Zoonotic Escherichia coli is a potential Driver of Antimicrobial Resistance among Pastoralist Communities in Uganda: A Laboratory based Cross sectional Study

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Research article

Keywords: Antimicrobial resistance, Zoonotic E. coli, Pastoralist communities

Posted Date: October 29th, 2019

DOI: https://doi.org/10.21203/rs.2.16491/v1

Abstract

Background Non-prescribed use of antimicrobials in Agriculture incurs a transfer risk of resistant pathogens to humans, complicating treatment. The aim of this study was to determine the potential of Zoonotic E. coli to serve as drivers of antimicrobial resistance among animals and humans in pastoralist communities in Kasese district, so as to protect the community.

Materials and methods A laboratory based cross-sectional study was done using archived E. coli isolates previously obtained from humans in pastoralist communities of Kasese district, Uganda. Recovery of the isolates was done by conventional culture, and Identification by biochemical methods, serotyping and PCR. Antimicrobial resistance profiling was done by using Kirby Bauer disc diffusion method. The isolates were also screened for resistance mechanisms including Extended Spectrum β-lactamase (ESBL), Carbapenemase and AmpC production using disc diffusion based methods.

Results The prevalence of Enterohemorrhagic E.coli (EHEC) was 16% (28/180). These EHEC isolates belonged to phylogroups; B 1 (94%, 26/28), B2 (3%, 1/28) and A (3%, 1/28). The most prevalent virulence gene was Stx1 (100%, 28/28) followed by Stx2e (94%, 26/28); none of the isolates was Stx2 positive. Highest resistance was seen to Cotrimoxazole (89%, 25/28), Tetracycline (71%, 20/28), Ampicillin (65%, 18/28) and Nitrofurantoin (28%, 8/28), these are the most commonly used antimicrobials in the agricultural sector in Uganda. Minimal resistance was observed to the antimicrobials that are commonly used in human medicine especially β-lactams, β-lactam+inhibitors and Carbapenems. Of the 28 zoonotic E. coli isolates, 17%, (5/28) were ESBL positive and among these 1 (3%, 1/28) was a Carbapenemase producer.

Conclusion There is a high prevalence of highly pathogenic and resistant zoonotic E. coli among humans in pastoralist communities in Uganda. We suspect that these pathogens, along with their AMR genes, were acquired from animals because they largely contained the animal specific Vero toxin gene VT2e and majority belonged Pylo-group B 1 which has been documented as the most common EHEC phylo-group inhabiting domestic animals. We recommend that studies involving relatedness of drug resistant isolates from humans and animals should be conducted to ascertain the role of enterohemorrhagic E. coli in the zoonotic spread of antimicrobial resistance in pastoralist communities.

Introduction

Globally, the incidence of antimicrobial resistant pathogens is increasingly manifesting its self [1, 2]. This has continued to make the treatment of infections caused by these pathogens very difficult and expensive, imparting a great risk of death [3]. Uncontrolled use of antimicrobials in farming is a major factor causing the emergence of resistant bacterial pathogens [4]. The emergent zoonotic strains may pass on to humans via the food chain [5-7]. At present, *E. coli* O157:H7 is one of major pathogens implicated in the zoonotic spread of antimicrobial resistance (AMR) globally [8]. In East Africa, and

Uganda in particular, antimicrobial resistant *E. coli* with the potential for transmission from animals to humans are commonly implicated in human diarrheal diseases [9, 10].

In Kasese district, western Uganda, farmers in and around Queen Elizabeth National park graze domestic animals within the Park. The interactions between domestic animals, wild animals and humans create a porous interface for zoonotic transfer of drug resistant pathogens [11]. This district has for long suffered outbreaks of antimicrobial resistant diarrheal diseases caused by zoonotic bacterial agents [12]. Though studies about these zoonotic pathogens have been done [9, 13], little is known about the prevalence of antimicrobial resistant *E.coli* 0157:H7, and the potential role of this strain in the zoonotic spread of antimicrobial resistance among pastoralist communities in this area.

This study aimed to determine the prevalence, population structure, and antimicrobial resistance profiles of zoonotic *E. coli,* among bacterial isolates obtained from humans in pastoralist communities of Kasese district: to ascertain whether *E. coli* is a potential zoonotic driver of antimicrobial resistance in such settings, in order to guide AMR stewardship.

