

ORIGINAL RESEARCH ARTICLE

PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF *ESCHERICHIA COLI* O157:H7, *SALMONELLA*, AND *SHIGELLA* SPECIES FROM STOOL SPECIMENS-SAMPLES OF PATIENTS WITH DIARRHEA AT BENJAMIN MKAPA HOSPITAL

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ABSTRACT

Background

~~Enterobacteraceae-Enterobacteriaceae~~ is among the large group of ~~gram-gram~~-negative rod bacteria in which it comprises ~~of-*Escherichia coli*, *Salmonella*, *Shigella*~~, and many other species whose natural habitat is ~~the~~ intestinal tract of humans and animals. ~~The-d~~Diarrheal diseases are a major problem worldwide caused by bacterial pathogens particularly in developing countries. ~~Previous-A previous~~ study reported a prevalence of 9.30% Extended Spectrum Beta Lactamases (ESBLs) producing Enterohemorrhagic *Escherichia coli* O157:H7 that revealed a high (100%) resistance to gentamicin, tetracycline, trimethoprim-~~/sulphamethaxazole-sulphamethoxazole~~, and amoxicillin/ clavulanic acid.

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Methods

A ~~laboratory-laboratory~~-based ~~cross-cross~~-sectional study was conducted at BMH. A convenient sampling method was used to enroll 308 patients with diarrhea in the study after consenting for ~~a period of~~ 12 months. Stool samples were collected into ~~an~~ acceptable clean sterile stool container and transported Microbiology department for investigation. Analysis of data was performed using Statistical Package for Social Sciences (SPSS) version 17.0.

Results:

Out of 308 participants, 61.0% (188) were female whereas 39.0% (120) were male. The age group of between 18 to 45 years had ~~a~~ larger number of participants recruited in ~~the~~ study 257 (83.4%) and less number 4 (1.2%) in ~~the~~ age group above 60 years old. About 5.2 (16/308) percent of stool ~~specimen samples~~ processed were positive with pathogenic bacteria whereas 94.8 (292/308) percent were negative (no pathogenic bacteria isolated) growth. The study presented two pathogenic bacteria species named ~~*Escherichia-E. coli* strain O157:H7 and *Salmonella typhi*~~ that were isolated from stool ~~samples specimen~~ of patients with diarrhea and ~~who~~ attended the hospital for treatment. The prevalence of ~~*Salmonella-S. typhi*~~ was 3.2% (10/308) whereas ~~*Escherichia-E. coli* strain O157:H7~~ was 1.9% (6/308) out of 308 stool ~~samples specimens~~-processed. The *in vitro* drug resistance patterns of ~~ampicillin-Ampicillin was-were~~ observed to be high 9 (90%) followed by Amoxicillin/-Clavulanate and tetracycline of which both had 6 (60%) ~~resistant-resistance~~ to ~~*Salmonella-S. typhi*~~. (Table 4).

Conclusion

The study is currently insisting laboratory practitioners ~~to~~ carry out ~~an~~ investigation of *Escherichia coli* strain O157:H7 as ~~a~~ routine test in parallel with other enteric pathogens.

Keywords: ~~Prevalence;~~ Antimicrobial; resistance; ~~Patterns;~~ ~~*Escherichia; C. coli* O157:H7; O157:H7;~~ ~~*Salmonella;*~~ and ~~*Shigella;*~~ ~~s~~Species; ~~Stool; Specimens; Patients;~~ diarrhea; ~~Tanzania;~~ Benjamin Mkapa; ~~Hospital.~~

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1.0 INTRODUCTION

~~Enterobacteraceae-Enterobacteriaceae~~ is among the large group of ~~gram-gram~~-negative rod bacteria in which it comprises ~~of-*Escherichia coli*, *Salmonella*, *Shigella*~~, and many other species whose natural habitat is ~~the~~ intestinal tract of humans and animals [1]. ~~The-d~~Diarrheal diseases are a major problem worldwide caused by bacterial pathogens particularly in developing countries [2,3]. The enteropathogenic *Escherichia coli* affecting humans are ~~categorised-categorized~~ into Shiga ~~toxin-toxin~~-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC); enteroaggregative *E. coli* (EAEC), entero-toxicogenic *E. coli*

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(ETEC), diffusely adherent *E. coli* (DAEC), and entero-invasive *E. coli* (EIEC) including the *shigella* *Shigella* and Salmonella species are common bacteria that causes diarrhea [1,2,4,5].

