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Prevalence and Antimicrobial Resistance Patterns of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* Species from Stool Samples of Patients with Diarrhea at Benjamin Mkapa Hospital

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Authors' contributions

This work was carried out in collaboration among all authors. Author ABC involved in designing the study, reviewed and provided major inputs of the manuscript. Author RSM involved in designing the study, performed collection of data, laboratory experiments, analysis of data and developed the manuscript. Author BL involved in data collection and reviewed the manuscript. Author BCK involved in performing laboratory tests. Author WS involved in data collection. Author CEK involved in data collection. Author SJS involved in performing laboratory tests. Author MLM involved in performing laboratory tests. Author JBL reviewed the manuscript. Author JDC involved in performing laboratory tests. Author CAM involved in performing laboratory tests. Author HEK reviewed the manuscript. Author EJS performed analysis of data. Author LEM reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Enterobacteriaceae is among the large group of gram-negative rod bacteria in which it comprises *Escherichia coli, Salmonella, Shigella*, and many other species whose natural habitat is the intestinal tract of humans and animals. Diarrheal diseases are a major problem worldwide caused by bacterial pathogens particularly in developing countries. *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/sulphamethoxazole, and amoxicillin/ clavulanic acid.

Methods: A laboratory-based cross-sectional study was conducted at BMH. A convenient sampling method was used to enroll 308 patients with diarrhea in the study after consent for 12 months. Stool samples were collected into an acceptable clean sterile stool container and transported to 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 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 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 *E. coli* strain O157:H7 and *Salmonella typhi* that were isolated from stool samples of patients with diarrhea and who attended the hospital for treatment. No any *shigella* species was isolated in the study. The prevalence of *S. typhi* was 3.2% (10/308) whereas *E. coli* strain O157:H7 was 1.9% (6/308) out of 308 stool samples processed. The *in vitro* drug resistance patterns of Ampicillin were observed to be high 9 (90%) followed by Amoxicillin/Clavulanate and tetracycline of which both had 6 (60%) resistance to *S. typhi*.

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

Keywords: Antimicrobial resistance; Escherichia coli O157:H7; Salmonella and Shigella species; diarrhea; Tanzania.

1. INTRODUCTION

Enterobacteriaceae is among the large group of gram-negative rod bacteria in which it comprises *Escherichia coli, Salmonella, Shigella*, and many other species whose natural habitat is the intestinal tract of humans and animals [1]. Diarrheal diseases are a major problem worldwide caused by bacterial pathogens particularly in developing countries [2,3]. The enteropathogenic *Escherichia coli* affecting humans are categorized into Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC); enteroaggregative *E. coli* (EAEC), entero-toxigenic *E. coli* (ETEC), diffusely adherent *E. coli* (DAEC), and enteroinvasive *E. coli* (EIEC) including the Shigella and Salmonella species are common bacteria that causes diarrhea [1,2,4,5].

Antibiotics are drugs that kill and prevent the multiplication or growth of microbes. They are the most common antimicrobial agents prescribed in hospitals worldwide [1,6,7,8,9]. 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 [1,10].

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

The increase in the rate of antimicrobial drugs resistance in our countries requires further efforts to classify specific causes and practices that aggravate the problem. Whether such factors are professional, infrastructural, social, or personal is not yet fully known [1].

The increase of antibiotics resistance to currently used antibiotics for both pathogenic and commensally bacteria of gram-negative rods are species-specific extended-spectrum beta-lactamase (ESBL) rate about 24.4% with resistances rate to gentamicin, tetracycline, sulphamethoxazole / trimethoprim, and ciprofloxacin were significantly higher among ESBLs isolates than non-ESBL isolates [7,11] and about 90% in different places reported that Co-trimoxazole, Ampicillin, Gentamycin, and Penicillin are commonly emerging resistance in these areas [7,12,9].

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. [13-17].

There is little doubt that *E. coli* O157:H7 strain 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 [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.

2. 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-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 staff. 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, 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-based cross-sectional study was carried out at BMH to determine pathogenic bacterial in stool samples of the patient. A convenient sampling method was used to enroll 308 patients with diarrhea in the study after consent for 12 months. Trained personnel was assigned to collect 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 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 (SOPs) [1].