Materials And Methods

Study design

A Laboratory based cross-sectional study was conducted from January to August 2017 in the department of medical microbiology at Makerere University College of health Sciences (MakCHS).

Study population

One hundred and eighty (180) *E. coli* isolates archived in the medical microbiology laboratory (MakCHS), were used. The isolates had been obtained from patients of all ages and sexes, with fever and/or diarrhea, among pastoralist communities in Kasese district, Uganda.

Culture and identification of Zoonotic *E. coli*

The *E. coli* isolates were recovered from storage by culturing on blood agar followed by sub culturing on Sorbitol MacConkey agar. Upon inoculation onto the solid media, plates were incubated at 37 ^{0}C for 18-

24 hours. Presumptive identification was done using; colony characteristics, gram stain and Biochemical methods including citrate, urea, and triple sugar iron and Sulphur indole motility. Further identification was done by Serotyping, and virulence genotyping using conventional PCR.

Serotyping: The translucent *E. coli* colonies from sorbitol MacConkey, following biochemical identification, were sub-cultured on Mueller Hinton agar and subjected to serotyping as described by Kok *et al*, 1996 at Uganda Central Public laboratories (CPHL) using rapid diagnostic *E. coli* 0157 antisera (Difco Laboratories, Detroit, USA).

Virulence genotyping: Further identification of enterohemorrhagic *E. coli* was done via detection of at least one of the three Shiga-like toxin (Verotoxin) genes namely (*VT1*, *VT2* and *VT2e*). Virulence genotypes were determined by conventional PCR using DNA *BIORAD* T100^{TM} thermal cycler (Medline Scientific Uganda). Specific primer sets as published by Pas *et al.*, 2000, were used in simplex PCR. Amplification was set at 30 cycles of denaturation at 94^{o}C for 60 sec, annealing at 54^{o}C for 90 sec, extension at 72^{o}C for 90 sec and post-extension at 72^{o}C for 10 min. The Positive control ATCC 35401 and negative controls; *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 used were procured from Kenya Medical Research Institute. Agarose gel electrophoresis was performed followed by visualization using a UV-trans illuminator.

Phylotyping of zoonotic *E. coli*

The zoonotic *E. coli* isolates were categorized into phylogroups by Multiplex PCR using DNA *BIORAD* T100^{TM} thermal cycler. The primers that were used are those published by Ramadan *et al.* 2016. Amplification was set at 34 cycles of denaturation at 94^{o}C for 60 sec, annealing at 57^{o}C for 90 sec, extension at 70^{o}C for 90 sec and post-extension at 70^{o}C for 10 min. The amplification targeted two Phylotyping genes; *chuA, yjaA* and the DNA fragment TspE4.C2. Agarose gel electrophoresis was performed followed by visualization using a UV-trans illuminator. A Positive control, ATCC 35401 and the negative control *K. pneumoniae* ATCC 700603 that were used were procured from Kenya Medical

Research Institute. Phylogenetic groups were deduced from the PCR results using a phylogenetic identification key [8].

The Kirby Bauer disc diffusion method was used in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines. Pure colonies of *E. coli* 0157:H7 (18 - 24 hour growth) were emulsified in normal saline and 0.5 McFarland concentration of inoculum was prepared. This was spread on Mueller Hinton agar and antibiotic discs applied followed by incubation at 35˚C for 18 - 24 hours. Isolates were regarded as susceptible, intermediate or resistant according to the inhibition zone around the disc. The antibiotics tested include; Ampicillin (10 mg), Amoxicillin+Clavulanate (10 mg), Ceftazidime (30 mg), Ceftriaxone (30 mg), Cefotaxime (30 mg), Cefepime (30 mg), Imipenem (10 mg), Ertapenem (10 mg), Amikacin (30 mg), Nalidixic acid (30 mg), Gentamicin (10 mg), Ciprofloxacin (5 mg), Tetracycline (30 mg), Nitrofurantoin (300 mg) and Trimethoprim-Sulphurmethoxazole (23.5 mg). These were tested against in accordance with the treatment guidelines of the Ministry of Health in Uganda [14]. Disk diffusion based methods were used to determine the mechanisms underlying antimicrobial resistance, including; ESBL, MBL, AmpC and Carbapenemase production. Data analysis was done using SPSS versions 12.