Antibiotics are the drugs that kill and prevent the multiplication or growth of microbes. They are the most common antimicrobial agents prescribed in hospitals worldwide [1,7,8,10,12]. Use-The use of similar or identical antibiotics in humans has come under increasing scrutiny by regulators concerned that bacteria resistant to animal antibiotics will infect people and resist treatment with similar human antibiotics, leading to excess illnesses and deaths. Scientists, regulators, and interest groups in the United States and Europe have urged bans on non-therapeutic and some therapeutic uses of animal antibiotics to protect human health [1,6].

Previous-A previous study reported a prevalence of 9.30% Extended Spectrum Beta Lactamases (ESBLs) producing Enterohemorrhagic *Escherichia-E. coli* O157:H7 that which demonstrated revealed a high (100%) resistance to gentamicin, tetracycline, trimethoprim—/—sulphamethaxazole sulphamethoxazole, and amoxicillin/ clavulanic acid [1].

The increase in the rate of antimicrobial drugs resistance in our countries calls-requires for morefurther efforts to identifyclassify specific causes and practices that aggravate the problem. Whether such factors are professional, infrastructural, social, or personal is not yet fully known it remains to be found out [1].

The increase of antibiotics resistance to currently used antibiotics for both pathogenic and commensally bacteria of gram-negative rods are species-species-specific extended-extended-spectrum beta-lactamase (ESBL) rate about 24.4% with resistances rate to gentamicin, tetracycline, sulphamethaxazolesulphamethoxazole/trimethoprim, and ciprofloxacin were significantly higher among ESBLs isolates than non-ESBL isolates [8,9] and about 90% in difference-different places reported that Co-trimoxazole, Ampicillin, Gentamycin, and Penicillin are commonly emerging resistance in these areas [8,11,12].

It is known that many factors such as incompatibility, overdose, counterfeit products as well as the abuse of drugs in animal agriculture promote the development of antibiotic resistance. Many factors are known to promote the development of antibiotic resistance: Noncompliance, under dosing, counterfeit products, and also misused of the drugs in animal agriculture [13-17].

There is little doubt that *Escherichia-E. coli* strain O157:H7 strain are-is one of the common pathogenic bacteria infecting patients daily at the Benjamin Mkapa Hospital. However, although *Salmonella typhi*, *S. paratyphi* and *Shigella* species are common bacteria that infect patients, *E. coli* O157:H7 is also a current factor that increases the burden in the society and continues to cause complications. However, *Salmonella typhi*, *Salmonella paratyphi* and *Shigella* species are the common bacteria paid attention for infecting patients. *Escherichia coli* strain O157:H7 is a current agent increasing burden and continue to pose complication in community [1].

In this study, it was aimed to determine the prevalence and antimicrobial resistance patterns of pathogenic *E. coli* O157:H7 strain, *Salmonella* and *Shigella* species in stool samples of patients with diarrhea in Benjamin Mkapa Hospital. Thus, the results of the study will help us to obtain the basic information to routinely identify the pathogenic *E. coli* O157:H7 in parallel with *Salmonella* and *Shigella* species, and also provide us with awareness of the increasing patterns of antibiotic resistance of isolates in diarrheal patients at Benjamin Mkapa Hospital. The study aimed to determine the prevalence and antimicrobial resistance patterns of pathogenic *Escherichia coli* strain O157:H7, *Salmonella* and *Shigella* species in stool specimen of patients with diarrhea at Benjamin Mkapa Hospital.

The results of this study will aid us to get basolone information in order to identify pathogenic *Escherichia coli* strain O157:H7 routinely in parallel with *Salmonella* and *Shigella* species. The results will also alert us on the increasing drug resistance patterns of isolates in patients with diarrhea at Benjamin Mkapa Hospital.

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2.0 MATERIALS AND METHODS

2.1 Study Site

The study was conducted at Benjamin Mkapa Hospital (BMH) Laboratory in Dodoma, the capital city of Tanzania. The Benjamin Mkapa Hospital is a ~~super-super~~-specialized hospital for serving patients in the central zone and all over the country in Tanzania. The hospital is a huge complex with over four hundred beds capacity, hundreds of outpatients, and over five hundred staffs. A study involved patient samples that were collected from patients from various hospital departments such as Internal medicine, Surgery, Urology, Pediatric, and child care unit, Obstetrics & ~~gynecology~~Gynecology, Ophthalmology, ENT (Ear, Nose & Throat), Dermatology, medical ward, surgical ward, physiotherapy unit, labor ward, Dialysis unit, Kidney transplant unit, Outpatient & inpatient department.

2.2 Study Design and Sampling Method

A ~~laboratory-laboratory~~-based ~~cross-cross~~-sectional study was carried out at BMH to determine pathogenic bacterial in stool ~~samples specimens~~ of the patient. A convenient sampling method was used to enroll 308 patients with diarrhea in the study after consenting for ~~a period of~~ 12 months. A ~~+~~trained personnel was assigned to collect ~~ed~~ samples continuously from the start day till the estimated sample size of 308 ~~was~~ reached. Each stool sample was assigned a serial sample number that ~~were was~~ linked to the patient's file.

