of them were showed multidrug resistance. Out of that, 10(52.6%) of *E.coli* isolate were multidrug resistant, 6(31.5%) of them resistance to 3 antimicrobials and 4(21.1%) of them resistance to 4 antimicrobials. Regarding, *Salmonella* isolate, 5(35.7%) of the isolates showed multidrug resistant, 4(28.6%) of them showing resistance to 3 antimicrobials and 1(7.1%) to 4 antimicrobial. Also, *Shigella* isolates showed multidrug resistance for 5 (45.5%) of which 4 (36.4%) of the isolates showed resistant to 4 antimicrobials and the remaining 1(9.1%) were resistant to 4 antimicrobials. But 3 (15.8%) *E.coli*, 3(21.4%) *Salmonella* and 2(18.2%) of *Shigella* were susceptible to all tested antimicrobial (Table 5).

Table 5. Resistance Antibiogram of *Escherichia coli*, *Salmonella*, and *Shigella* species of fewer than five chidren at Mettu Karl Comprehensive Specialized Hospital Southwest Oromia, Ethiopia from June to August, 2023

Number of antimicrobial resistance	Resistance antibiogram	Resistance isolates n (%)		
		E coli spp (n=19)	Salmonella (n=14)	Shigella (n=11)
RO	None	3 (15.8)	3(21.4)	2(18.2)
R1	Amp	2 (10.5)	1(7.1)	1(9.1)
	AUG	-	-	-
	SXT	1(5.3)	1(7.1)	-
R2	Amp , AUG	-	1(7.1)	-
	Amp, SXT	3 (15.8)	2(14.3)	2(18.2)
	Amp ,CIP		1(7.1)	1(9.1)
R3	Amp, AUG,SXT			-

	Amp, SXT,CRO	2(10.5)	2(14.3)	1(9.1)
	Amp, SXT,GN	3(15.8)	-	-
	Amp, SXT,CZ	1(5.3)	2(14.3)	3(27.3)
R4	Amp ,AUG,SXT,CRO		1(7.1)	1(9.1)
	Amp ,SXT,CIP,GEN	4(21.1)	-	-
R5	AUG,CIP,SXT,CRO,AZT,	-	-	-

Key: Amp-Ampicillin, ,SXT-Cotrimoxazole, AUG- Agumentin, AZT- azithromycin, GEN-Gentamycin, CRO-ceftriaxone, CIP-Ciprofloxacillin, RO-none resistance, R1-resistance for one antimicrobial, R2-resistance for two antimicrobial, R3-resistance for three antimicrobial, R4-resistance for four antimicrobial, R6- resistance for six antimicrobial

Discussion

The overall prevalence of *E.coli, Salmonella,* and *Shigella* obtained in the current study was 44 (11.52%). Current study is comparable with study conducted in Bahir Dar with prevalence of enteric bacteria 13.1%, and in Jimma, southwest Ethiopia which counts 8.4% (24,34) but lower than previous findings from Hawassa Town, South Ethiopia which counted 22.5%, Nigeria 68.5%, and Kenya 46.0% (21,23,27). This might be partly due to true difference of the bacterial infection in the area and also seasonal variations.

According to this study's results, children between 13 to 24 months old are more susceptible to diarrhea diseases and enteropathogenic bacteria as demonstrated in (Table 2). Studies done at Hawassa, Nigeria and Gambella agree with this finding that children ages between 13 to 24 months old are more susceptible to diarrhea diseases (21,27,29). This is probably because of youngsters at this age attempt to masticate everything's with their mouths and also more likely to play in soil where they may come into contact with faeces.

In current study, 19(5.0%) of children were found to be infected with *E.coli*, 14(3.7%) were infected with *Salmonella* species and 11(2.9%) with *Shigella* species. The prevalence of *Salmonella* and *Shigella* in the current study is comparable with previously reported in 2014 in Ambo town, Ethiopia which was 3.8% (30).

