Antimicrobial Resistance & Infection Control

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High rate of multi-drug resistant *Escherichia coli* isolated from patients with urinary tract infections in Ifakara-Tanzania: implications for empirical antibiotic treatment guidelines and stewardship programs

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Abstract

Background Antimicrobial resistance (AMR) in Sub-Saharan Africa is a significant health threat, with limited data guide treatment. This study investigates multi-drug resistant *Escherichia coli* in urinary tract infections (UTIs) in rural Tanzania to guide empirical treatment strategies.

Methods A cross-sectional prospective study of adults with UTIs was conducted at St. Francis Regional Referral Hospital in Ifakara, Tanzania, from September 2021 to August 2023. Urine culture isolates underwent routine diagnostics in Tanzania and *E. coli* isolates underwent whole-genome sequencing in Switzerland.

Results Of 1055 patients, 248 (23.5%) had positive urine cultures, with *E. coli* as predominant pathogen ($n=87$; 55.7%). Extended-spectrum beta-lactamase-producing *E. coli* (ESBL-E) was identified in 20 (23.0%) isolates, primarily sequence type ST167 carrying CTX-M-27. All ESBL-E cases (20/20, 100.0%) and half of non-ESBL-E cases (29/58, 50.0%) received empiric antibiotics to which the isolates were documented as resistant. ESBL-E showed higher resistance to cotrimoxazole (100.0%) and ciprofloxacin (90.0%) latter recommended for complicated UTIs in Tanzania's Standard Treatment Guidelines (STG) compared to non-ESBL-E. All ESBL-E isolates were susceptible to nitrofurantoin, as recommended by STG for uncomplicated UTIs, and fosfomycin showed potential alternative for complicated cases.

Conclusion Nearly one-quarter of *E. coli* isolates causing UTIs were ESBL-E, predominantly ST167 harboring $bla_{CTX-M-27}$. Notably, nitrofurantoin remained effective for uncomplicated UTIs, similarly, fosfomycin emerged as a viable alternative. However, ciprofloxacin, despite being recommended in local guidelines for complicated UTIs, showed no efficacy. The genetic similarity between human and environmental isolates underscores the critical need for a One Health approach to tackle antimicrobial resistance (AMR) in the region.

Keywords Multi-drug resistant *Escherichia coli*, Urinary tract infections, Rural-Tanzania, Treatment guidelines

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Introduction

Antimicrobial resistance (AMR) is a global threat in the 21st century, leading to longer hospital stays, higher healthcare expenses, and increased mortality rates [1]. Without effective intervention, projections indicate an annual toll of 10 million deaths and a staggering economic burden of up to 100 trillion USD by 2050, with a disproportionate impact on low and middle-income countries (LMIC) [1, 2].

AMR is a complex and interconnected challenge across various hosts and ecosystems, encompassing humans, animals, food sources, and the environment [3, 4]. Human-related factors such as globalization, international travel, lack of novel antimicrobial agents, poverty, and indiscriminate antimicrobial use contribute to the spread of bacterial resistance genes, emphasizing the importance of the "One Health" approach [5].

The World Health Organization (WHO) has urged countries to develop action plans to address AMR [6]. In Africa there is a lack of comprehensive epidemiological data on AMR, particularly in Sub- Saharan Africa [7]. Tanzania, in particular, has received limited research attention concerning AMR, with scarcity of data on urinary tract infections (UTIs). Previous studies highlight the prevalence of ESBL *Escherichia coli* (*E. coli*) strains causing UTIs in Tanzania, underscoring the necessity of far-reaching national programs to combat antibiotic resistance [8].

Investigations in Tanzania reveal high rates of antibiotic utilization and prescription within hospital settings, with resistance proportions exceeding 50% for third-generation cephalosporins and approximately 10% for last-resort antibiotics like meropenem [9].

Our study aimed to comprehensively investigate the epidemiology of AMR in *E. coli* in urine isolates from patients in rural Tanzania. We sought to determine the underlying mechanisms of antibiotic resistance, identify factors predisposing individuals to MDR *E. coli* infections, and examine current antimicrobial therapy practices. The study's broader goal was to inform and advance local infection control practices, providing evidence-based guidance for healthcare practitioners in selecting appropriate antimicrobial treatments.

Methods

Study setting and patients

This cross-sectional prospective study was conducted at St. Francis Regional Referral Hospital (SFRRH) in Ifakara, Tanzania, between September 2021 and August 2023. SFRRH is a 371-bed teaching hospital serving approximately 16,480 inpatients and 100,000 outpatients annually (Fig. 1). In- and outpatients aged 18 years or older with clinical evidence of UTI based on Infectious Diseases Society of America (IDSA) guidelines [10], who had signed an informed consent form, were included in the study. All bacterial isolates from urine samples underwent routine analysis in Tanzania. Our study focused specifically on *E. coli* isolates from confirmed UTI cases. These *E. coli* isolates underwent more detailed analysis, including confirmation with MALDI-TOF and whole genome sequencing.

Sample and data collection and management

Patients received instructions from a clinician or registered nurse to collect a mid-stream urine sample of five to ten millilitres into a sterile container (Urine container Mshale, Dar es salaam, Tanzania). The collected samples were promptly delivered to the Microbiology laboratory at SFRRH within a time frame of two hours.

Structured questionnaires gathered socio-demographic and clinical data. Variables included age, gender, UTI history, pregnancy status, antibiotic use in the past two months, and companion animals. Furthermore, additional variables were gathered, including the identification of bacterial species and their resistance patterns. Data management and analysis plans were developed at SFRRH in Tanzania and HOCH, Cantonal Hospital of St. Gallen in Switzerland, respectively.

