

Original Research Article

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

ABSTRACT

Aim: We aimed to determine the prevalence and drug resistance patterns of ESBL producing Enterohemorrhagic *Escherichia coli* strain O157:H7 (EHEC O157:H7) isolated from cattle and humans in Moshi, northern Tanzania.

Method: A comparative cross sectional study was carried out to enroll 200 cattle recto-fecal specimens and 107 human stool specimens in Moshi, northern Tanzania. A convenient sampling method was used to enroll human patients with diarrhea or abdominal complaints and rectal fecal specimens from cattle upon consenting a patient, guardian or owner of the cattle. Bacterial isolation, identification and antimicrobial susceptibility testing were performed based on the standard operating procedures (SOPs). The EHEC O157:H7 were phenotypically confirmed by using MacConkey agar with sorbitol (SMCA) whereas, the suspected ESBL producing strains of EHEC O157:H7 were further confirmed by using the Minimum inhibitory concentration (M. I. C.) Test Strip.

Results: About 20 (10.0%) out of 200 recto-fecal specimens collected from cattle were positively confirmed ESBL producing EHEC O157:H7, whereas 10 (9.30%) out of 107 human specimens were confirmed ESBL producing EHEC O157:H7 ($\chi^2=0.961$, $P= 0.002$). Twelve (6.0%) of the cattle specimens were ESBL producing non-EHEC O157:H7 whereas 21 (19.6%) of human specimens confirmed as ESBL producing non-EHEC O157:H7. A high number of non-EHEC O157:H7 isolates was found in male 13 (20.3%) than in females 8(18.6%) (OR= 1.506, 95% CI= 0.534-4.236). Both cattle (n=20) and human (n=10) isolates revealed high (100%) resistance against Trimethoprim/ sulfamethoxazole and Tetracycline.

Conclusion: Our study revealed that ESBL producing *E. coli* is common among human patients as well as cattle in Moshi, Tanzania. The study is supporting 'ONE HEALTH' approach which ensure a

sustainable control and reduction of transmission and spread of drug resistant bacteria in humans and cattle.

Keywords: *Antimicrobial Resistance; Beta-lactamase; Extended Spectrum; Escherichia coli O157:H7*

INTRODUCTION

ESBL is currently a burden of global concern to humans as well as animals and the ecosystem at large. This is due to zoonotic spread of disease between animals and humans [1-3]. ESBL confer resistant to penicillin, cephalosporin and aztreonam including other antimicrobial agent such as trimethoprim-sulfamethoxazole, aminoglycosides and quinolones [4, 5] and are posing a major therapeutic challenge today in the treatment of patients [6].

ESBL producing *E. coli* is among the most important strains of public health concern due to their resistance to antibiotics [7-14]. ESBL producing *E. coli* are isolated with increasing frequency in animal and human specimens [15]. *E. coli* plays an important source of resistance gene and can transmit ESBL gene to other bacteria by conjugation [16].

The transmission of ESBL producing *E. coli* or ESBL gene between animals and humans is currently a subject of intensive discussion due to the zoonotic spread of resistance strains between humans and animals [1-3, 17, 18]. This can results into interface that enables transmission of antibiotic resistance genes between two strains or bacterial species [19, 20].

Several studies have addressed the risk factors for ESBL carriage that could arise from traveling to areas with a high number of cases within the last one-year, hygienic behavior, antibiotic consumptions, hospitalization genetic factors and keeping of pet animals [19, 21-28]. Irrational use of antibiotics in clinical medicine and animal husbandry has suggested to be a major factor in the emergence and dissemination of antimicrobial resistance in animals and humans [29] leading to the development of multi-drug resistance of bacteria [30-32].

A recent study in Tanzania has documented that CTX-M-15 were dominant in ESBL among the clinical isolates and were associated with significant morbidity as well as mortality [33, 34]. Moreover, the growing problem of antibiotic resistance were observed with varying ESBL alleles in animals [15, 19, 35].