Results

Prevalence of zoonotic *E. coli*

A total of 180 human derived *E. coli* isolates were analyzed. Of these, 28 (16%) were found to be zoonotic *E. coli.* (*E. coli* 0157:H7). Prevalence of Vero toxin genes among the zoonotic isolates was 100% (28/28), 93% (26/28) and 0% (0/28) for *VT1*, *VT2e* and *VT2* respectively (Figure 1a)

Phylotyping of zoonotic *E. coli*

Of the 28 zoonotic *E. coli* isolates, 26 (94%) were of phylogroup B_1, 1(3%) belonged to phylogroup B_2 and 1(3%) belonged to phylogroup A (Figure 1b).

Antimicrobial resistance profiling

Resistance profiles to antimicrobial agents commonly used in Agriculture

Of the 28 isolates of *E.coli* 0157:H7 identified, 89% (25/28) were resistant to Cotrimoxazole (SXT), 71% (20/28) were resistant to Tetracycline (TE), 65% (18/28) were resistant to Ampicillin (AMP), 28% (8/28)

were resistance to Nitrofurantoin (F). 25% (7/28) and 14 % (4/28) were intermediate to Ampicillin and Nitrofurantoin respectively, none of the isolates were intermediate to Cotrimoxazole and Tetracycline (fig 2).

Resistance to other antimicrobial agents

Resistance to Cefuroxime, Ceftazidime, Cefotaxime, Cefepime and Amoxicillin-clavulanic was 7%, 4%, 7% 7% and 4% respectively; 29% (8/28) of the isolates showed intermediate resistance to Cefepime (a 4^{th} generation cephalosporin). For Amoxicillin-clavulanic acid (AMC), a β lactamase inhibitor, 25% (7/28) were resistant while 4% (1/28) were intermediate; Multidrug resistance (MDR) was observed in 79% (23/29) of the EHEC isolates. Seventeen percent (17%, 5/28) were ESBL positive; 4% (1/28) were positive for Carbapenemase production, none was positive for AmpC production and Metallo β lactamase production. For Gentamicin (CN), resistance of 7% (2/28) was seen in the isolates, none of the isolates was resistant to Amikacin (AK). 4% (1/28) were resistant to Nalidixic acid In addition to this 11% (3/28) were intermediate and 11% (3/28) of the isolates were resistant to Ciprofloxacin (Figure 2).

Five out of twenty eight, 5/28 (16%) of the zoonotic *E. coli* isolates were ESBL positive and 1/28 (3%) was Carbapenemase positive.

Highest resistance was observed to Trimethoprim-sulphurmethoxazole followed by Tetracycline and then Ampicillin. Least resistance was observed in Ceftazidime, Imipenem and Nalidixic Acid. None of the Isolates was resistant to Cefepime, Ertapenem and Amikacin.

Discussion

We report a 16% prevalence of zoonotic *E. coli* in this study. Most of these zoonotic isolates harbored Vero toxin genes *VT1 and VT2e*. Of the 28 zoonotic *E. coli* isolates, 94% belonged to phylogroup B_1, 3% belonged to phylogroup B_2 and 3% belonged to phylogroup A. Highest resistance in these isolates was seen to Cotrimoxazole followed by Tetracycline and Ampicillin. The most common mechanism underlying antimicrobial resistance was Extended Spectrum β-lactamases (ESBL).

The 16% prevalence of zoonotic *E. coli* reported in this study is higher than the prevalence reported in an earlier study conducted in Western Uganda by Majalija *et al*, 2008 which reported a prevalence of 8.5%. This rise may be partly attributed to the increasing number of people settling and grazing animals in Queen Elizabeth National park. The domestic animals interact with wild animals and end up spreading such pathogens to humans.

