



## **High Prevalence of Antibiotic Resistant *Escherichia coli* Serotype O157: H7 among Pastoral Communities in Rural Uganda**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author AW performed the laboratory work, data entry and analysis, and wrote the primary draft of the manuscript. Author JSI supervised the laboratory work, advised on the data analysis plan and was a major contributor in writing the manuscript. Author HKM participated in data analysis and writing of the manuscript. Author CFN participated in construction of the research idea, supervision of laboratory work and manuscript writing. Author DA participated in conducting laboratory work. Author HK provided some of the laboratory supplies and was a major contributor in writing the manuscript. Author 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.*

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## 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 (AMR) 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. Kirby Bauer disc diffusion method was adopted for AMR profiling. Isolates were screened for resistance mechanisms including Extended Spectrum  $\beta$ -lactamase (ESBL), Carbapenemases 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<sub>1</sub> (94%, 26/28), B<sub>2</sub> (3%, 1/28) and A (3%, 1/28). All the 28 EHEC isolates possessed the virulence gene *stx1*, 26 of the 28 EHEC isolates contained the virulence gene *stx2e*, but none of the 28 possessed the virulence gene *stx2*. 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  $\beta$ -lactams,  $\beta$ -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, drug resistant *E. coli* O157:H7 among humans in pastoralist communities in Uganda. We suspect that these were acquired from animals because they mostly contained vero toxin gene *vt2e* which is animal specific, predominantly swine. Also majority of these EHEC isolates belonged to phylo-group B<sub>1</sub> which has been documented to inhabit domestic animals. We recommend future studies to investigate relatedness of drug resistant isolates from humans and animals to ascertain the zoonotic spread of resistant enterohemorrhagic *E. coli* in pastoralist communities.

**Keywords:** Antibiotic resistance; *Escherichia coli* O157:H7; pastoralist communities; PCR.

## ABBREVIATIONS

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

## 1. 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 [10]. Though studies about these zoonotic pathogens have been done [9,12], little is known about the prevalence of antimicrobial resistant *E.coli* O157: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.

## 2. MATERIALS AND METHODS

### 2.1 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).

### 2.2 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.

### 2.3 Culture and Identification of *E. coli* 0157

The *E. coli* isolates used in this study had been stored in glycerol at -80°C since December 2015. The 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°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 sulfur indole motility. Further identification was done by Serotyping, and by characterization of virulence genes using conventional PCR [26].

### 2.4 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). To 1 drop of sterile normal saline on a slide was added one drop of free falling antisera containing monoclonal antibodies to the *E. coli* 0157 and H7 antigens, a pure colony of each test isolate from Mueller-Hinton agar was then emulsified into the mixture. The slides with contents were then

rocked in circular motion for 60 seconds and observed to check for visible agglutination.

### 2.5 Characterization of Virulence Genes

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™ 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°C for 60 sec, annealing at 54°C for 90 sec, extension at 72°C for 90 sec and post-extension at 72°C for 10 min. The Positive control ATCC 35401 and negative controls; *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 used were obtained from Kenya Medical Research Institute. Agarose gel electrophoresis was performed followed by visualization using a UV-trans illuminator.

### 2.6 Phylotyping of *E. coli* 0157

The potentially zoonotic *E. coli* isolates, i.e. *E. coli* 0157 were categorized into phylogroups by Multiplex PCR using DNA BIORAD T100™ thermal cycler. The primers that were used are those published by Pass et al., 2000. Amplification was set at 34 cycles of denaturation at 94°C for 60 sec, annealing at 57°C for 90 sec, extension at 70°C for 90 sec and post-extension at 70°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].

### 2.7 Antimicrobial Resistance Profiling

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 0.85% physiological 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 µg), Amoxicillin+Clavulanate (10 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg), Cefepime (30 µg), Imipenem (10 µg), Ertapenem (10 µg), Amikacin (30 µg), Nalidixic acid (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Tetracycline (30 µg), Nitrofurantoin (300 µg) and Trimethoprim-Sulfamethoxazole (23.5 µg). These were tested against in accordance with the treatment guidelines of the Ministry of Health in Uganda [13]. 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.