Currently there is little information known about the prevalence and antimicrobial resistance patterns of ESBL producing EHEC O157:H7 in cattle that is a common reservoir of EHEC and interact with humans in day-to-day lives, although a lot of information is available in human medicine [28, 36].

Therefore, this study aimed to compare and provide baseline information on the prevalence and antimicrobial resistance pattern of ESBL producing EHEC O157:H7 in cattle and humans from the same area in Moshi urban Tanzania. The findings of this study highlighted the importance of 'ONE HEALTH' approach which will ensure a sustainable control and hence reduction of transmission and spread of drug resistant bacteria in humans and cattle.

METHODS

Study design and sampling process

This was a comparative cross sectional study carried out in Moshi, Tanzania. Moshi is a region located in northern Tanzania. The study was conducted from October 2014 to May 2015 and involved 200 cattle recto-fecal specimens and 107 human stools specimens. The sample size of 107 human and 200 cattle was estimated using Kish Leslie (1965) formula and using previous prevalence of 3.6% and 0.9% of *E. coli* O157:H7 for human and cattle, respectively [19, 37].

A convenient sampling method was used to enroll 107 patients with diarrhea or abdominal complaints and 200 specimens from cattle upon consenting a patient, guardian or owner of the cattle. Study participants were enrolled continuously from the start till the estimated sample size was reached. Both patient's and cattle's recto-fecal specimens were taken by a skilled personnel to KCMC Clinical Laboratory for analysis.

Specimen collection

Two hundred (200) cattle recto-fecal specimens were collected using sterile cotton swabs into the Stuart transport medium and immediately transported to the KCMC clinical laboratory for further processing in Microbiology Department. Human stool specimens (107) were collected in a clean container and transported to the laboratory for further processing.

Isolation and identification of *E. coli*

Stool and recto-faecal specimens inoculated onto MacConkey (MCA) and Blood agar (BA) using a standard inoculating loop for culture. The incubation period was 18-24 hours, aerobic atmosphere at 37 °C. A single colony picked up to subculture onto a new plate of MCA. Gram-negative bacilli isolated were identified based on their colony morphology and characterized by performing gram staining following biochemical tests using Kligler Iron agar (KIA), Sulfide Indole Motility (SIM), Simmon's citrate, Urea, Lysine Iron agar (LIA) medium, Indole and Oxidase test.

Phenotypic detection of EHEC O157:H7

E. coli isolates from pure colony were cultured onto Sorbitol MacConkey agar (SMCA) and incubated for 18-24 hours aerobic atmosphere at 37 °C. The EHEC O157:H7 appeared colorless on SMCA while non EHEC O157:H7 appeared pink.

Phenotypic detection of ESBL producing EHEC O157:H7

E. coli isolates were previously sub cultured onto a new plate of MCA before performing ESBL detection in order to reduce heavy growth. Isolates resistant to the first or second line antibiotics were selected to test for the third generation cephalosporins. A pure colony selected was spread onto Muller Hinton agar (MHA) using a sterile cotton swab. The third generation cephalosporins applied on top of inoculum to determine the ESBL producing EHEC O157:H7 among isolates. Isolates which were resistant or intermediately susceptible to any of the third generation cephalosporins such as Ceftazidime (30µg), Cefotaxime (30µg), and Cefepime (30µg) were selected for ESBL confirmation using Minimum inhibitory concentration (M.I.C.) Test Strip (Liofilchem®, Italy) as per manufacturer's instruction as previously described [4].

The M.I.C. Test Strip confirmed suspected ESBL producing strains of EHEC O157:H7. Cefotaxime/cefotaxime + Clavulanic acid (CTX/CTL), Ceftazidime/ceftazidime + Clavulanic acid (CAZ/CAL) and Cefepime/Cefepime + Clavulanic acid (FEP/FEL) strips are intended to confirm the presence of clavulanic acid inhibitable ESBL enzymes in EHEC O157:H7. The test organisms were considered an ESBL producer if M.I.C. CTX ≥ 0.5 and CTX/CTL ratio ≥ 8 ; CAZ ≥ 1 and the CAZ / CAL ratio ≥ 8 ; and FEP ≥ 0.25 and FEP/FEL ratio ≥ 8 .