The high prevalence of Vero toxin genes *VT1* (100%) and *VT2e* (93%) observed in our study is in agreement with Omisakin *et al,* 2003, and reflects the high pathogenic nature of our zoonotic *E. coli* study isolates [15]. These Vero toxin genes mediate the virulence factors that enhance the organism's potential

to cause disease [8, 16-18]. Vero toxin gene *VT2e* is animal specific and is common among isolates that have encountered the animal host [19]; hence its high prevalence in our study suggests a possible zoonosis. Furthermore, Pylo-group B_1, observed as the most prevalent phylo-type circulating among individuals in our study population has been documented as the most common enterohemorrhagic *E. coli* phylo-group inhabiting domestic animals [19, 20]. This affirms our earlier assumption that these isolates are potential conduits for the zoonotic spread of antimicrobial resistance to humans who interact frequently with the animals (the one health concept).

Highest resistance was observed to; Trimethoprim-sulphurmethoxazole, Tetracycline, Ampicillin and Nitrofurantoin: these have been documented as the most commonly used antimicrobial agents in the agricultural sector in Uganda [21]. These finding are consistent with studies done elsewhere on similar isolates whose source was animals [22]. In addition, these studies also reported a possible transmission of the resistance in these isolates to humans. The implication of this is that the resistance seen in our zoonotic *E. coli* study isolates may be of agricultural origin; however this remains speculative.

Our study successfully proved that 16% of the *E. coli* isolates obtained from humans in this pastoralist community are zoonotic and highly resistant to antibiotics, using conventional culture and molecular methods. The zoonotic nature of these pathogens implies that they could have originated from animals from which they potentially transmit resistance to humans. We were unable to compare our study isolates with drug resistant isolates obtained from animals to certainly infer zoonotic transmission of antimicrobial resistance in this setting.

The high prevalence of resistant, enterohemorrhagic *E.coli* is of utmost significance because these bacteria have been reported as the most common causative agents of diarrheal disease, a major cause of death in humans especially children under five years [8, 23]. The linkage of this serotype to zoonosis creates a risk for the development of MDR phenotypes when the already resistant zoonotic strains get exposed to new classes of antimicrobial agents while in the human host. The high antimicrobial resistance burden observed in this study setting is of global public health concern because the number of international tourists visiting Queen Elizabeth National park in this area continues to rise amidst the expanding local populations of animals and humans in this locality [24-26]. This global connectivity, which is now associated with the rapid worldwide spread of infectious agents and their resistance genes [24], implies that the AMR observed in our study may consequently spread to the rest of the world.

Conclusions

There is high prevalence of highly pathogenic and resistant zoonotic *E. coli* pathogens among humans in pastoralist communities in Uganda. We suspect that these were acquired from animals where the resistance observed in the human host may have originated. We therefore recommend that studies

involving isolates from humans and animals should be conducted, and relatedness of the resistant isolates from the two host groups investigated, to confirm the role of enterohemorrhagic *E. coli* in the zoonotic spread of antimicrobial resistance in this setting. A one health approach should be used to establish the drivers of MDR spread in pastoralist communities.

Abbreviations

AMR: Antimicrobial Resistance; ATCC: American type culture collection; CLSI: Clinical and laboratory standard Institute; ESBL: Extended-spectrum ß-lactamase; MDR: Multi-drug resistance; SPSS: Statistical package for social sciences

Declarations

Ethical approval and consent to participate

The study was approved by the School of Biomedical Sciences, Higher Degrees Research and Ethics Committee (HDREC) of Makerere University. Study participants had consented for storage of the isolates and this study obtained a waiver of consent from HDREC.

Consent for publication

Consent for publication was approved by the School of Biomedical Sciences, Higher Degrees Research and Ethics Committee (HDREC) of Makerere University.

Availability of data and material

Datasets generated and/or analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

There were no competing interests in this study.

Funding

This research was funded by; Makerere University/UVRI Immunity and Infection research, Uganda Virus Research Institute, St. Andrews University (UK) and Uganda Virus Research Institute.

Authors' contributions

“AW Performed the laboratory work, data entry and analysis, and wrote the primary draft of the manuscript.

“JSI Supervised the laboratory work, advised on the data analysis plan and was a major contributor in writing the manuscript.

“HK provided some of the laboratory supplies and was a major contributor in writing the manuscript.

“CFN was a major contributor in writing and editing the manuscript.