### 3. RESULTS AND DISCUSSION

#### 3.1 Results

##### 3.1.1 Prevalence of *E. coli* 0157

A total of 180 human derived *E. coli* isolates were analyzed. Of these 180 human derived *E. coli* isolates, 28 (16%) were found to be potentially zoonotic *E. coli*. (*E. coli* 0157:H7). Prevalence of Vero toxin genes among the *E. coli* 0157 isolates was 100% (28/28), 93% (26/28) and 0% (0/28) for *mvt1*, *mvt2e* and *mvt2* respectively (Fig. 1a).

##### 3.1.2 Phylotyping of *E. coli* 0157

Among the 28 *E. coli* 0157 isolates that were identified from the archived 180 human derived *E. coli* isolates, 26 (94%) belonged to phylogroup B<sub>1</sub>, 1(3%) belonged to phylogroup B<sub>2</sub> and 1(3%) belonged to phylogroup A (Fig. 1b).

##### 3.1.3 Antimicrobial resistance profiling

###### 3.1.3.1 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).

###### 3.1.3.2 Resistance to other antimicrobial agents

Resistance to the antibiotics; Cefuroxime, Ceftazidime, Cefotaxime, Cefepime and Amoxicillin-clavulanic was 7%, 4%, 7% 7% and 4% respectively, while resistance to Cefepime (a 4<sup>th</sup> generation cephalosporin) was intermediate in 29% (8/28) of the *E. Coli* 0157:H7 isolates. 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 (Fig. 2).

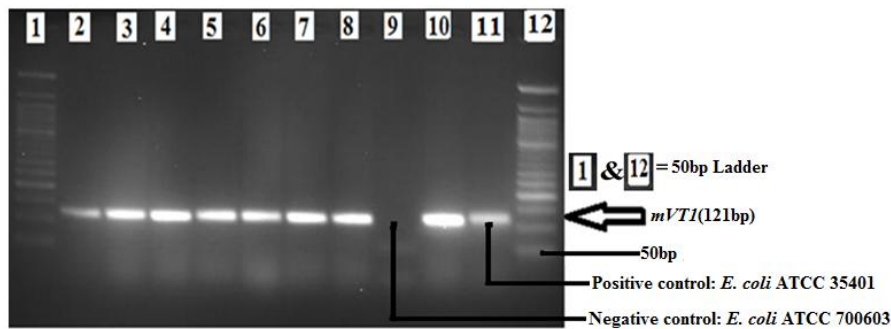
Five out of twenty eight, 5/28 (16%) of the *E. coli* 0157:H7 isolates were ESBL positive and 1/28 (3%) was Carbapenemase positive.

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.

#### 3.2 Discussion

We report a 16% prevalence of potentially zoonotic *E. coli* (*E. coli* 0157:H7) in this study. Most of these zoonotic isolates harbored Vero toxin genes *vt1* and *vt2e*. Of the 28 *E. coli* 0157:H7 isolates, 94% belonged to phylogroup B<sub>1</sub>, 3% belonged to phylogroup B<sub>2</sub> 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).

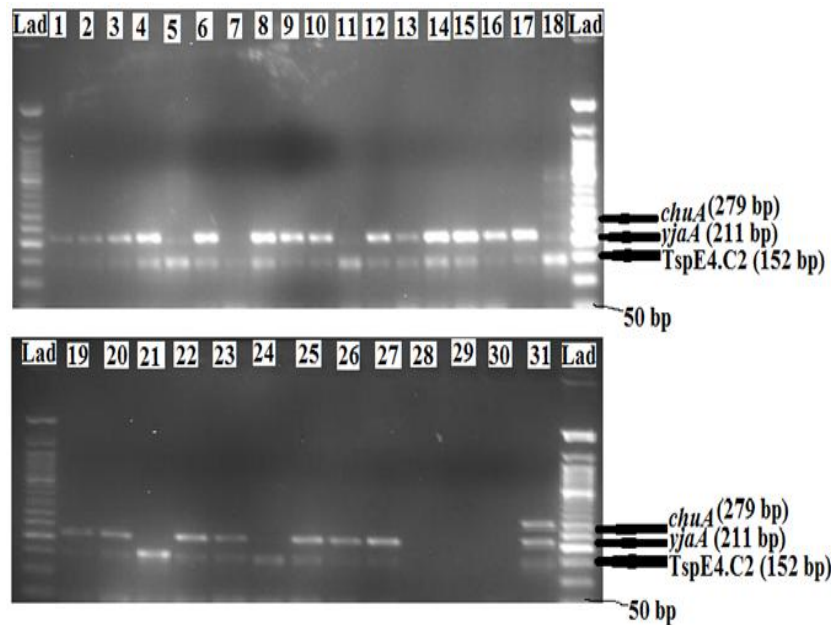
The 16% prevalence of *E. coli* 0157 reported in this study is higher than the prevalence reported in an earlier study conducted in Western Uganda by Majalija et al., 2008 which reported a prevalence of 8.5%. This rise may be partly attributed to the increasing number of people settling and grazing animals in Queen Elizabeth