Laboratory procedures

In Tanzania:

Bacterial culture, isolation, and identification procedures adhered to the standard operating protocols of SFRRH. Mid-stream urine samples were collected aseptically and streaked on Blood, Cystine Lactose Electrolyte Deficient agar (CLED), and/or MacConkey agar (Liofilchem, Roseto degli Abruzzi (Te), Italy) using a calibrated 0·01 mL inoculating loop. Cultures underwent incubation for 18 to 24 h at 37 °C. Agar plates exhibiting pure growth equivalent to or exceeding 10^5 colony-forming units (CFU/mL) were categorized as having significant growth. Plates with no visible colonies were reported as having no bacterial growth. Results showing growth $\leq 10^2$ CFU/mL but below the threshold for significant growth were considered as insignificant growth. Cultures displaying more than two different types of organisms were classified as mixed growth, considered contaminated and patients were requested to re-collect and provide a new sample, if possible, to ensure accurate results. The pure bacterial isolates underwent further identification based on colonial characteristics, microscopic features following Gram's stain, and biochemical tests. Gram-negative isolates were identified through oxidase, Triple Sugar Iron (TSI), sulphur indole and motility (SIM), urease, citrate test, Voges–Proskauer (VP), and Methyl red test. The pure isolates were then inoculated onto nutrient agar slants, incubated for 18 h at 37 °C, and subsequently preserved in trypticase soy broth (TSB)

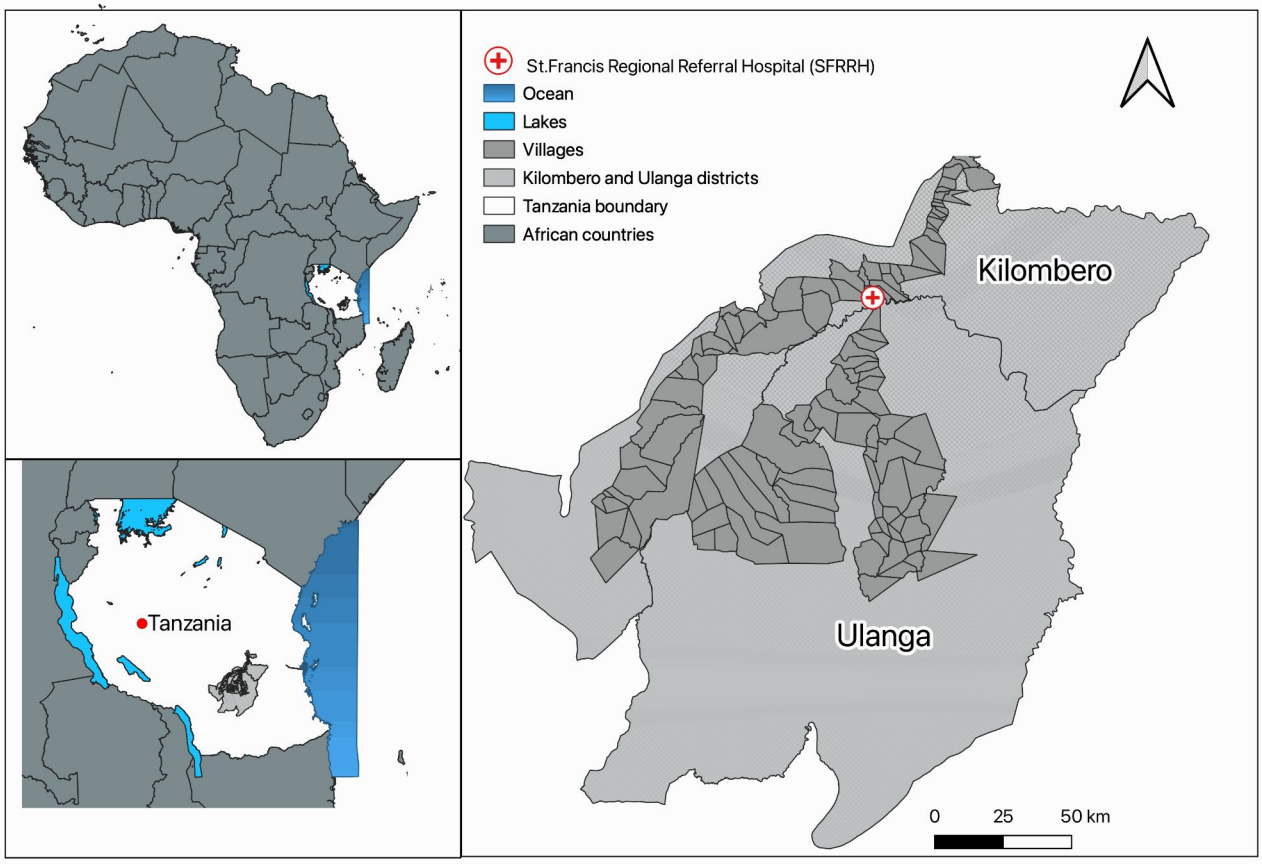

Fig. 1 Map showing the location of St. Francis Regional Referral Hospital (SFRRH), Ifakara-Tanzania

containing 20% glycerol at -80 °C. The bacteria were retrieved for shipment by using a sterile loop to scrape them off from the frozen bacterial glycerol stock. They were then streaked onto a nutrient agar plate and incubated for 24 h at 37 °C. After this, they were collected using a swab with Stuart transport system (Trust lab, China) and shipped at room temperature to the Laboratory of Experimental Infectious Diseases, HOCH, Cantonal Hospital of St. Gallen in Switzerland for advanced analysis.