2.3 Sample Collection

~~Stool samples were collected using sterile disposable stool containers as described by Mkala and Azizi [1] and then transported to the Microbiology department for processing according to current standard operating procedures. The stool specimens were collected using sterile disposable stool container as described by Mkala and Azizi [1]. And transported to Microbiology department for processing following existing standard operating procedures (SOPs).~~

2.4 Isolation and Bacterial Identification

The stool samples were inoculated onto Blood Agar (BA) and MacConkey Agar (MCA) for culture using a sterile wire loop and incubated for ~~a period of~~ 18-48 hours aerobic atmosphere at 37^oC. ~~A single colony of each sample was subcultured on fresh Salmonella-Shigella Agar (SSA) and MacConkey Agar with Sorbitol (SMCA) plate. A single colony of each sample was picked up to subculture onto newly plate of Salmonella-Shigella Agar (SSA) and MacConkey Agar with Sorbitol (SMCA).~~ Identification of ~~Escherichia E. coli were was done performed~~ using biochemical tests such as KIA, SIM, citrate, urea, LIA, and oxidase test after gram staining technique following existing SOPs. A single to three singly colonies of ~~Escherichia E. coli~~ were picked up to inoculate onto SMCA agar using sterile wire loop and incubated for 18-48 hours aerobic atmosphere at 37^oC for identification of ~~Escherichia E. coli strain O157:H7~~ that appear colorless on SMCA agar whereas, ~~non non-Escherichia coli strain O157:H7~~ appear pink.

2.5 Antimicrobial Susceptibility Testing (AST)

~~Each strain of E. coli O157:H7 and approximately two to three pure colonies of S. typhi were suspended in physiological sterile normal saline using a sterile cotton swab to compare the turbidity of 0.1 McFarland Equivalent standards before spreading on Muller Hinton Agar (MHA). About two to three pure colonies of each Escherichia coli strain O157:H7 and Salmonella typhi were stabbed in physiological sterile normal saline using sterile cotton swab to compare turbidity of 0.1 McFarland Equivalent standards before spreading onto Muller Hinton Agar (MHA). Thereafter, the suspended isolates were spread onto MHA using a sterile cotton swab, and antimicrobial discs were applied over the inoculum to identify drug resistance patterns of commonly used antibiotics disksstabbed isolates were spread onto MHA using sterile cotton swab and antimicrobial disks were applied on top of inoculums to determine the drug resistance patterns of commonly used antibiotics disks named Ampicillin (10 µg), Amoxicillin/Clavulanic acid (30 µg), Gentamycin (10 µg), Trimethoprim/Sulphamethoxazole (5/25 µg), Tetracycline (30 µg), Ciprofloxacin Amikacin (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Imipenem (10 µg), Nalidixic acid (30 µg), Piperacillin-Tazobactam (100/10 µg), and Chloramphenicol (30 µg) by Kirby-Bauer disc diffusion methods among the isolated Escherichia E. coli strain O157:H7 and Salmonella S. typhi described in the previous studiesy [1,18]. After 18-24 hours of incubation at 37°C, the diameter of the zone of inhibition were was measured using a millimeter scale around each antimicrobial disk on the under the surface of the plate. The zone size around each antimicrobial disk was interpreted as sensitive, intermediate, or resistant according to Clinical and Laboratory Standards Institute (CLSI) guidelines [19].~~

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Quality Control

Reference strains *Escherichia-E. coli* ATCC 25922 for gram-gram-negative and *Staphylococcus aureus* ATCC 25923 for gram-gram-positive bacteria were used for Microbiological quality controls of staining, culture, identification, and antimicrobial susceptibility testing procedures [1,-18].

2.7 Data Analysis

Data analysis was performed using *Statistical Package for Social Sciences (SPSS) version 17.0* software. Descriptive statistics of crosstabs were used to summarize the antimicrobial resistance pattern of bacterial isolates in tables. Chi-square test was used to compare proportions and frequency of occurrence bacterial isolates and drug resistance patterns of isolates across populations. A *p-value* ≤ 0.05 was considered *significant measure of statistically significant*.