Prevalence of *E.coli was* 19 (5.0%). The finding of this study was comparable with previous reports with studies done at Bahir Dar with prevalence of 6.1% (24). On the other hand, it was found to be higher than similar study conducted in Bangladesh with prevalence of 1.45% (17). But lower than studies done at South Africa with prevalence of 13.9% (22), at Tehran, Iran 16% (19), at Hawassa 22.2% (27), Addis Ababa with prevalence of 24.1% (26), Kenya

36.4% (23), at Ghana 49.1% (20), at Pakistan with prevalence 60% (18), at Debra Berhan 62.7% (25),and at Nigeria with prevalence of 62.8% (21).

The prevalence of *Salmonella* in this study was 14(3.7%). The finding of the current study was comparable with previous reports at Pakistan with prevalence of 3% (18), at Bahir Dar 3.1% (24), at Addis Ababa 3.95% (26), 21,22), at Gambella 2.3%(29), at Kenya 2.4%(23), at Hawassa 2.5% (27), at Bangladesh with prevalence of 4.34% (17) but higher than studies done at Ambo with prevalence of 1.3% (30), at South Africa and Abuja, Nigeria with prevalence of 1.7% . The current study is lower than a study conducted at Debra Berhan 6.7%(25), at Arba Minch (28), Ghana (20), and Iran (19) with prevalence of 12.6%, 23.9% and 42% respectively. The variability of isolation of *Salmonella* may be attributable to the difference in study areas and period, because the features of the disease vary from place to place and time to time community awareness about personal and environmental hygiene and socioeconomic elements.

The prevalence of *Shigella* in current study was found to be 11(2.9%). This was comparable with study done at Ambo which was 2.5% (30), at Kenya with prevalence of 3.2% (23), at Debra Berhan and Bahir Dar with prevalence 4.0% (24,25), at Bangladesh 4.34% (17). Contrarily, the prevalence *Shigella* in the current study is lower as compared to studies done at Arba Minch 4.8%, Abuja, Nigeria and South Africa 5.5%, Gambella 6% (21,22,28,29). Also lower than the study conducted at Hawassa 7%, at Addis Ababa 9.1%, at Ghana 10.2%, and at Tehran, Iran 40% (19,20,26,27). The lower prevalence of *E.coli*, *Salmonella,* and *Shigella* could be due to different factors including, variation of socio-demographic and behavioral characteristics, methods used in isolation of pathogens, and the presence of other enteric bacterial causing diarrhea.

Among parasitic infections, *Giardia lamblia* and *Entamoeba* identified in this study were 6(1.6%) and 2(0.5%) respectively. The study is comparable to the study in Ambo with a prevalence of *Giardia lamblia* (3.3%), followed by *Entamoeba* at (2.1%) (30) but it is lower than the study conducted in Gambella with *Giardia lamblia* 28(50.9%), *E. histolytica/dispar* 13(23.6%). This may be because of their nature for easily transmitting in the community and accessibility as well as utilization of latrine (29).

The resistance of enteric pathogens to currently used antimicrobial agents has increased all over the world as a result of the widespread use of antimicrobials. There are reports from Arba Minch, Gambella and Jimma on multiple antimicrobial resistances among strains of pathogenic *E.coli*, *Salmonella,* and *Shigella* species in Ethiopia (28,29,34).

All *E.coli*, *Salmonella* and *Shigella* species isolates from current study displayed resistance to one or more antimicrobials including Ampicillin, Cotrimoxazole, and Cefazolin. Among the isolates there were no resistances for Imipenem, Cefepime and except 2(10.5%) a few resistances for Cefotaxime which comparable with studies done at Debre Berhan, Addis Ababa and Ambo Town (25,26,30).