Antibiotic susceptibility testing (AST)

All ESBL producing EHEC O157:H7 isolates were selected to determine their susceptibility patterns against commonly used antibiotic disks by the Kirby-Bauer disk diffusion method as described by Clinical Laboratory Standards Institute (CLSI).³⁸ The antibiotic disks used were ampicillin (10µg), amoxicillin/clavulanic acid (30µg), gentamycin (10µg), trimethoprim/sulphamethoxazole (5/25 ug), tetracycline (30µg), norfloxacin (30µg), ciprofloxacin (30µg), amikacin (30ug), ceftriaxone (30µg), ceftazidime (30µg), and cefotaxime (30µg). ESBL producing EHEC O157:H7 that shown a zone of inhibition of ≤ 25 mm for ceftriaxone and ceftazidime were selected for ESBL confirmation, according to guidelines of CLSI [38].

Quality control

Reference strains *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 for gram negative and gram positive bacteria, respectively, were used for quality control of Microbiological procedures (staining, culture, identification and antimicrobial susceptibility testing). In house control organism for positive ESBL was used in this study.

Data analysis

All data were entered into Excel and exported to Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, USA) for analysis based on the study objectives. Chi square tests were used to compare the significant difference in distribution of categorical variables using proportion. Fisher's test was used to measure association between age and ESBL colonization by performing linear regression. Statistical significant were considered at the probability (p) value of less than 0.05

RESULTS

Demographic and Characteristics of specimens

This study enrolled 307 specimens. Among these, 200 (65.1%) were cattle recto-fecal specimens and 107 (34.9%) were human stool specimens. Out of 107 human specimens, 64 (59.8%) were isolates from males whereas, 43 (40.2%) were isolates from females. A high number 22 (20.6%) of human involved were aged above 60 years old whereas, only 8 (7.50%) were aged between 51-60 years (Table 1).

Prevalence of ESBL producing EHEC O157:H7 in cattle and humans

Of the 200 recto-fecal specimens collected from cattle, 20 (10.0%) were positively confirmed ESBL producing EHEC O157:H7, whereas 10 (9.30%) out of 107 human specimens used were confirmed ESBL producing EHEC O157:H7 ($X^2=0.961$, $P= 0.002$). There was a high number 7(10.9%) of isolates in males although statistically was not significant for ESBL producing EHEC O157:H7 compared to females (OR= 0.961, 95% CI= 0.191-4.824). There was a highest prevalence 3(6.9%) of ESBL producing EHEC O157:H7 isolates in the age group of less than 5 years. There were no isolates of ESBL producing EHEC O157:H7 among age group of between 31-40 and 51-60 years (Table 1). The trend line for ESBL producing EHEC O157:H7 was decreasing from age below five years to age above 60 year (F test= 5.3706, $P= 0.0683$, $df= 1$)

Table 1: Prevalence of ESBL producing EHEC O157:H7 in cattle and humans

Variable		ESBL Producing EHEC O157:H7			
		Frequency (%)	N (%)	X^2	<i>P</i> -Value
Specimen source	Cattle	200 (100)	20 (10.0)	-	-
	Human	107 (100)	10 (9.30)	0.961	0.002
Gender	Female	43 (40.20)	3 (6.90)	0.961	0.191-4.824
	Male	64 (59.8)	7 (10.9)		
	Total	107 (100)	10 (9.30)		
Age groups	<5	19 (17.8)	04 (21.10)	0.349	7.814
	6-10	10 (9.30)	01 (10.0)		
	11-20	15 (14.0)	01 (6.60)		
	21-30	14 (13.1)	01 (7.10)		
	31-40	09 (8.40)	00 (00.0)		
	41-50	10 (9.30)	02 (20.0)		

51-60	08 (7.50)	00 (00.0)
>60	22 (20.6)	01 (4.50)
Total	107 (100)	10 (9.30)