In Switzerland, the isolates were assigned study IDs and subsequently streaked on Luria Bertani agar (Sigma Aldrich, USA), followed by incubation at 37 °C for 24 h. The following day, individual colonies were selected and then suspended in separate 1.5mL tubes containing 1 mL of Luria Broth (Sigma Aldrich, USA) with 10% glycerol and stored in a -80 °C freezer for future experiments.

Bacterial identification and susceptibility testing were conducted on all isolates. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) was employed for bacterial identification, utilizing the BDAL 9.0 database (MALDI Biotype Smart System, Bruker Daltonics, Bremen, Germany).

For susceptibility testing, a BD™ Phoenix instrument (Becton Dickinson, Sparks, MD, USA) with an NMIC-474 cartridge was utilized and E-test stripes (bioMérieux, Marcy-l'Étoile, France) were used for ESBL confirmation were necessary. Antimicrobial susceptibility testing data were interpreted following the respective European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (version 12.0 in 2022).

Genomic DNA isolation (Nucleic acid extraction)

Genomic DNA (gDNA) was extracted from all ESBL and Non-ESBL *E. coli* derived from single colonies on plates. The QiaAMP Mini Kit (QIAGEN, Hilden, Germany) was employed for DNA extraction, following the manufacturer's instructions, and the concentration was determined using the Nanodrop OneC spectrophotometer (Thermo Fisher Scientific, Massachusetts, United States). The extracted DNA was eluted in 50–100 µL of sterile water, and the DNA templates were stored at -20 °C until further analysis.

Whole genome sequencing and analysis of sequencing data

The genomic DNA (gDNA) extracts of *E. coli* were sent for whole genome sequencing (WGS) at the Functional Genomic Center Zurich, at the University of Zürich and the Swiss Federal Institute of Technology. 1 ng of DNA from each sample was tagmented using Illumina Nextera XT according to standard protocol. Nextera adapters containing Unique Dual Indices (UDI) were added by PCR. The libraries were double-sided size selected and the quality and quantity of the libraries were validated using the Fragment Analyzer (Agilent, Santa Clara, California, USA). The libraries were normalized to 10nM in Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20, and pooled equimolar. After library quantification, libraries were prepared for loading according to the NovaSeq workflow with the NovaSeq6000 Reagent Kit (Illumina, Catalog No. 20012865). Cluster generation and sequencing were performed on a NovaSeq6000 System with a run configuration of paired-end at 2 × 150 bp. Illumina paired-end (PE) reads underwent quality checks using FastQC (v0.11.9) [11] and FastQScreen (v0.14.1) [12]. Adapter sequences and low-quality read ends (identified with a sliding window of 4 bp and a base quality lower than Q20) were trimmed away using Fastp (v0.20.0). Only trimmed reads above the quality cutoff (Q20) and read length threshold (18 bp) were retained for downstream analysis. Trimmed and filtered reads were mapped to the reference genome (Ensembl *Escherichia_coli* K12 MG1655 ASM584v2) using bowtie2 (v2.4.2). Variants were identified using samtools (v1.11)/bcftools(v1.11) [13]. Hierarchical cluster dendrogram based on pairwise identity-by-state (IBS) values from SNP data for all samples was computed using SNPRelate (v1.30.1). A phylogeny tree from SNP data was constructed using mash tree (v1.4.6) and combined with metadata using itol (v6). Trimmed and filtered reads were also assembled using Spades (v3.15.5) [14]. Assembled contig sequences were annotated using prodigal (v2.6.1). Annotated protein sequences were compared to the Swiss-Prot (downloaded on 2023/02/10) using blastp (ncbi-blast v2.12.0+) for functional annotation. Phylogroups of assembled genomes were determined using the Clermont Typing pipeline (v20.03). *E.coli* Achtman MLST database was downloaded from pubmlst [15]. Annotated gene sequences were compared to the MLST database (downloaded on 2023/03/15) using blastn (ncbi-blast v2.12.0+). A customized perl script was used to identify the MLST profile, associated ST, and clonal complex. In silico serotyping was performed using SerotypeFinder (v2.0.1). Fim type was determined using FimTyper (v1.1) [16] by comparing assembled contig sequences against the FimTyper database using blast (2.12.0+). Antibiotic resistant genes were predicted using fARGene (Fragmented Antibiotic Resistance Gene iENntifiEr) (v0.1) [17] and blastp (2.12.0+) comparison against the Comprehensive Antibiotic Resistance Database (CARD v3.2.6) [18]. Contigs of plasmid origin were predicted using plasmidfinder (v2.1.6) and plaScope [19].

Sequences were in addition compared to the sequences of *E. coli* found in the aquatic environment and Nile perch in Lake Victoria, Tanzania, as reported by Baniga et al. [20] which were deposited on Genbank.

Data analysis

Categorical variables were summarized as frequencies with their percentages and compared between patients with ESBL *E. coli* and non-ESBL-E using Fisher's exact test.

Continuous variables were presented as means [standard deviation (SD)] and median [interquartile range] and compared with the presence or absence of ESBL *E. coli* using the Wilcoxon rank sum test.

Variables for the multivariable logistic regression were selected based on clinical relevance and previous literature identifying potential risk factors for ESBL *E. coli* infections. The analysis included factors such as age, gender, and history of UTI to determine whether there were associations between ESBL strains and these risk factors. Other variables were considered for the model, such as companion animals, diabetes and past antibiotic use, though due to limited data availability for these variables, they needed to be excluded.

Results with $p < 0.05$ were considered statistically significant. Statistical analyses were performed using R (version 4.2.2) [21].