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3.0 RESULTS

3.1 Demographic features of study participants

~~Of the 308 patients who participated in the study, 188 (61.0%) were female and 120 (39.0%) were male. Out of 308 participants, 61.0% (188) were female whereas 39.0% (120) were male. Among the participants in the study, those aged between 18 and 45 years constituted the largest majority with 257 patients (83.4%), while those over 60 years old constituted the least majority with 4 (1.2%). The age group of between 18 to 45years had larger number of participants recruited in the study 257 (83.4%) and less number 4 (1.2%) in age group above 60 years old. The majority of the patients included in the study consisted of 177 (57.5%) participants residing in the Dodoma region, followed by 49 (15.9%) and 25 (8.1%) participants residing in the Singida and Manyara regions, respectively, while only 1 (0.3%) participant (0.3%) was from the Kigoma region. The majority 177 (57.5%) of study participant recruited in study came from Dodoma region following Singida region 49 (15.9%), Manyara region 25 (8.1%) and fewer number 1 (0.3%) of patients recruited lived in Kigoma region (Table 1).~~

Table 1. Demographic features of patients attended BMH for treatment

Demographic	Description	Frequency	Percentage (%)
Age	Below 18	14	4.5
	18-45	257	83.4
	46-60	33	10.7
	Above 60	4	1.2
	Total	308	100.0
Sex/Gender	Male	120	39.0
	Female	188	61.0
	Total	308	100.0
Residence	DODOMA	177	57.5
	SINGIDA	49	15.9
	MOROGORO	18	5.8
	IRINGA	22	7.1
	MANYARA	25	8.1
	ARUSHA	6	1.9
	TABORA	7	2.3
	SHINYANGA	3	1.0
	KIGOMA	1	0.3
	Total	308	100.0

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3.2 Frequency of Bacterial growth

About 5.2 (16/308) percent of stool *specimen samples* processed were positive with pathogenic bacteria whereas 94.8 (292/308) percent were negative (no pathogenic bacteria isolated) growth. *There was an equal growth of percentage for both males and females, although not statistically significant for bacterial*

growth ($\chi^2=1.373$, $P=0.968$). There was equal percent isolate (growth) for both male and female although statistically was not significant for bacterial growth ($\chi^2=1.373$, $P=0.968$). While pathogen was observed in 11 (68.7%) patients from Dodoma, no pathogen was observed in patients from Morogoro, Iringa, Manyara and Kigoma. More isolates were observed in participants from Dodoma 11 (68.7%) and no isolate 0 (0.0%) from Morogoro, Iringa, Manyara and Kigoma regions. There was no pathogenic bacterial growth in the group over 60 years of age, while pathogen was observed in 13 (81.2%) patients aged 18-45 years. Meanwhile, there was no growth of pathogenic bacteria in age group above 60 years old, however the age group of 18 to 45 years presented larger number 13 (81.2%) of bacterial growth (Table 2).

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Table 2. Growth of Bacteria in patients attended BMH for treatment

Demographic	Description	No Pathogen Isolated	Growth of pathogen	Total
Age	Below 18	13 (4.5%)	1 (6.3%)	14 (4.5%)
	18-45	244 (83.5%)	13 (81.2%)	257 (83.5%)
	46-60	31 (10.6%)	2 (12.5%)	33 (10.7%)
	Above 60	4 (1.4%)	0 (0.0%)	4 (1.3%)
	Total	292 (100.0%)	16 (100.0%)	308 (100.0%)
Sex/Gender	Male	112 (38.4%)	8 (50.0%)	120 (39.0%)
	Female	180 (61.6%)	8 (50.0%)	188 (61.0%)
	Total	292 (100.0%)	16 (100.0%)	308 (100.0%)
Residence	DODOMA	166 (56.8%)	11 (68.7%)	177 (57.5%)
	SINGIDA	47 (16.1%)	2 (12.4%)	49 (15.9%)
	MOROGORO	18 (6.2%)	0 (0.0%)	18 (5.8%)
	IRINGA	22 (7.5%)	0 (0.0%)	22 (7.1%)
	MANYARA	25 (8.6%)	0 (0.0%)	25 (8.1%)
	ARUSHA	5 (1.7%)	1 (6.3%)	6 (2.0%)
	TABORA	6 (2.1%)	1 (6.3%)	7 (2.3%)
	SHINYANGA	2 (0.7%)	1 (6.3%)	3 (1.0%)
	KIGOMA	1 (0.3%)	0 (0.0%)	1 (0.3%)
Total	292 (100.0%)	16 (100.0%)	308 (100.0%)	

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3.3 Bacterial isolates in patients who attended BMH for treatment