142 ESBL, Extended Spectrum Beta-Lactamases; EHEC, Enterohemorrhagic *Escherichia coli*

143 Prevalence of ESBL producing Non-EHEC O157:H7 in cattle and humans

144 Twelve (6.0%) of the cattle specimens were ESBL producing non-EHEC O157:H7 whereas 21 (19.60%)
 145 of human specimens confirmed as ESBL producing non-EHEC O157:H7. A high number of non-EHEC
 146 O157:H7 isolates was found in male 13 (20.3%) than in females 8(18.6%) (OR= 1.506, 95% CI= 0.534-
 147 4.236). More isolates 5 (35.7%) of ESBL producing non-EHEC O157:H7 was found in the age group 21-
 148 30 years old. None of isolates was found in age between 6-10 years old ($X^2=0.349$, $P= 7.814$) (Table 2).
 149 The trend line of ESBL producing non-EHEC O157:H7 was increasing with age (F test= 14.3966, $P=$
 150 0.013, df= 1).

151 **Table 2: Prevalence of ESBL producing Non-EHEC O157:H7 in cattle and humans**

Variable		ESBL Producing Non-EHEC O157:H7			
		Frequency (%)	N (%)	X^2	P-Value
Specimen source	Cattle	200 (100)	12 (6.0)	-	-
	Human	107 (100)	21 (19.6)	0.438	0.602
Gender				OR	CI
	Female	43 (65.1)	8.0 (18.6)	1.505	0.534-4.236
	Male	64 (20.8)	13 (61.9)		
	Total	107 (100)	21 (19.6)		
Age groups	<5	19 (17.8)	03 (15.8)	X^2	P-Value
	6-10	10 (9.30)	00 (00.0)	0.349	7.814

11-20	15 (14.0)	03 (20.0)
21-30	14 (13.1)	05 (35.7)
31-40	09 (8.40)	02 (22.2)
41-50	10 (9.30)	03 (30.0)
51-60	08 (7.50)	02 (25.0)
>60	22 (20.6)	03 (13.6)
Total	107 (100)	21 (19.6)

ESBL, Extended Spectrum Beta-Lactamases; EHEC, Enterohemorrhagic *Escherichia coli*

Antimicrobial resistance patterns of ESBL producing EHEC O157:H7 in cattle and humans

Both cattle (n=20) and human (n=10) isolates revealed a high (100.0%) resistance against Trimethoprim/sulfamethoxazole, Tetracycline and Amoxicillin/clavulanic acid. Hundred percent (100%) of the cattle isolates were resistant to Norfloxacin whereas 90.0% of human isolates were resistant to Norfloxacin. Resistance against Gentamicin was higher 100% in human isolates than 75.0% in cattle isolates. The resistance against Amikacin was 80.0% in cattle isolates as compared to 60.0% of human isolates. About 80% of human and cattle isolates were resistance against Ciprofloxacin (Table 3).

Table 3: Antimicrobial resistance patterns of ESBL producing EHEC O157:H7 in cattle and humans

Antimicrobials	Frequency (%)				
	of resistant in	χ^2	P-Value	OR	95% CI
CATTLE					
Trimethoprim/sulfamethoxazole	20 (100%)	10.615	0.000	0.692	0.589-0.814
Norfloxacin	20 (100%)	31.789	0.000	0.512	0.380-0.691
Ciprofloxacin	16 (80%)	38.624	0.000	0.031	0.008-0.117
Tetracycline	20 (100%)	14.294	0.000	0.661	0.551-0.794
Gentamicin	15 (75%)	11.127	0.000	0.167	0.054-0.513
Amoxicillin/clavulanic acid	20 (100%)	21.467	0.000	0.600	0.478-0.752

Amikacin	16 (80%)	44.184	0.000	0.289	0.146-0.574
HUMANS					
Trimethoprim/sulfamethoxazole	10 (100%)	1.371	0.242	0.677	0.531-0.874
Norfloxacin	09 (90.0%)	5.685	0.017	0.592	0.388-0.903
Ciprofloxacin	08 (80.0%)	3.342	0.068	0.212	0.037-1.211
Tetracycline	10 (100%)	-	-	-	-
Gentamicin	10 (100%)	4.359	0.037	0.615	4.540-0.831
Amoxicillin/clavulanic acid	10 (100%)	1.889	0.169	0.667	0.518-0.859
Amikacin	06 (60.0%)	10.472	0.001	0.295	0.088-0.992

