ORIGINAL RESEARCH ARTICLE

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

ABSTRACT

Background

Enterobactereceae is among the large group of gram negative rod bacteria in which it comprises of *Escherichia coli,* Salmonella*,* Shigella and many other species whose natural habitat is intestinal tract of humans and animals. The diarrheal diseases are a major problem worldwide caused by bacterial pathogens particularly in developing countries. 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 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 consenting for a period of 12 months. Stool samples were collected into 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 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. About 5.2 (16/308) percent of stool specimen 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 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 *Salmonella typhi* was 3.2% (10/308) whereas *Escherichia coli* strain O157:H7 was 1.9% (6/308) out of 308 stool specimens processed. The *in vitro* drug resistance patterns of ampicillin was observed to be high 9 (90%) followed by Amoxicillin/ Clavulanate and tetracycline of which both had 6 (60%) resistant to *Salmonella typhi* (Table 4).

Conclusion

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

Keywords: Prevalence; Antimicrobial; Resistance; Patterns; *Escherichia; Coli;* O157:H7; Salmonella; Shigella; Species; Stool; Specimens; Patients; Diarrhea; Benjamin Mkapa; Hospital.

1.0 INTRODUCTION

Enterobactereceae is among the large group of gram negative rod bacteria in which it comprises of *Escherichia coli,* Salmonella*,* Shigella and many other species whose natural habitat is intestinal tract of humans and animals [1]. The 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 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 entero invasive *E. coli* (EIEC) including the shigella and Salmonella species are are common bacteria that causes diarrhea [1,2,4,5].

Antibiotics are the drugs that kill and prevent multiplication or growth of microbes. They are the most common antimicrobial agents prescribed in hospitals worldwide [1,7,8,10,12]. Use of similar or identical antibiotics in human 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 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 and amoxicillin/ clavulanic acid [1].

The increase in the rate of antimicrobial drugs resistance in our countries calls for more efforts to identify specific causes and practices that aggravate the problem. Whether such factors are professional, infrastructural, social or personal 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 specific extended spectrum beta-lactamase (ESBL) rate about 24.4% with resistances rate to gentamicin, tetracycline, sulphamethaxazole/trimethoprim and ciprofloxacin were significantly higher among ESBLs isolates than non-ESBL isolates [8,9] and about 90% in difference places reported that Co-trimoxazole, Ampicillin, Gentamycin, and Penicillin are commonly emerging resistance in these areas [8,11,12].

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 coli* strain O157:H7 are common pathogenic bacteria infecting patients daily at the Benjamin Mkapa Hospital. However, Sal*monella typhi, Salmonella paratyph*i 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].

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.

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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.

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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 specialized hospital for serving patients in 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, 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 specimens of 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 collected samples continuously from the start day till the estimated sample size 308 reached. Each stool sample was assigned a serial sample number that were linked to the patient's file.

2.3 Sample Collection

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 sterile wire loop and incubated for a period of 18-48 hours aerobic atmosphere at 37^0C. 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 coli* were done 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 coli* were picked up to inoculate onto SMCA agar using sterile wire loop and incubated for 18-48 hours aerobic atmosphere at 37 ^{0}C for identification of *Escherichia coli* strain O157:H7 that appear colorless on SMCA agar whereas, non *Escherichia coli* strain O157:H7 appear pink.

2.5 Antimicrobial Susceptibility Testing (AST)

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, stabbed 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 (10µg), Amoxicillin/Clavulanic acid (30µg), Gentamycin (10µg), Trimethoprim/Sulphamethoxazole (5/25 ug), Tetracycline (30µg), Ciprofloxacin Amikacin (30ug), Ceftriaxone (30µg), Ceftazidime (30µg), Imipenem (10µg), Nalidixic acid (30µg), Piperacillin-Tazobactam (100/10µg), and Chloramphenicol (30µg) by Kirby Bauer disc diffusion methods among the isolated *Escherichia coli* strain O157:H7 and Salmonella typhi described in previous study [1,18]. After 18-24 hours of incubation at 37°C, the diameter of zone of inhibition were measured using a millimeter scale around each antimicrobial disk on the under 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].

Quality Control

Reference strains *Escherichia 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 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 measure of statistical significant.

3.0 RESULTS

3.1 Demographic features of study participants

Out of 308 participants, 61.0% (188) were female whereas 39.0% (120) were male. 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 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**

3.2 Frequency of Bacterial growth

About 5.2 (16/308) percent of stool specimen processed were positive with pathogenic bacteria whereas 94.8 (292/308) percent were negative (no pathogenic bacteria isolated) growth. There was equal percent isolate (growth) for both male and female although statistically was not significant for bacterial growth (X^2=1.373, P=0.968). More isolates were observed in participants from Dodoma 11 (68.7%) and no isolate 0 (0.0%) from Morogoro, Iringa, Manyara and Kigoma regions. 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).

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%)**

3.3 Bacterial isolates in patients attended BMH 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 *Salmonella typhi* was 3.2% (10/308) whereas *Escherichia coli* strain O157:H7 was 1.9% (6/308) out of 308 stool specimens processed. 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.

Table 3. Bacteria isolated in patients attended BMH for treatment

Demographic	Description	*Salmonella typhi* (%)	*Escherichia coli* strain 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)**

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

The *in vitro* drug resistance patterns of ampicillin was observed to be high 9 (90%) followed by Amoxicillin/ Clavulanate and tetracycline of which both had 6 (60%) resistant to *Salmonella typhi* (Table 4). The imipenem, Chlorampenicol and Piperacillin/ Tazobactam were not resistant to *Salmonella typhi.* Meanwhile, 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).

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

Antimicrobials	Resistance patterns (%) to *Salmonella Typhi*	Resistance patterns (%) to *Escherichia coli* 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)	-	-	-	-
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

4.0 DISCUSSION

This was the first survey conducted 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 study observed a higher prevalence of *Salmonella typhi* (3.2%) following *Escherichia coli* strain O157:H7 (1.9%) in stool specimens of patients.

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 by Mkala and Azizi in 2017 [1]. These advices come due to current existing protocol of regarding *Escherichia coli* strain O157:H7 as rare disease.

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].

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 though eating [1,18].

The study has reported a high prevalence of *Salmonella typhi* than other bacteria species. This was probably due to 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 well particularly in village and use 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 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, *Escherichia coli* strain O157:H7 were highly resistant to tetracycline and ampicillin that was revealed to other studies. This was probably due to irrational use of antimicrobials in both human and cattle [1,22]. Meanwhile, ampicillin slightly resembled resistant to both *Salmonella typhi* and *Escherichia coli* strain O157:H7 suggesting that all species might have acquired the same resisting mechanism to the same antimicrobial.

5.0 LIMITATIONS OF STUDY

The study lacked reagent for performing gene sequencing. There was 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 continuous provide relevant health education to encourage 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 coli* strain O157:H7 as currently has been increasing in the community.

7.0 CONCLUTION

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

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 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 of the Helsinki and Good laboratory practices that Confidentiality was kept for all information gathered from study.

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