In the study, two pathogenic bacteria, *E. coli* O157:H7 and *S. typhi*, were isolated from stool samples of patients with diarrhea who applied to the hospital for treatment. The study presented two pathogenic bacteria species named *Escherichia coli* strain O157:H7 and *Salmonella typhi* that were isolated from stool specimen of patients with diarrhea and attended the hospital for treatment. The prevalence of *S. typhi* in 308 stool samples was 3.2% (10/308), while *E. coli* O157:H7 was 1.9% (6/308). The prevalence of *Salmonella typhi* was 3.2% (10/308) whereas *Escherichia coli* strain O157:H7 was 1.9% (6/308) out of 308 stool specimens processed. Of the 16 bacterial isolates, 10 (62.5%) were *S. typhi* and 6 (37.5%) were *E. coli* O157:H7. No pathogen was found in patients from Morogoro, Iringa, Manyara and Kigoma regions. Most of whom were in the 18-45 age group, while *S. typhi* (8/80%) and *E. coli* O157:H7 (5/83%) were observed, no pathogen was observed in the group over 60 years of age (Table 3). As described in Table 4, while of the detected 10 *S. typhi* isolates, 6 (60%) were in male and 4 (40%) were in female patients, of the detected 6 *E. coli* O157:H7 isolates, 2 (33%) were in male patients and 4 (67%) were in female patients.

Out of 16 bacterial isolates, 10 (62.5%) were *Salmonella typhi* and 6 (37.5%) were *Escherichia coli* strain O157:H7. It was observed that no any bacterial isolates in patient from Morogoro, Iringa, Manyara and Kigoma region. However of the high number of both *Escherichia coli* strain O157:H7 (5/83%) and *Salmonella typhi* (8/80%) in age group 18 to 45 years, there was no bacterial isolate in age group above 60 years (Table 3). Male had more isolates of *Salmonella typhi* 6 (60%) as compared to female 4 (40%). On the other hand, male had less number 2 (33%) of *Escherichia coli* strain O157:H7 than female 4 (67%) as described in Table 4.

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Table 3. Bacteria isolated in patients attended BMH for treatment

Demographic	Description	<i>Salmonella S. typhi</i> (%)	<i>Escherichia E. coli strain</i> O157:H7 (%)	Total (%)
Age	Below 18	1 (10)	0 (0)	1 (6)
	18-45	8 (80)	5 (83)	13 (81)
	46-60	1 (10)	1 (17)	2 (13)
	Above 60	0 (0)	0 (0)	0 (0)
	Total	10 (100)	6 (100)	16 (100)
Sex/Gender	Male	6 (60)	2 (33)	8 (50)
	Female	4 (40)	4 (67)	8 (50)
	Total	10 (40)	6 (100)	16 (100)
Residence	DODOMA	7 (70)	4 (66)	11 (69)
	SINGIDA	1 (10)	1 (17)	2 (13)
	MOROGORO	0 (0)	0 (0)	0 (0)
	IRINGA	0 (0)	0 (0)	0 (0)
	MANYARA	0 (0)	0 (0)	0 (0)
	ARUSHA	1 (10)	0 (0)	1 (6)
	TABORA	0 (0)	1 (17)	1 (6)
	SHINYANGA	1 (10)	0 (0)	1 (6)
	KIGOMA	0 (0)	0 (0)	0 (0)
	Total	10 (100)	6 (100)	16 (100)

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3.4 Antimicrobial Resistance Patterns of *Salmonella S. typhi* and *Escherichia E. coli strain* O157:H7 in patients attended at BMH for treatment

The *in vitro* drug resistance patterns of Ampicillin ~~was-were~~ observed to be high 9 (90%) followed by Amoxicillin/Clavulanate and ~~tetracycline-Tetracycline~~ of which both had 6 (60%) ~~resistant-resistance~~ to *Salmonella S. typhi* (Table 4). The ~~imipenem-Imipenem~~, Chloramphenicol and Piperacillin/Tazobactam were not resistant to *Salmonella S. typhi*. ~~Meanwhile~~ ~~On the other hand~~, ~~Tetracycline was highly resistant to E. coli O157:H7 in 6 (100%) patients followed by Ampicillin and Trimethoprim/Sulphamethoxazole in 5 (83%) patients, whereas Imipenem and Piperacillin/Tazobactam had no resistance to E. coli O157:H7~~ tetracycline was highly resistant 6 (100%) to *Escherichia coli strain* O157:H7 followed by 5 (83%) both ampicillin and Trimethoprim/Sulfamethoxazole and there was no resistance of imipenem and Piperacillin/Tazobactam to *Escherichia coli strain* O157:H7 (Table 4).