163 ESBL, Extended Spectrum Beta-Lactamases; EHEC, Enterohemorrhagic *Escherichia coli*; CI, Confidence
164 Interval

165 **Antimicrobial resistance patterns of ESBL producing Non-EHEC O157:H7 in cattle and**
166 **humans**

167 Isolates from humans and cattle were highly (100%) resistant to Trimethoprim/sulfamethoxazole. The
168 resistance against Norfloxacin was higher 100% in cattle isolates compared to 90% in human isolates.
169 Only 91.7%, 41.7% and 83.3% of cattle isolates were resistant to Tetracycline, Gentamicin and
170 Amoxicillin/clavulanic acid respectively as compared to human isolates that were highly resistant to all of
171 the three antimicrobials (Tetracycline, Gentamicin and Amoxicillin/clavulanic acid). In cattle, all isolates
172 were resistant to Amikacin but, only 60% isolates in humans were resistant to Amikacin (Table 4).

173 **Table 4: Antimicrobial resistance patterns of ESBL producing non-EHEC O157:H7 in cattle and**
174 **humans**

Antimicrobials	Frequency (%)				
	of resistant in	X ²	P-Value	OR	95% CI
CATTLE					
Trimethoprim/sulfamethoxazole	12 (100%)	8.931	0.003	0.815	0.726-0.915
Norfloxacin	12 (100%)	28.157	0.001	0.657	0.517-0.835
Ciprofloxacin	11 (91.7%)	37.667	0.001	0.014	0.002-0.120

Tetracycline	11 (91.7%)	6.480	0.011	0.103	0.013-0.830
Gentamicin	05 (41.7%)	0.167	0.683	1.288	0.382-4.343
Amoxicillin/clavulanic acid	10 (83.3%)	0.956	0.328	0.462	0.095-2.242
Amikacin	12 (100%)	38.571	0.000	0.571	0.415-0.788
HUMANS					
Trimethoprim/sulfamethoxazole	21 (100%)	6.995	0.008	0.644	0.533-0.779
Norfloxacin	21 (90.0%)	24.322	0.000	0.475	0.343-0.658
Ciprofloxacin	20 (80.0%)	24.874	0.000	0.022	0.003-0.180
Tetracycline	21 (100%)	4.146	0.042	0.661	0.566-0.796
Gentamicin	19 (100%)	3.126	0.077	2.600	0.054-1.251
Amoxicillin/clavulanic acid	21 (100%)	3.127	0.077	0.682	0.578-0.804
Amikacin	16 (60.0%)	10.391	0.001	0.607	0.433-0.852

175 ESBL, Extended Spectrum Beta-Lactamases; EHEC, Enterohemorrhagic *Escherichia coli*; CI, Confidence
 176 Interval

177 DISCUSSION

178 This was a first survey carried out to determine the prevalence and drug resistance patterns of ESBL
 179 producing EHEC O157:H7 in humans and cattle in Moshi, northern Tanzania. ESBL producing *E. coli* are
 180 isolated with increasing frequency in cattle and human specimens. The study observed a higher
 181 prevalence of ESBL producing EHEC O157:H7 in cattle than in humans, although, there was a higher
 182 prevalence of ESBL producing non-EHEC O157:H7 in human than in cattle.

183 A high prevalence of ESBL producing EHEC O157:H7 in cattle was possibly due to cattle being the
 184 common reservoir of EHEC. The cattle might have ingested contaminated water or pastures during the
 185 rainy season as water flows on soil where cattle feed and drinks. A higher ESBL producing non-EHEC
 186 O157:H7 in humans may possibly due to previous exposure of people to second or third generation
 187 cephalosporin and nosocomial infections [25] in the previous few months when patients attended or
 188 admitted in hospital. This predict that, there might be a cross transmission of a resistance strain between
 189 cattle and humans resulting to spreading in both cases. One of the evidence is due to discharge of

190 sewage from human toilets into flowing water that in turns contaminate soil, grazing pastures or water for
191 cattle.