Ethical considerations

Ethical clearance was obtained from the National Health Research Committee of the Tanzania National Institute for Medical Research with certificate number NIMR/HQ/R.8a/Vol. IX/3759. Informed consent was obtained in Kiswahili. Clinicians or registered nurses offered detailed study explanations to illiterate individuals, with a chosen witness present; those willing to participate expressed consent through a thumbprint. Additionally, culture and antimicrobial susceptibility testing results were promptly communicated to guide rational treatment.

Results

Sociodemographic and clinical characteristics of study participants

We enrolled 1,055 eligible patients meeting Infectious Diseases Society of America (IDSA) criteria for urinary tract infection (UTI) (Fig. 2). Out of these, 788 [74·7%] were outpatients, 710 [67·0%] were females, and the median age was 33 years [IQR: 25–52]. Bacterial growth was observed in 248 [23·5%], with 156 [62·9%] identified

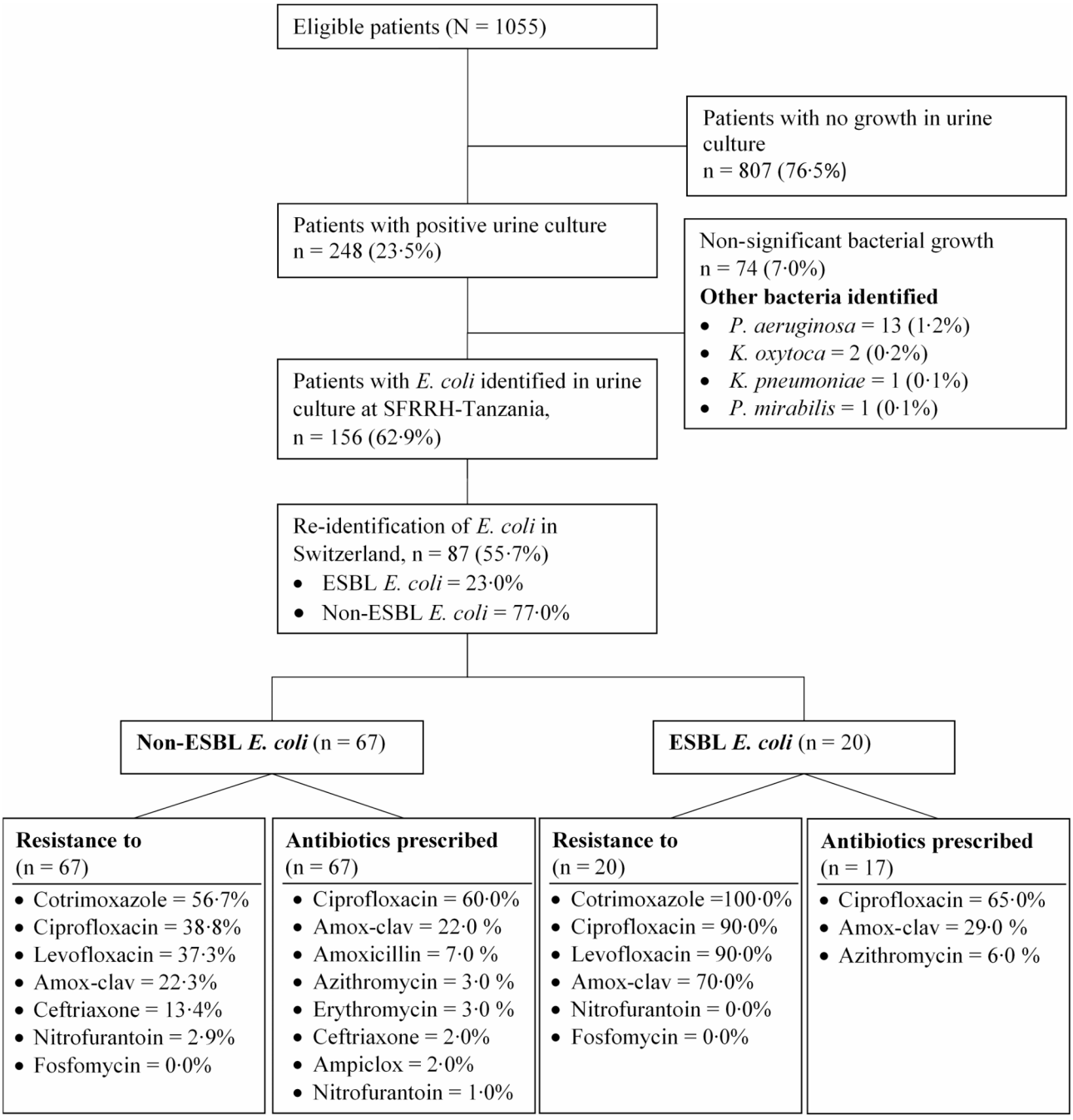

Fig. 2 Flow chart of bacteria identification, resistance and antibiotic treatment

as *E. coli*. After analysis in Switzerland, 87 [55·7%] of isolates were confirmed as *E. coli*, and other identified organisms included *Citrobacter freundii* (20 [12·8%]), *Pseudomonas aeruginosa* (20 [12·8%]), *Stenotrophomonas maltophilia* (11 [7·0%]), *Klebsiella pneumonia*e (7 [4·5%]) and various other organisms (details in Table S1).

Genomic diversity and clonality of *E. coli*

Among *E. coli*, 23·0% [*n* = 20] were ESBL producers. Notably, ST167 was the predominant sequence type in ESBL-producing strains, representing 55·5% [*n* = 11]. In the non-ESBL-producing group, ST131 was the prevailing sequence type, accounting for 23·8% [*n* = 16].