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Table 4. Antimicrobial Resistance Patterns to *Salmonella S. typhi* and *Escherichia E. coli* strain O157:H7

Antimicrobials	Resistance patterns (%) to <i>Salmonella S. Typhi</i>	Resistance patterns (%) to <i>Escherichia E. coli</i> strain O157:H7	X ²	P-value	OR	95% CI
Amikacin	4 (40)	1 (16)	0.950	0.330	0.300	0.025-3.626
Imipenem	0 (0)	0 (0)	-	-	-	-
Chloramphenicol	0 (0)	1 (16)	1.778	0.182	1.200	0.839-1.716
Gentamicin	4 (40)	1 (16)	0.950	0.330	0.300	0.025-3.625
Piperacillin/-Tazobactam	0 (0)	0 (0)	-	-	-	-
Ampicillin	9 (90)	5 (83)	1.52	0.696	0.556	0.028-10.933
Nalidixic Acid	2 (20)	1 (16)	0.027	0.869	0.800	0.057-11.298
Amoxicillin/-Clavulanate	6 (60)	3 (50)	0.152	0.696	0.667	0.087-5.127
Ceftazidime	3 (30)	3 (50)	0.640	0.424	2.333	0.287-18.965
Ceftriaxone	3 (30)	3 (50)	0.640	0.424	2.333	0.287-18.965
Ciprofloxacin	2 (20)	3 (50)	1.571	0.210	4.000	0.431-37.108
Tetracycline	6 (60)	6 (100)	3.200	0.074	0.600	0.362-0.995
Trimethoprim/Sulfamethoxazole	4 (40)	5 (83)	2.861	0.091	7.500	0.621-90.646

X²: *Chi-Chi*-square; CI: Confidence Interval; OR: Odds Ratio

4.0 DISCUSSION

This study was the first survey conducted to determine the prevalence and antimicrobial resistance patterns of pathogenic *Escherichia E. coli* strain O157:H7, *Salmonella*, and *Shigella* species in stool specimen samples of patients with diarrhea at Benjamin Mkapa Hospital. The study observed a higher prevalence of *Salmonella S. typhi* (3.2%) following *Escherichia E. coli* strain O157:H7 (1.9%) in stool specimens samples of patients.

The presence of *E. coli* O157:H7 in stool specimens from patients with diarrhea is an extremely important issue that deserves attention as scientists and medical practitioners seek further effort and study on pathogenic *E. coli* O157:H7 as a routine test in a basic laboratory. The presence of *Escherichia coli* strain O157:H7 in stool specimen of patients with diarrhea is alarming. Scientists and medical practitioners to put more effort and work on pathogenic *Escherichia coli* strain O157:H7 as routine test in basic laboratory as advised earlier suggested by Mkala and Azizi in 2017 [1]. These This advice suggestion come due to derive from the currently existing protocol of regarding *Escherichia E. coli* strain O157:H7 as a rare disease.

Hence, it is not routinely performed in the laboratory for patient treatment, not for research purposes worldwide. The study also revealed the presence of *E. coli* O157:H7 as observed in previous studies, although the WHO did not announce that it would include *E. coli* O157:H7 in routine testing, but is still present in many patients and continues to threaten health. Therefore, it is globally not routinely performed in laboratory for patient treatment rather than research purposes. The study is also revealed the presence of *Escherichia coli* strain O157:H7 as observed in previous studies even though the WHO has not announced to incorporate *Escherichia coli* strain O157:H7 in routine tests but still exist in many patient and continuing to pose threatening of health [1,20,21]. On the other hand, since *E. coli* is among the normal flora of the gastrointestinal tract in both humans and animals, it has been reported in previous studies that beef is a source of *E. coli* and can be transmitted to humans due to the habit of eating beef [1,18].

The study has reported a high prevalence of *S. typhi* than other bacteria species. This was probably due to the environmental behavior of people to acquire infectious agents from contaminated water as the

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study was conducted in both seasons of rain and dry season. People tend to fetch rainwater from the well particularly in the village and use it for various activities including cooking drinking, washing clothes, and other activities in which may probably infect people. However, people of age group 18-45 years has a larger number of bacterial isolate than others, this was probably due to daily activities resulting in exposure to the risk factors of fetching water especially female and caregivers of cattle for male in which may get the infection through contacting fecal from cattle. Meanwhile, people of the age group above 45 and below 18 years might have less contact with risk factors.

~~Since *E. coli* is among the normal flora of the gastrointestinal tract in both humans and animals, it has been reported in previous studies that beef is a source of *E. coli* and can be transmitted to humans due to the habit of eating beef. Since *Escherichia coli* is among of the normal flora of gastrointestinal tract of both human and animal, people might have acquired from habit of eating beef meat as reported in previous studies that cattle is a common reservoir of *Escherichia coli* and may be transferred to human through eating [1,18].~~

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~~The study has reported a high prevalence of *Salmonella S. typhi* than other bacteria species. This was probably due to the environmental behavior of people to acquire infectious agents from contaminated water as the study was conducted in both seasons of rain and dry season. People tend to fetch rain water from the well particularly in the village and use it for various activities including cooking drinking, washing clothes, and other activities in which may probably infect people. However, people of age group 18-45 years has a larger number of bacterial isolate than others, this was probably due to daily activities resulting in exposure to the risk factors of fetching water especially female and caregivers of cattle for male in which may get the infection through contacting fecal from cattle. Meanwhile, people of the age group above 45 and below 18 years might have less contact with risk factors.~~