“BBA Was the senior advisor and supervisor in the study, availed the necessary funds, was a major contributor in writing the manuscript and performed final editing of the manuscript.

All authors read and approved the final manuscript."

Acknowledgements

Not applicable"

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Figures

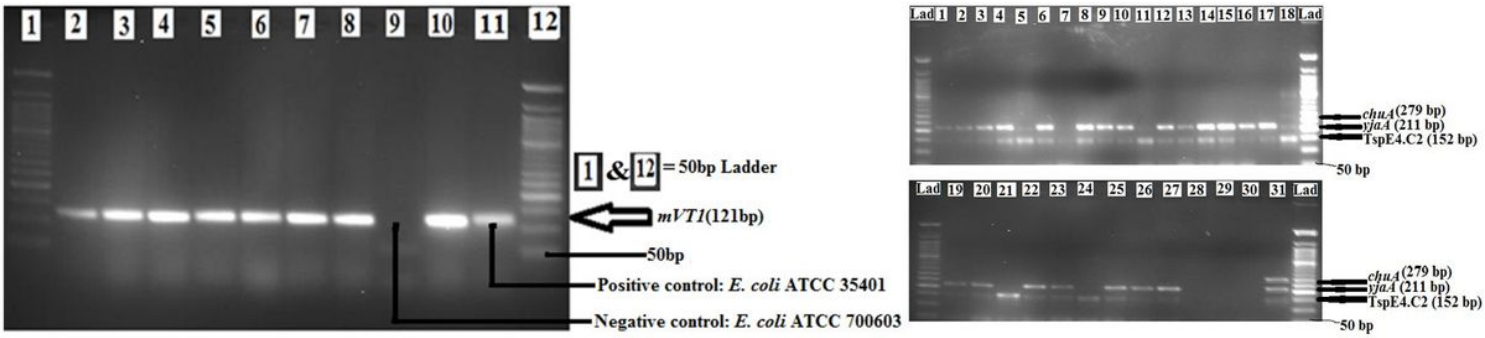

Figure 1

A representative Electrophoretic gel Image for the PCR detection of Vero toxin gene mVT1 among E. coli 0157: H7 isolates obtained from pastoralist communities in Kasese district, Uganda Key: From left to right; Positive reaction with target gene (EHEC; mVT1, 121bp) is in lanes 2 to 8, 10; Lane 11 is a Positive control ATCC 35401, while lane 9 is a negative control of K. pneumoniae ATCC 700603. Lanes 1 and 12 is 50bp Ladder. Legend: The gel image in Figure 1(a) shows the presence of Vero toxin gene (mVT1) in the study isolates when a light band corresponding to the 121bp (signifying a specific positive reaction) is observed in the lane where a particular isolate was assayed. Absence of such a band means absence of the gene mVT1 in the isolate. A representative Electrophoretic gel image for phylogenetic analysis of EHEC Isolates from pastoralist communities in Kasese district, Uganda. Key: From left to right; Positive reaction with only 2 target genes (EHEC; yjaA, 211bp & TspE4.C2, 152bp) is in lanes 1 to 6, 8 to 17, 19 & 20 and 22 &23, 24 to 27; Positive reaction with only 1 target gene (EHEC; TspE42, 152bp) is in lanes 7, 21 &24. Positive reaction with 3 target genes (EHEC; chuA, 179bp; yjaA, 211bp & TspE42, 152bp) is in lane 18. In lane 28 there is no reaction with any of the target genes for this isolate hence phylogroup A; 29 is a Blank (plain PCR water); 30 is a negative control of K. pneumoniae ATCC 700603; 31 is a Positive control ATCC 35401; Lanes Lad contain 50bp Ladder. Legend: The electrophoretic gel image in figure 1(b) shows the presence or absence of the three Phylotyping genes; chuA, yjaA and TspE4.C2) for E. coli populations. A gene is considered as present in the isolate when a light band corresponding to the size of that specific gene amplicon on the molecular ladder is observed in the lane where a particular isolate was assayed.

Absence of a band representing a particular amplicon size of the gene means absence of the gene (chuA, 179bp; yjaA, 211bp & TspE42, 152bp) in the isolate.