CTX-M-27, the primary ESBL enzyme, constituted 70·0% [*n* = 14] of cases, followed by CTX-M-15 at 30·0% [*n* = 6]. The ST167 ESBL-producing *E. coli* exhibited clonality, as confirmed by phylogenetic tree analysis. Additionally, our investigation uncovered a shared clonal lineage between these ESBL-producing *E. coli* strains and those found in the aquatic environment and Nile perch

(*Lates niloticus*) in Lake Victoria, Tanzania, as reported by Baniga et al. [20] (Fig. 3).

Among the 67 non-ESBL *E. coli* isolates, diversity was notable, spanning 31 different STs. The most prevalent ST was ST131, identified in 23·8% [*n* = 16] of the isolates, followed by ST10 at 10·4% [*n* = 7] (Fig. 4).

Plasmid analysis in the 20 ESBL-E isolates showed diversity in replicons, with IncFIB being the most common (18 [90·0%]), followed by IncFIA (16 [80·0%]), Inc FII (12 [60·0%]), and col 156 (12 [60·0%]). Within this subset, fimH54 was the predominant type in (13 [65·0%]) of isolates. Non-ESBL isolates revealed IncFIB (AP001918) as the most frequent replicon (26 [38·8%]), followed by IncFII (pRSB107) (19 [28·4%]). The quinolone resistance gene *qnrS1* was predominant among non-ESBL isolates (8 [12·0%]) (Fig. 5, Table S2, Table S3, Table S4).

Virulence factor distribution among *E. coli*

Table S5 illustrates the distribution of virulence factors (VFs) among 87 sequenced *E. coli* isolates, highlighting key genes involved in adherence, toxin production, iron acquisition, and immune evasion. Adherence-related genes were predominant, with 85 (98%) curli structural gene A (*csgA)*, 79 (91%) putative autotransporter adhesin (*yehA*), and 78 (90%) fimbrial adhesin H *(fimH)* frequently detected. Toxin production was driven by 63 (72%) Hemolysin E (*hlyE*) and 29 (33%) secreted autotransporter toxin (*sat*).

Antibiotic resistance, treatment outcomes and risk factors in *E. coli* utis

Among the 87 *E. coli* isolates, notable resistance to cotrimoxazole (44 [50·6%]) and quinolones (43 [49·4%]) was observed. All isolates were susceptible to fosfomycin, and only (2 [2·3%]), all non-ESBL) were resistant to nitrofurantoin, as shown in (Table S6, Table S7).

In clinical data, among 87 patients with *E. coli* in urine, (3 [3·4%]) had recent (past two months) antibiotic use, (55 [63·2%]) had a history of UTI. A limited number disclosed animal interactions: (8 [9·2%]) with chickens, (6 [6·9%]) with dogs, (5 [5·7%]) with cattle, and (2 [2·3%] with cats (Table S8). Notably, none of these factors were statistically significant for ESBL presence. Multivariable analysis, considering the small numbers (*n* = 87), included age, gender, and UTI history in the model (Table S9).