~~However people of age group 18-45 years has larger number of bacterial isolate than other, this was probably due to daily activities resulting to exposing to the risk factors of fetching water especially female and care givers of cattle for male in which may get infection through contacting fecal from cattle. Meanwhile, people of the age group above 45 and below 18 years might have less contact to risk factors.~~

~~Furthermore, *E. scherichia coli* strain O157:H7 were was highly resistant to Tetracycline and Ampicillin that was was revealed to in other studies. This was probably due to the irrational use of antimicrobials in both humans and cattle [1,22]. Consequently, Ampicillin showed close resistance to both *S. typhi* and *E. coli* O157:H7, suggesting that all strains may have acquired the same resistance mechanism for the same antimicrobial. Meanwhile, ampicillin Ampicillin slightly resembled resistant resistance to both *Salmonella S. typhi* and *Escherichia E. coli* strain O157:H7 suggesting that all species might have acquired the same resisting mechanism to the same antimicrobial.~~

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5.0 LIMITATIONS OF STUDY

The study lacked a reagent for performing gene sequencing. There was an unequal number of participants in age groups, gender, and residence in order to get a correlation of isolates and drug resistance patterns.

6.0 RECOMMENDATIONS

The nation is advised to continuously provide relevant health education to encourage the use of prescribed drugs in order to reduce the burden of increasing antimicrobial resistance in the community as described by [1]. It has been advised to perform routinely culture for pathogenic *Escherichia E. coli* strain O157:H7 as currently has been increasing in the community.

7.0 CONCLUSIONCONCLUSION

The BMH Laboratory was not performing culture to identify pathogenic *Escherichia E. coli* strain O157:H7 as a routine test. Therefore, the study is currently insisting laboratory practitioners to carry out an investigation of *E. scherichia coli* strain O157:H7 as a routine test in parallel with other enteric pathogens, however, *Salmonella S. typhi*, *Salmonella S. paratyphi*, and *Shigella* species are the common bacteria paid attention for infecting patients [1].

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CONSENT

Each study participant signed a consent form for voluntary agreeing to be involved in the study.

ETHICAL ISSUES

The study was approved by Central Zone Health Research Ethics Review Committee (CZHREC) and Benjamin Mkapa Hospital authority will grand-grant permission to conduct this study at Benjamin Mkapa Hospital allowed conduction of the study in Benjamin Mkapa Hospital Laboratory. The study complied with the principals-principles of the Helsinki and Good laboratory practices that Confidentiality was kept for all information gathered from the study.