**Fig. 1a. 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.

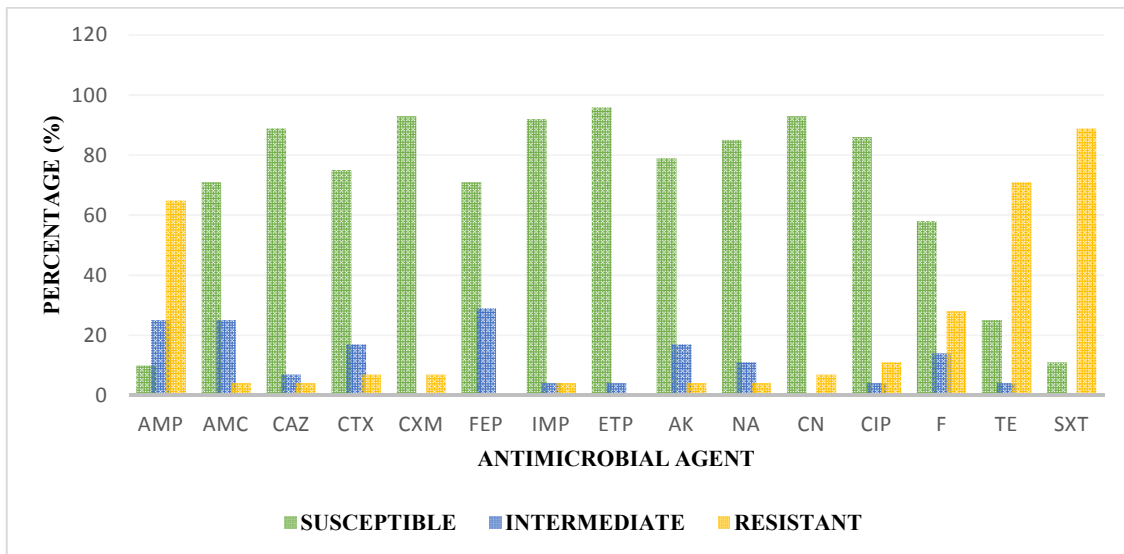
**Legend:** The gel image in Figure 1(a) shows the presence of Vero toxin gene (*mVT1*) in the study isolates when a light band corresponding to the 121bp (signifying a specific positive reaction) is observed in the lane where a particular isolate was assayed. Absence of such a band means absence of the gene *mVT1* in the isolate.



**Fig. 1b. 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 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



**Fig. 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 Fig. 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)*

National park. The domestic animals potentially 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 *E. coli* 0157 study isolates [14]. These Vero toxin genes mediate the virulence factors that enhance the organism's potential to cause disease [8,15-17]. Vero toxin gene *vt2e* is animal specific and is common among isolates that have encountered the animal host [18]; hence its high prevalence in our study suggests a possible zoonosis. Furthermore, phylo-group B<sub>1</sub>, 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 [18,19]. This affirms our earlier assumption that these isolates might be 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 [20]. These finding are consistent with studies done elsewhere on similar isolates whose source was animals [21]. 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 *E. coli* 0157 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 *E. coli* 0157 strain 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 [8,22]. 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 [23-25]. This global connectivity, which is now associated with the rapid worldwide spread of infectious agents and their resistance genes [23], implies that the AMR observed in our study may consequently spread to the rest of the world.

#### 4. CONCLUSION

There is high prevalence of highly pathogenic and resistant *E. coli* O157 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.

#### CONSENT

Waiver of consent for this research was approved by the School of Biomedical Sciences, Higher Degrees Research and Ethics Committee (HDREC) of Makerere University. This study did not recruit human participants but used archived isolates.

#### ETHICAL APPROVAL

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.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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