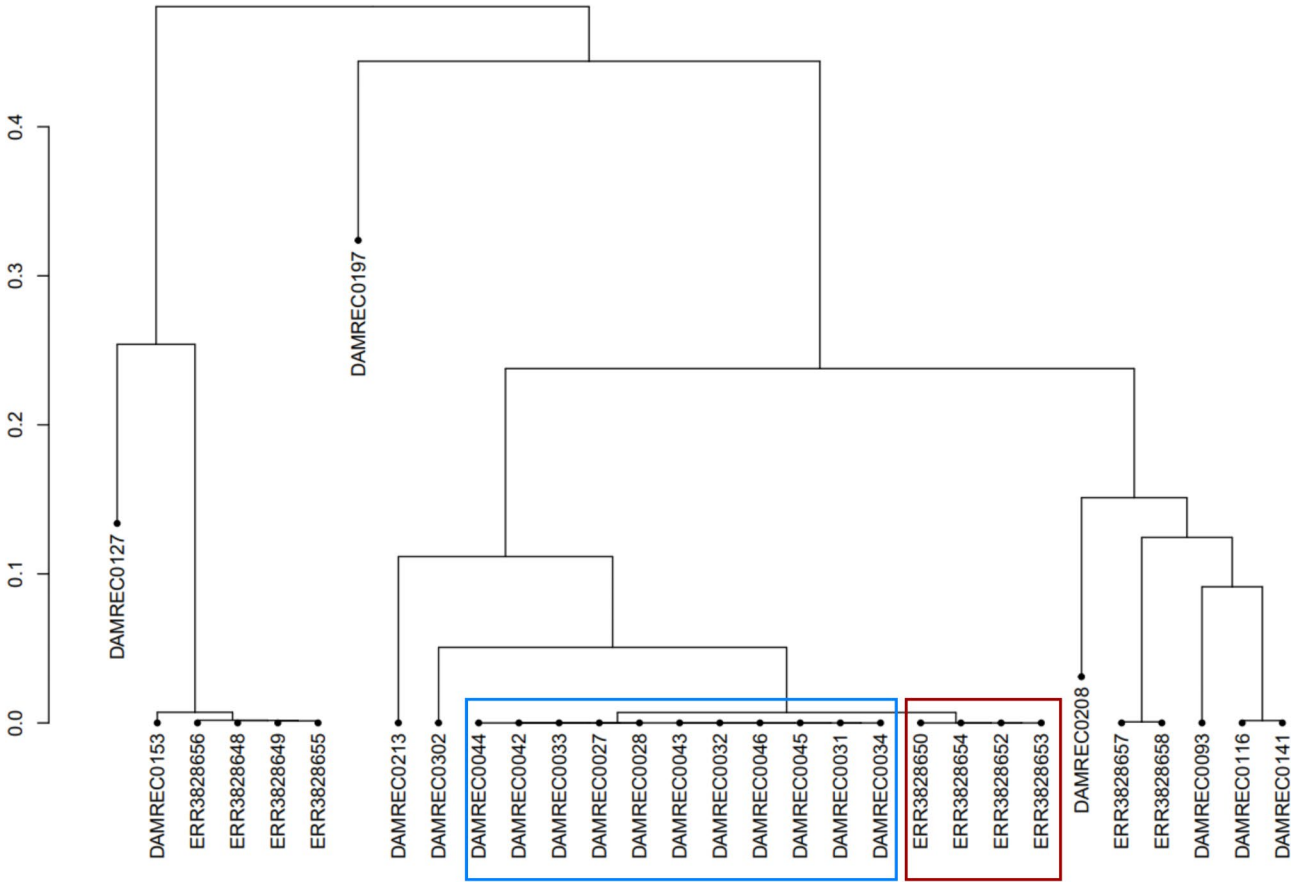

Fig. 3 Phylogenetic tree based on SNPs showing the clonal relationship among our ESBL *E. coli* isolated from UTI patients (blue rectangle) and ESBL *E. coli* from the aquatic environment of Lake Victoria-Tanzania (red square)

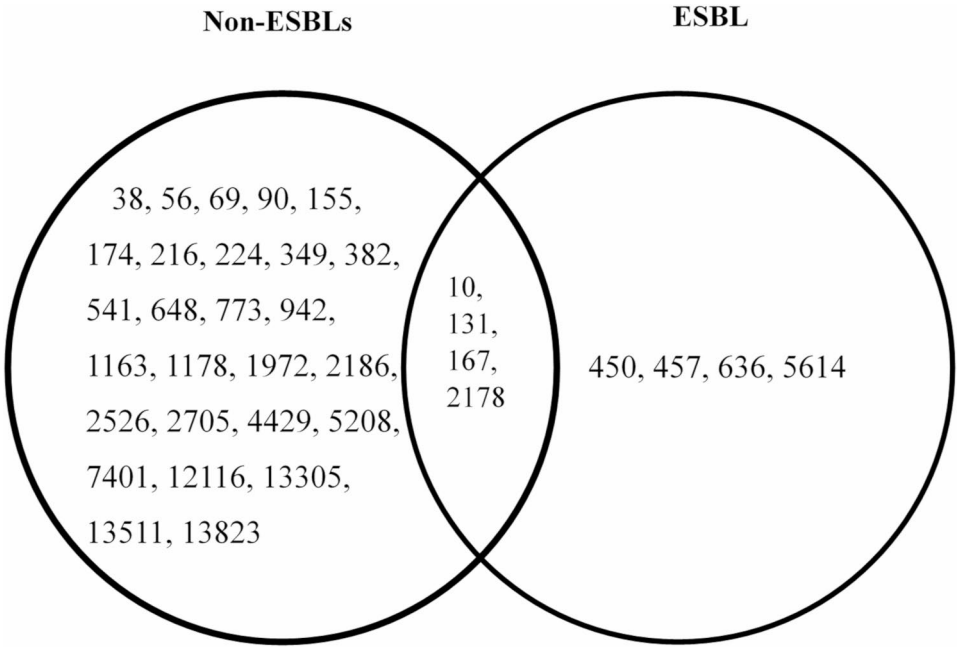

Fig. 4 STs of Non-ESBL and ESBL *E. coli* Clones

Among 87 diagnosed with *E. coli* - UTI, (72 [82·7%]) received antibiotic treatment, with (6 [6·9%]) receiving multiple antibiotics. In the subset of 67 patients with a single antibiotic, amoxiclav or ciprofloxacin were predominant (15 [22·0%] and (40 [60·0%], respectively). Additionally, (5 [7·5%]) received amoxicillin, (2 [2·9%]) azithromycin, and (2 [2·9%]) erythromycin. Among the 20 patients with ESBL-producing *E. coli*, (13 [65%]) were treated with ciprofloxacin, (6 [29·0%]) with amoxiclav, and (1 [6·0%]) with azithromycin (Fig. 2). All patients received empiric antibiotic treatment before culture and antimicrobial susceptibility testing (AST) results were available. According to the resistance phenotype, none of the ESBL *E. coli* were empirically treated with an adequate antibiotic. Only (34 [50·0%]) of patients with non-ESBL *E. coli* were treated with an antibiotic that was tested as susceptible. Once the AST results were obtained, they were promptly communicated to the clinicians to guide potential adjustments in therapy.

The genomic sequences are deposited in the European Nucleotide Archive (ENA). The accession and secondary accession numbers of the study are PRJEB71751 and ERP156535, respectively.

Discussion

This study investigated *Escherichia coli* isolates causing urinary tract infections (UTIs) in participants living in rural Tanzania, aiming at determining resistance, molecular mechanisms of resistance, predisposing factors, and antimicrobial practices.

Among the *E. coli* isolates exhibiting ESBL production in our study, ST167 carrying $bla_{\text{CTX-M27}}$ was predominant, marking its first identification in this rural setting. This finding distinguishes our results from previous studies in Tanzania, where *E. coli* ST131 carrying $bla_{\text{CTX-M-15}}$ has been the most frequently reported, particularly in fecal samples from street children [22]. Reports of *E. coli* ST167 in Tanzania are scarce, and this sequence type has been associated with IncFIA type plasmids [23].

Globally, *E.coli* ST131 carrying $bla_{\text{CTX-M15}}$ is recognized as the dominant clone associated with multi-drug resistance and widespread dissemination in both community and hospitals [24, 25]. The unexpected predominance of ST167 carrying $bla_{\text{CTX-M27}}$ in our study raises important questions about its emergence and spread. This finding suggests a potential shift in the local epidemiology of ESBL-producing *E. coli*, which may be influenced by several factors such as environmental reservoirs, human interactions and movements, and regional antibiotic use patterns.