192 Moreover, prevalence of ESBL producing non-EHEC O157:H7 carriage in human shown in this study
193 (19.6%) is relatively higher than 15% found in Bugando Mwanza, Tanzania. This is showing an increase
194 of ESBL carriage among people in hospitals and community [28].

195 Presence of ESBL producing EHEC O157:H7 in cattle might be a direct uptake of resistance strains from
196 humans particularly during rainy season where cattle drinks contaminated water or pastures. It was
197 evidence by the discharge of sewages from human toilets into flowing water that directly contaminated
198 the soil, grazing pastures or drinking water for cattle. On top of that, cattle as are common reservoir might
199 have transmitted infections to humans due to direct contact with them during cleaning of cages, feeding
200 or milking especially males who are normally involved in such activities.

201 The general trends ESBL producing was increasing with age. There was a higher prevalence of ESBL
202 producing non-EHEC O157:H7 in the active age particularly at age groups 21-30 years. This was possibly
203 due to independent decision of using antibiotics resulting to development of multidrug resistance. This
204 phenomenon approved by a recent study that suggested the irrational use of antibiotics in clinical
205 medicine and animal husbandry to be a major factor in the emergence and dissemination of antimicrobial
206 resistance in animals and humans [29]. Children under five years had higher prevalence of ESBL
207 producing EHEC O157:H7 due to vulnerability of children to contaminated soil especially in the crawling
208 stage. This stage is very risk to children because of a habit to put in the mouth everything including soil.

209 Both human and cattle isolates were highly resistant to advance generation cephalosporin cefotaxime
210 and other antimicrobial agents [25]. This was in harmony with the recently findings that noted resistance
211 to advance generation cephalosporin [4]. All cattle and humans isolates were highly resistant to
212 trimethoprim/ sulfamethoxazole, Tetracycline and Ampicillin perhaps due to previous misuse of this
213 antibiotic [29].

214 Furthermore, all isolates were resistant to each of the tested antimicrobial agents, including the
215 aminoglycosides possibly due to co-transfer of resistance from one bacterial species to another as ESBL

216 are plasmid mediated [39, 40-42]. The study has shown co-resistance of ESBL producing *E. coli* against
217 most of antibiotics used, which was consistent with reports of recent studies [8, 43].

218 We recommended other similar studies involving a wide range of sites and more tests for profiling the
219 level of drug resistance, Multidrug resistance as well as identifying marker for resistance. Moreover, there
220 is a need to carry out a study to assess the possible factors resulting to Multidrug resistance in human
221 from consumption of antibiotic in food producing animals.

222 The nation should provide health education to people on the usefulness of good hygiene to reduce
223 spread of infection and the effects of using un-prescribed drugs in both humans and cattle. It would be
224 important to identify ESBL producers as routine in the hospitals and to direct appropriate antibiotics for
225 adequate treatment as to save patients and control from spreading infection to other health people or
226 cattle.

227 **CONCLUSION**

228 ESBL producing *E. coli* strains are believed to be widely distributed among cattle and human. Regulation
229 of Veterinary antibiotic prescription has to receive greater attention to control selection of antimicrobial
230 resistant strains in both cattle and human. The study is supporting 'ONE HEALTH' approach which will
231 ensure a sustainable control and hence reduction of transmission and spread of drug resistant bacteria in
232 humans and cattle.

233 **ETHICAL APPROVAL**

234 Ethical approval for this study was granted by the Kilimanjaro Christian Medical University College
235 Research Ethics and Review Committee (CRERC) with research ethical clearance number 669. The
236 CRERC approved both the human and animal components of this study. Written consent forms that
237 contain all the information about the study were provided to all study participants including cattle's owner
238 prior to the specimen collection.

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