The highest prevalence antimicrobial resistance of *E.coli* spps against Ampicillin (89.5%) observed was comparable with the study done at Addis Ababa which counts 83.6% (26), at Pakistan (93.5%) (18), at Korea resistance was 78% (31), at South Africa (98%) (32). It is higher than a study done in Kenya which counts 55.2% (23).This may be show Ampicillin resistance strain is widespread in study area.

Antimicrobial resistance of *E.coli* spp in the current study against cotrimoxazole was (73.7%) which comparable with a study done at Bangladesh which counts 80%(17). The current study is higher than study conducted in Kenya with cotrimoxazole resistance rate 61.4% (23), in Addis Ababa which counts 62.3%(26) but lower than study done at South Africa, with Cotrimoxazole resistance (96%) (32). *E.coli* was susceptible for Imipenem 94.7%, cefepime and cefotaxime 89.5%, augumentin 68.4%, azithromycin 73.7%, ciprofloxacin 57.9% and ceftazidime 47.4%. This is comparable with study done in Pakistan, Korea, South Africa Kenya and Addis Ababa (18,23,26,31,32). This may be due to the different laboratory techniques used for susceptibility test.

In current study resistance of *Salmonella* species to ampicillin, Ceftriaxone and cotrimoxazole was 85.7%, 64.3%, and 57.1% respectively. Accordingly, a high level of *Salmonella* resistance was observed for ampicillin (85.7%) which is comparable with a study done in Kenya which counts 77.8% (23), in Mekelle which counts 89.5% (33), but lower than study done at Ambo 100%(30) and higher than study conducted in Iran 50% (19). Level of resistance for cotrimoxazole was 64.3% which is a relatively similar pattern of resistance reported from study done at Mekelle 57.9% (33), Ambo 66.7% (30) but higher than study done in Bangladesh 40%(45). In contrarily to this, study done at Kenya showed that cotrimoxazole was 100% susceptible for *Salmonella* species (23). In the current study *Salmonella* was 100% susceptible for imipenem, cefepime, 85.7% susceptible for azithromycin, 78.6% susceptible for ciprofloxacin which is comparable study done at Bangladesh, Pakistan, Jimma, Ambo and Addis Ababa (17),(30),(34),(18),(26).However, augumentin, ceftazidime, cefotaxime, cefazolin was shown more than one-fourths of resistance for *Salmonella* species.

The highest prevalence Antimicrobial resistance of *Shigella* spp against Ampicillin was (72.7%) This was comparable with the study done on *Shigella* isolates in Ambo which counts 83.3% (30) The current study was lower than study in Mekelle which counts 88.9% (33), in Tehran, Iran which counts 92% (19), in Jimma which counts 100% (34). This study was higher than a study done in Kenya which counts 28.6% (23). Antimicrobial resistance of *Shigella* spps against cotrimoxazole was (72.7%) which lower than a study done in Tehran, Iran (93.0%) (19), in Jimma and Bangladesh 100% (17,34). The study was higher than study done in Kenya 47.6% (23), in Ambo which counts 50% resistance for cotrimoxazole (30), in Mekelle 55.6% (33). Among the *Shigella* species 100% susceptible for Imipenem, 81.8% susceptible for cefepime and more than three-fourths (72.7%) were susceptible cefotaxime, ciprofloxacin, ceftriaxone, augumentin and azithromycin which is comparable to studies conducted in Ambo, Jimma, and Kenya.(23,30,34). The difference may be due to susceptibility methods used.

Conclusion and Recommendation

Conclusion

The overall prevalence of enteric bacterial isolates in this study was 11.52%. This study indicates that *E.coli, Salmonella,* and *Shigella* species are among the most pathogenic bacteria identified in children with diarrhea less than five years old examined in the study area. These isolated bacteria were susceptible to Imipenem, cefepime, and cefotaxime. But those bacteria were showed the highest resistance for ampicillin followed by cotrimoxazole and cefazolin indicating that no longer potent in the treatments of *E.coli, Salmonella,* and *Shigella* among children with diarrhea aged fewer than five years old diagnosed in the study area. Though still at low levels, the major concern from this finding is the emerging resistance of enteric pathogens that were observed for ceftriaxone, ceftazidime, azithromycin, and ciprofloxacin. Multiple antimicrobial resistances were high among the isolated bacteria. From total of 44 isolated bacteria, 20(45.5%) of them were showed multidrug resistance.