A connection to contaminated fish from Lake Victoria [20] was established through SNP analysis (Fig. 3). Baniga and colleagues [20] had also observed a high occurrence of ESBL *E. coli* in fish from the local fish market, which was attributed to fecal cross-contamination from fish handlers and vendors in Mwanza. This observed connection may be attributed to human factors, such as individuals visiting families or working in both Mwanza and Ifakara, consequently having the potential to be exposed to contaminated fish from the local market in Mwanza and subsequently becoming gut colonizers. *E. coli* ST

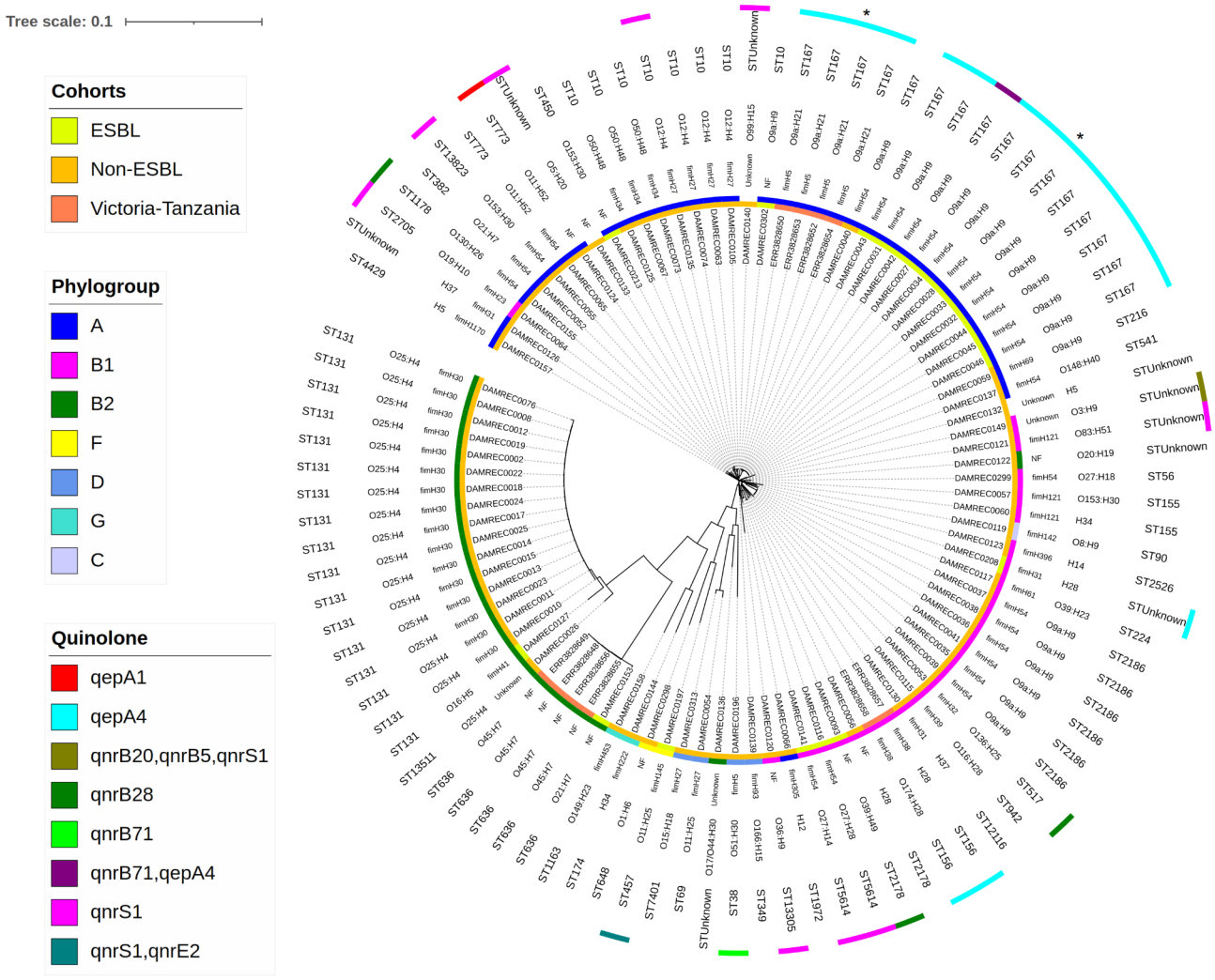

Fig. 5 SNP-based phylogenetic tree of 97 *E. coli* (20 ESBL-*E. coli*, 67 Non-ESBL *E. coli* and 10 *E. coli* isolates from Lake Victoria [20]. From centre to periphery, the different layers correspond to the isolate name, cohorts, the phylogroup, the *fimH* allele, the serotype, the sequence type (according to the Achtman scheme), the quinolone resistance gene. In the layer dedicated to ST type, ST167 ESBL *E. coli* from our study closely related to those of fish are annotated with a star

167, carrying NDM-5 carbapenemases, has been shown to spread across regions, such as Tanzania and Finland [23, 26], affecting humans, animals, and the environment.

Environmental reservoirs, such as contaminated aquatic sources like Lake Victoria, may facilitate the emergence and persistence of ST167. Moreover, regional variations in selective pressure, particularly differences in antibiotic use between rural Ifakara and urban centers like Mwanza, could contribute to the selection and dissemination of distinct clones such as ST167. Future research is imperative to explore the intricate interplay of human, animal, and environmental health in the dissemination of resistant pathogens mainly ST167. Ongoing surveillance initiatives should be established for continual monitoring of ST167 prevalence in both human and food samples. The efficacy of public health interventions, including educating fish sellers on safe handling practices and informing the local population about the risks associated with contaminated fish, warrants thorough assessment.