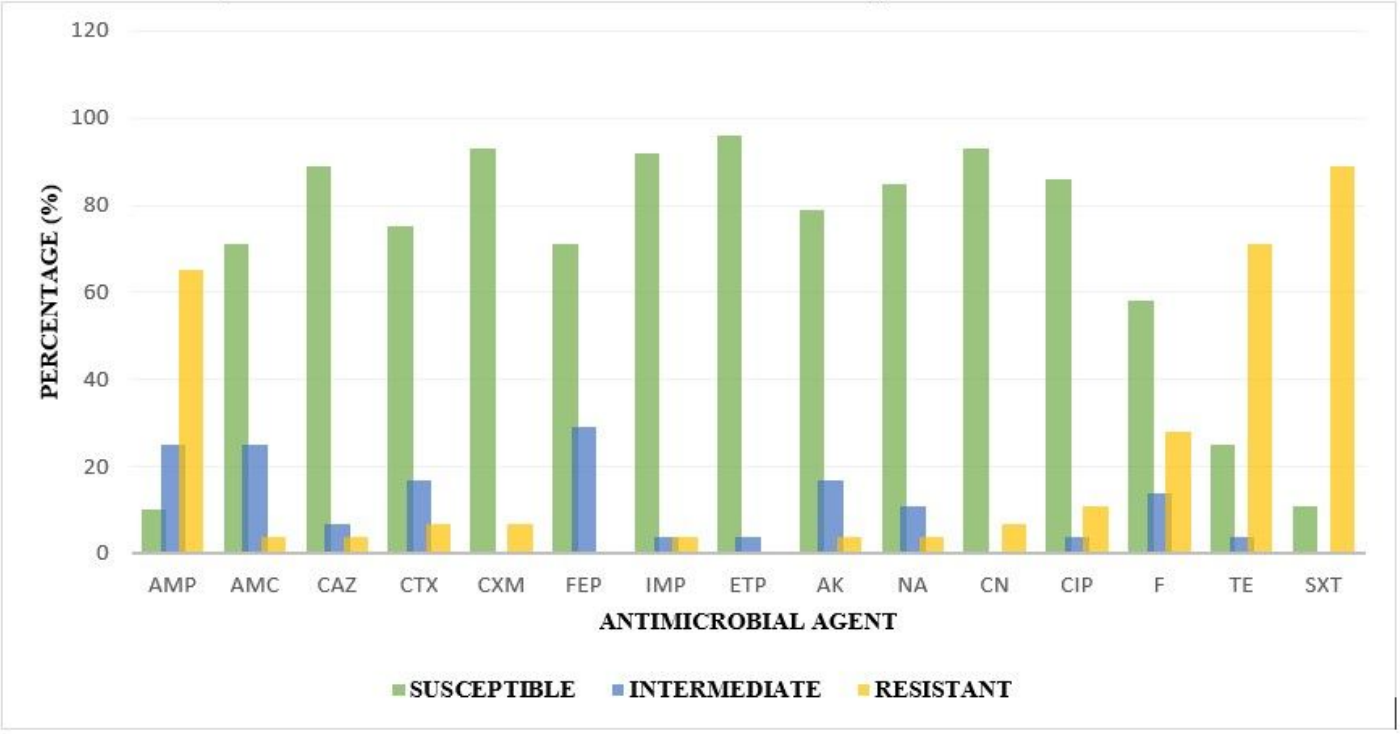

Figure 2

A graph showing the antimicrobial resistance profiles of zoonotic E. coli isolates obtained from humans in pastoralist communities in of Kasese district, Uganda Legend: The bar graph in figure 2 demonstrates the percentages of resistance, intermediate and susceptibility of the zoonotic E. coli isolates to the antimicrobial agents namely; Ampicillin (AMP), Amoxicillin/Clavulanic acid (AMC), Cefuroxime (CXM), Ceftazidime (CAZ), Cefotaxime (CTX), Aztreonam (ATM), Cefepime (FEP), Imipenem (IPM), Ertapenem (ETP), Amikacin (AK), Nalidixic acid (NA), Gentamicin (CN), Ciprofloxacin (CIP), Tetracycline (TE), Nitrofurantoin (F) and Trimethoprim-Sulphurmethoxazole (SXT).