REFERENCES

1. Mkala, R. S and Azizi, K, A. (2017). Prevalence and Antimicrobial Resistance Patterns of Extended Spectrum Beta Lactamase Producing Enterohemorrhagic *Escherichia coli* Strain O157:H7 from Cattle and Humans in Moshi, Northern Tanzania. *Microbiology Research Journal International*. 19(3): 1-10.
2. Fereshteh Jafari, Mohammad Hamidian, Maryam Rezadehbashi, Michael Doyle, Siavosh Salmanzadeh-ahrabi, Faramarz Derakhshan, and Mohammad Reza Zali, (2009) Prevalence and antimicrobial resistance of diarrheagenic *Escherichia coli* and *Shigella* species associated with acute diarrhea in Tehran, Iran. *The Canadian journal of infectious diseases & medical microbiology*. vol. 20,3 (2009): e56-62. doi:10.1155/2009/341275
3. Wilson G, Easow JM, Chiranjoy M, Shivananda PG. Isolation and antimicrobial susceptibility of *Shigella* from patients with acute gastroenteritis in western Nepal. *Indian J Med Res*. 2006;123:146–50. [PubMed] [Google Scholar]
4. Paresh K. Virpari, J. B. Nayak, H. C. Thaker, M. N. Brahmabhatt (2013). Isolation of pathogenic *Escherichia coli* from stool samples of diarrhoeal patients with history of raw milk consumption. *Veterinary World*, EISSN: 2231-0916.
5. Tchaptchet, S. and Hansen, J. (2011). The Yin and Yang of host-commensal mutualism. *Gut Microbes*.2: 347–352.
6. Ibrahim ME, Bilal NE, Hamid ME. (2012). Increased multi-drug resistant *Escherichia coli* from hospitals in Khartoum state, Sudan. *Afr. Health Sci*. 2012;12:368- 375.
7. Lee K, Lim S., Choi H, Lim S, Song J, and An D (2014). Plasmid-mediated AmpC β -lactamase (CMY-2) gene in *Salmonella typhimurium* isolated from diarrheic pigs in South Korea, 7(1), 1–4.
8. Mshana SE, Kamugisha E, Mirambo M, Chakraborty T, and Lyamuya EF (2009). Prevalence of multiresistant gram-negative organisms in a tertiary hospital in Mwanza Tanzania. *BMC Res Notes* 2009, 2:49.
9. WHO, (2012). The evolving threat of antimicrobial resistance: options for action. World Health Organization 2012. Geneva, Switzerland. ISBN 978 92 4 150318 1.
10. Li R, Lai J, Wang Y, Liu S, Li Y, Liu K, Shen J, and Wu C (2013). Prevalence and characterization of *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China. *Int J Food Microbiol* 2013, 163:14–18.
11. Kajeguka D.C, Nambunga P.P, Kabissi F, Benjamin Kamugisha B, Kassam N, Nyombi B, Mataro C, Venance Maro V, Chilongola J.O. (2015). Antimicrobial resistance patterns of phenotype Extended Spectrum Beta-Lactamase producing bacterial isolates in a referral hospital in northern Tanzania. *Tanzan. J. Health Res*. 2015; Volume 17, Number.
12. Mshana SE, Matee M, and Rweyemamu M (2013). Antimicrobial resistance in human and animal pathogens in Zambia, Democratic Republic of Congo, Mozambique and Tanzania: An urgent need of a sustainable Surveillance system. *Ann-clinmicrob.com/content/12/1/28*.
13. Aiyegoro O, Adewusi A, Oyedemi S, Akinpelu D, and Okoh IA (2011). Interactions of antibiotics and methanolic crude extracts of *Azelaia africana* (Smith) against drug resistance bacterial isolates. *Int. J. Mol. Sci.*, 12: 4477- 4487.
14. Byarugaba DK, Kisame R, and Olet S (2011). Multi-drug resistance in commensal bacteria of food of animal origin in Uganda. *Afr. J. Microbiol. Res*. 5(12): 1539-1548.
15. Moyane JN, Jideani AI, Aiyegor OA (2013). Antibiotics usage in food-producing animals in South Africa and impact on human: Antibiotic resistance. *African Journal of Animal production and Husbandry* Vol. 1 (1), pp. 001-008
16. Thaller MC, Migliore L, Marquez C, Tapia W, Cedeno V, Rossolini M, and Gentile G. (2010). Tracking acquired antibiotic resistance in commensal bacteria of Galapagos Land Iguanas: No Man, No Resistance. *PLoS ONE* 5(2): e8989.
17. WHO, (2014). Antimicrobial Resistance: Global report on surveillance 2014. WHO media centre.

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18. Chandika, A. B., Mkala, R. S., Lugoba, B., Kipilipili, B. C., Saitot, W., Kamkunguru, C. E., Susu, S. J., Mkhosi, M. L., Lindi, J. B., & Matamba, L. E. (2021). Bacterial Contaminants on Exposed Surfaces and Their Antibiotic Sensitivity Patterns at the Benjamin Mkapa Hospital, Dodoma-Tanzania. *Asian Journal of Research in Infectious Diseases*, 7(1), 1-11. <https://doi.org/10.9734/ajrid/2021/v7i130205>
19. CLSI, (2011). Performance standards for antimicrobial susceptibility testing; informational supplement. CLSI document M100-S20. Clinical and Laboratory Standards Institute, Wayne, PA.
20. Lupindu AM, Olsen JE, Ngowi HA, Msoffe PLM, Mtambo MM, Scheutz F, et al. Occurrence and characterization of Shiga toxin-producing *Escherichia coli* O157:H7 and other non-sorbitol-fermenting *E. coli* in cattle and humans in urban areas of Morogoro, Tanzania. *Vector Borne Zoonotic Dis.* 2014;14503–510. DOI:10.1089/vbz.2013.1502.
21. Nelson E, Kayega J, Seni J, Mushi MF, Kidenya BR, Hokororo A, Zuechner A, Kihunrwa A, Mshana SE. Evaluation of existence and transmission of extended spectrum beta lactamase producing bacteria from post-delivery women to neonates at Bugando Medical Center, Mwanza-Tanzania. *BMC. Res. Notes.* 2014;7:279.
22. Chilongola J, Msoka E, Juma A, Kajeguka D.C, Semvua H, Kituma E, Kwigizile E, Nyombi B. (2015). Antibiotics Prescription Practices for Provisional Malaria Cases in Three Hospitals in Moshi, Northern Tanzania. *Tanzania. Journal of Health Research.* 2015; 17: 3.

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