Acronyms and Abbreviations

AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Tests
CLSI	Clinical And Laboratory Standards Institute
DAEC	Diffuse Adhering *Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
LMIC	Low and Middle-Income Countries
MAC	MacConkey agar
MDR	Multi-Drug Resistance
OPD	Outpatient Department
SFB	Selenite F broth
SOP	Standard Operating Procedures
Spp	Species
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

Acknowledgments

First of all, I would like to express my heartfelt thanks to the almighty God for allowing me to accomplish this thesis.

I would like to express my sincere and heartfelt gratitude to:

Salale University College of Health Sciences, Departments of Medical Laboratory Sciences and Oromia Health Bureau for their sponsoring me.

My deep gratitude goes to Mettu Karl Comprehensive Specialized Hospital laboratory for supporting me and providing laboratory reagents, materials and staff of hospital for their co-operation during laboratory work.

I would like to express my gratitude to Ethiopian Public Health Institute for providing me a known control strain.

I would like to acknowledge Pediatrics Wards of the hospital, for providing necessary clinical data and all nurses for their help during data collection period.

I would also like to acknowledge all study participants and their parents for their cooperation

Finally, I would also like to express my heartfelt thanks to all individuals who directly or indirectly contributed to the work of this thesis.

Declaration

Ethical Approval and consents of Study paticipants

Ethical clearance was obtained from the Ethical Review Committee (ERC) of Salale University College of Health Sciences with Ref. No. SLU-IRB/28/23. Permission letters was obtained from the Chief Executive Officer of MKCSH Hospital with Ref. No. MD/2874/015.All selected study participants signed the informed assent form. Parents/guardians of study participants were also informed about the purpose of the study, the importance of their participation, and their right to withdraw at any time. The privacy and confidentiality of the information given by each respondent was maintained, and name was not recorded. Culture results and antimicrobial susceptibility results were communicated to the hospital clinician as soon as the results had ready.

Author contributions; - SD. Had a substantial contribution from conception to the acquisition of data. All the authors had a great contribution to study design, analysis, and interpretation of the findings. **SD and AA** were involved in laboratory analysis. All drafted the manuscript. All authors revised the paper carefully for important intellectual contents. All authors read and approved the final manuscript.

Funding: - Oromia Health Bureau funded partially. But didnot have a role in study design, data collection and analysis, interpretation, or preparation of the manuscript.

Data availability:-

All relevant data are within this paper.

Competing interests: - There is no competing interest.