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 18-48 hours aerobic atmosphere at 37^0C. A single colony of each sample was subcultured on fresh *Salmonella-Shigella* Agar (SSA) and MacConkey Agar with Sorbitol (SMCA) plate. Identification of *E. coli* was performed using biochemical tests such as KIA, SIM, citrate, urea, LIA, and oxidase test after gram staining technique following existing procedures [1]. A single to three singly colonies of *E. coli* were picked up to inoculate onto SMCA agar using sterile wire loop and incubated for 18-48 hours aerobic atmosphere at 37^0C for identification of *E. coli* O157:H7 that appear colorless on SMCA agar whereas, non-Escherichia coli O157:H7 appear pink colour on SMCA [1].

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). Then, 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 antibiotic discs named Ampicillin (10 µg), Amoxicillin/Clavulanic acid (30 µg), Gentamycin (10 µg), Trimethoprim / Sulphamethoxazole (5/25 ug), Tetracycline (30 µg), Ciprofloxacin Amikacin (30 ug), 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 *E. coli* O157:H7 and *S. typhi* described in the previous studies [1,18]. After 18-24 hours of incubation at 37°C, the diameter of the zone of inhibition was measured using a millimeter scale around each antimicrobial disk 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].

2.6 Quality Control

Reference strains *E. coli* ATCC 25922 for gram-negative and *Staphylococcus aureus* ATCC 25923 for 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 SPSS 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 statistically.

3. 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. 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 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 was from the 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
	MANYARA	25	8.1
	IRINGA	22	7.1
	MOROGORO	18	5.8
	TABORA	7	2.3
	ARUSHA	6	1.9
	SHINYANGA	3	1.0
	KIGOMA	1	0.3
	Total	**308**	**100.0**

3.2 Frequency of Bacterial Growth

About 5.2 (16/308) percent of stool 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 (X^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.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 (Table 2).

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. No any *shigella* species was isolated in the study. The prevalence of *S. typhi* in 308 stool samples was 3.2% (10/308), while *E. coli* O157:H7 was 1.9% (6/308). 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.

3.4 Antimicrobial Resistance Patterns of *S. typhi* and *E. coli* O157:H7 in Patients Attended at BMH for Treatment

The *in vitro* drug resistance patterns of Ampicillin were observed to be high 9 (90%) followed by Amoxicillin/Clavulanate and Tetracycline of which both had 6 (60%) resistance to *S. typhi* (Table 4). The Imipenem, Chlorampenicol and Piperacillin/ Tazobactam were not resistant to *S. typhi.* 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 (Table 4).

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%)
	MANYARA	25 (8.6%)	0 (0.0%)	25 (8.1%)
	IRINGA	22 (7.5%)	0 (0.0%)	22 (7.1%)
	MOROGORO	18 (6.2%)	0 (0.0%)	18 (5.8%)
	TABORA	6 (2.1%)	1 (6.3%)	7 (2.3%)
	ARUSHA	5 (1.7%)	1 (6.3%)	6 (2.0%)
	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%)**

Table 3. Bacteria isolated in patients attended BMH for treatment

Demographic	Description	*S. typhi* (%)	*E. coli* 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)**

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

Antimicrobials	Resistance patterns (%) to *S. typhi*	Resistance patterns (%) to *E. coli* 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)	-	-	-	-
Chlorampenicol	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

X^2: Chi-square; CI: Confidence Interval; OR: Odds Ratio

4. DISCUSSION

This study was the first to determine the prevalence and antimicrobial resistance patterns of pathogenic *E. coli* O157:H7, *Salmonella*, and *Shigella* species in stool samples of patients with diarrhea at Benjamin Mkapa Hospital. The study observed a higher prevalence of *S. typhi* (3.2%) following *E. coli* O157:H7 (1.9%) in stool 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 as earlier suggested by Mkala and Azizi in 2017 [1]. This suggestion derive from the currently existing protocol regarding *E. coli* 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 [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].

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 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.

Furthermore, *E. coli* O157:H7 was highly resistant to Tetracycline and Ampicillin that was revealed 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.

5. 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 to get a correlation of isolates and drug resistance patterns.

6. CONCLUSION

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

The results of this study will aid us to get baseline 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.

7. RECOMMENDATIONS

The nation is advised to continuously provide relevant health education to encourage the use of prescribed drugs 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 *E. coli* O157:H7 as currently has been increasing in the community.

CONSENT

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

ETHICAL APPROVAL

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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