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1. References

1. World Health Organization. Diarrhea disease. WHO. 2017;
2. Khalil IA, Troeger C, Blacker BF, Rao PC, Brown A, Atherly DE et al. Morbidity and mortality due to shigella and enterotoxigenic Escherichia coli diarrhea: The Global Burden of Disease Study 1990–2016. Lancet Infect Dis. 2018;18(11):1229–40.
3. World Health Organization. Diarrheal disease fact Sheet. WHO. 2013;No. 330.
4. Nemeth V, Zulfiqar H PN. Diarrhea - StatPearls. NCBI Bookshelf StatPearls. 2019;1–10.
5. Barr W SA. Acute Diarrhea in Adults.In American Family Physician. 2014;89(3).
6. Lanata CF, Fischer-Walker CL, Olascoaga AC, Torres CX, Aryee MJ, Black RE. Global Causes of Diarrheal Disease Mortality in Children <5 Years of Age: A Systematic Review. PLoS One. 2013;8(9).
7. Black R LC. Epidemiology of diarrheal disease in developing countries. In: Infections of the Gastrointestinal Tract, editor. Blaser MJ, Smith PD, Ravdin JI, et al (Eds),. 2nd ed. Philadelphia: Lippincott Williams and Wilkins; 2002. 11–29.
8. Notario R, Borda N, Gambande T, Sutich E. Species and serovars of enteropathogenic agents associated with acute diarrheal disease in Rosario, Argentina. Vol. 38, Revista do Instituto de Medicina Tropical de Sao Paulo. 1996. 5–7.
9. Chiriac PC, Duceac L-D, Manole A, Poroch V, Delcea F, Stafie L, et al. Acute Bacterial Gastroenteritis as a Healthcare-Associated Infection in Pediatrics. Curr Heal Sci J. 2017;43(3):275–81.
10. Gustavo V, Batthyány L, Bianco MN, Pérez W, Pardo L, Algorta G, et al. Enteropathogens Associated with Acute Diarrhea in Children from Households with High Socioeconomic Level in Uruguay. Int J Microbiol. 2015;2015:8.
11. World Health Organization. Antimicrobial resistance: global report on surveillance. WHO. 2014;
12. Breurec S, Reynaud Y, Frank T, Farra A, Costilhes G, Weill FX LHS. Serotype distribution and antimicrobial resistance of human Salmonella enterica in Bangui, Central African Republic, from 2004 to 2013. PLoS Negl Trop Dis. 2019;13(12):7917.
13. Raza S, Tamrakar R, Bhatt C JS. Antimicrobial susceptibility patterns of Salmonella typhi and Salmonella paratyphi A in a tertiary care hospital. J Nepal Health Res Counc. 2012;10(22).
14. Kasanga M, Mudenda S, Siyanga M, Chileshe M, Mwiikisa MJ, Kasanga M et al. Antimicrobial susceptibility patterns of bacteria that commonly cause bacteremia at a

tertiary hospital in Zambia. Future Microbiology. 2020;15(18):1735–45.

15. Sjolund-Karlsson M, Joyce K, Blickenstaff K, Ball T, Haro J, Medalla FM, Fedorka-Cray P, Zhao S, Crump JA WJ. Antimicrobial susceptibility to azithromycin among Salmonella enterica isolates from the United States. Antimicrobial agents and chemotherapy. 2011;55(9):3985–9.
16. O'Neill J. Antimicrobial resistance: Tackling a crisis for the health and wealth of nations. 2014;1–2.
17. Uddin MS, Rahman M, Faruk O, Talukder A, Hoq I, Das S, et al. Bacterial gastroenteritis in children below five years of age : a cross - sectional study focused on etiology and drug resistance of Escherichia coli O157 , Salmonella spp ., and Shigella spp . Bull Natl Res Cent. 2021;45:138.
18. Quraishi F, Shaheen S, Memon Z, Fatima G. Culture and Sensitivity Patterns of Various Antibiotics Used for the Treatment of Pediatric Infectious Diarrhea in Children under 5 Years of Age: A Tertiary Care Experience from Karachi. Int J Clin Med. 2018;09(09):684–96.
19. Mahmoudi S, Pourakbari B, Moradzadeh M, Eshaghi H, Ramezani A, Haghi Ashtiani MT, et al. Prevalence and antimicrobial susceptibility of Salmonella and Shigella spp. among children with gastroenteritis in an Iranian referral hospital. Microb Pathog. 2017;109:45–8.
20. Afum T, Asandem DA, Asare P, Asante-Poku A, Mensah GI, Musah AB et al. Diarrhea-Causing Bacteria and Their Antibiotic Resistance Patterns Among Diarrhea Patients From Ghana. Front Microbiol. 2022;13.894319.
21. Cajetan IC, Nnennaya IR, Casmir AA, Florence IN. Enteric bacteria pathogens associated with diarrhoea of children in the federal capital territory Abuja, Nigeria. N Y Sci J. 2009;62–9.
22. Ledwaba S.E., Kabue J.P., Barnard T.G. TAN and PN. Enteric Pathogen Co-Infections in the Pediatric Population from rural Communities in the Vhembe District, South Africa. South African J Child Heal. 2018;12:170 – 174.
23. Kilongosi Webale M, Wanjala C, Guyah B, Shaviya N, Munyekenye GO, Lokamar Nyanga P et al. Epidemiological patterns and antimicrobial resistance of bacterial diarrhea among children in Nairobi city,Kenya. astroenterol Hepatol Bed Bench. 2020;12(3):238–46.
24. Balew M, Kibret M. Prevalence of enteric bacterial pathogens in diarrheic under-five children and their association with the nutritional status in Bahir Dar Zuria District, Northwest Ethiopia. BMC Nutr. 2023;9(1):1–9.

25. Tizazu Z, Zelelie TZ, Gebreyes DS, Tesema A, Craddock HA, Gishen NZ. Enteropathogens in Under-Five Children with Diarrhea in Health Facilities of Debre Berhan Town , North Shoa , Ethiopia. Ethiop J Heal Sci. 2019;29(2):203.
26. Mamuye Y, Metaferia G, Birhanu A, Desta K FS. Isolation and Antibiotic Susceptibility Patterns of Shigella and Salmonella among Under 5 Children with Acute Diarrhoea: A Cross-Sectional Study at Selected Public Health Facilities in Addis Ababa, Ethiopia. Clin Microbiol. 2015;4:186.
27. Mulatu G, Beyene G ZA. Prevalence of Shigella, Salmonella and Cmpylobacter species and their susceptibility patters among under five children with diarrhea in Hawassa town, South Ethiopia. Ethiop J Heal Sci. 2014;24(2):101–18.
28. Ameya G, Tsalla T, Getu F, Getu E. Antimicrobial susceptibility pattern , and associated factors of Salmonella and Shigella infections among under five children in Arba Minch , South Ethiopia. Ann Clin Microbiol Antimicrob. 2018;17(1):1–7.
29. Mekonnen GK, Mengistie B, Sahilu G, Kloos H, Mulat W. Etiologies of diarrhea and drug susceptibility patterns of bacterial isolates among under-five year children in refugee camps in Gambella Region , Ethiopia : a case control study. BMC Infect Dis. 2019;19:1008.
30. Tosisa W, Mihret A, Ararsa A, Eguale T, Abebe T. Prevalence and antimicrobial susceptibility of Salmonella and Shigella species isolated from diarrheic children in Ambo town. BMC Pediatr. 2020;20:91.
31. Cho SH, Lim YS, Park MS, Kim SH, Kang YH. Prevalence of antibiotic resistance in Escherichia coli fecal isolates from healthy persons and patients with diarrhea. Osong Public Heal Res Perspect. 2011;2(1):41–5.
32. Omolajaiye SA, Afolabi KO, Iweriebor BC. Pathotyping and Antibiotic Resistance Profiling of Escherichia coli Isolates from Children with Acute Diarrhea in Amatole
33. Gebreegziabher G, Asrat D, W/Amanuel Y, Hagos T. Isolation and Antimicrobial Susceptibility Profile of Shigella and Salmonella Species from Children with Acute Diarrhoea in Mekelle Hospital and Semen Health Center, Ethiopia. Ethiop J Health Sci. 2018;28(2):197–206.
34. Beyene, G. and Tasew H. Prevalence of intestinal parasite, Shigella and Salmonella species among diarrheal children in Jimma health center, Jimma southwest Ethiopia: a cross sectional study. Ann Clin Microbiol Antimicrob. 2014;13(1):1–10.
35. Clinical and laboratory standard Institute. M100 Performance Standards for Antimicrobial Susceptability Testing. Vol. 31th editi. 2021.