Of 1055 patients with UTI, 23·0% had ESBL-producing *E. coli* consistent with rates in Tanzanian tertiary hospitals [27]. Resistance to quinolones (up to 51·0%) and co-trimoxazole (68·0%) mirrors global trends, nitrofurantoin and fosfomycin showed high efficacy, in line with other studies conducted in Tanzania [28]. Although nitrofurantoin is listed as the first-choice antibiotic for uncomplicated cystitis in Tanzania's Standard Treatment Guidelines and National Essential Medicines List (STG-NEMLIT), most patients were treated with alternative agents such as amoxicillin or amoxicillin-clavulanic acid and ciprofloxacin. All empirically treated ESBL-*E. coli* cases received antibiotics to which the isolates were documented as resistant, highlighting a mismatch

between the prescribed empiric therapy and the susceptibility profiles of the pathogens. Additionally, only 50·0% of non-ESBL- *E. coli* UTIs were treated with susceptible antibiotics, highlighting the need for establishing or adapting antimicrobial treatment guidelines. Antibiotic resistance pattern variations may result from inappropriate antibiotic use among patients, as highlighted by the Holistic Approach to Unraveling Antibacterial Resistance (HATUA) study in Tanzania [29]. This study revealed a rapid increase in Antimicrobial Resistance (AMR) due to antibiotic misuse, including unsupervised use without prescriptions, improper dispensing by pharmaceutical providers, and inadequate diagnosis leading to incorrect prescriptions. These findings emphasize the immediate need for reassessment of the appropriateness of antibiotics, particularly for treating *E. coli* infections like urinary tract infections.

Risk factors traditionally associated with ESBL infections showed no significant associations, aligning with a study from Norway [30], reinforcing the consistency of these observations across different geographical regions. However, the sample size did not allow us to include important co-variables in our model.

Only 23·5% of urine samples showed significant bacterial growth, potentially influenced by factors such as infections other than UTI, symptom ambiguity and non-adherence to medication regimens encompassing the unsupervised use of antibiotics without prior consultation with healthcare providers [31] and handling and processing of urine samples [32].

Conventional microbiological techniques initially identified 62·9% of isolates as *E. coli*, with MALDI-ToF MS confirming 56·0% accuracy in Switzerland. This variation in findings underscores distinct methodological approaches employed at the two study sites highlighting the diagnostic challenges faced in resource-limited settings. In Tanzania, several factors contribute to the potential for misidentification, including limited availability of cutting-edge diagnostic tools such as MALDI-ToF MS, inconsistent access to standardized reagents, and a shortage of specialized personnel [33, 34]. These factors, combined with the reliance on conventional microbiological techniques, further emphasize the need to enhance diagnostic capabilities. Improving and standardizing bacterial identification protocols in Tanzania through the implementation of MALDI-ToF MS and advanced biochemical testing would be a significant step towards ensuring more accurate bacterial identification. In our investigation, the prevalence of ST131 was notably elevated in the subset of Non-ESBL *E. coli*, accounting for 23·8% of the isolates. This observation aligns with the findings from previous studies [35], which emphasized that a significant proportion of ST131 isolates do not produce Extended-Spectrum Beta-Lactamases (ESBLs), and fluoroquinolone resistance is particularly associated with this clonal lineage.

Studies on the prevalence of virulence genes in *Escherichia coli* isolates across Africa, including Tanzania, are limited. The high prevalence of adherence-related genes like *csgA*, *yehA*, and *fimH* in our sequenced *E. coli* isolates (98%, 91%, and 90% respectively) underscores the importance of biofilm formation and host cell attachment in the pathogenesis of UTIs. We also identified several additional virulence genes, including *fimH*, *traT*, *iutA*, and *hlyA*, which play crucial roles in adherence, serum resistance, and hemolysin production. These findings are consistent with systematic review study reporting similar virulence gene profiles among uropathogenic *E. coli* (UPEC) strains [36].

Conclusion

In conclusion, our study revealed a high prevalence of ESBL-producing *E. coli* and the dominance of the globally emerging ST167 clone among patients with UTIs in rural Tanzania. The coverage of antibiotics, particularly ciprofloxacin and amoxicillin-clavulanic acid, for treating ESBL *E. coli* UTIs raises significant concerns and emphasizes the need for a critical reassessment of antibiotic prescription practices in our setting.

Notably, all isolates exhibited susceptibility to older drugs such as fosfomycin and nitrofurantoin. These findings reinforce the continued effectiveness of nitrofurantoin, which is already recommended as a preferred first-line treatment for UTIs in the Tanzanian Standard Treatment Guidelines. Additionally, fosfomycin susceptibility results suggest its potential role as an alternative treatment option. These findings underscore the importance of revisiting and updating treatment guidelines to align with the susceptibility patterns observed in our study mainly in the recommendation for alternative second-line agents.

To address the growing concern of antimicrobial resistance (AMR), urgent measures are required, including the implementation of robust AMR surveillance in rural settings. This surveillance should focus on monitoring drug resistance patterns, strengthening laboratory diagnostic systems, and ensuring the appropriate treatment of urinary tract infections. These efforts will contribute significantly to the development and implementation of national AMR policy guidelines, promoting effective antibiotic use and combating the rising threat of antimicrobial resistance.

Our study is subject to several limitations. Firstly, within our clinical setting, certain patients were unaware of prior antibiotic exposure and history of urinary tract infections. We recognize the potential for recall bias, given the reliance on self-reported information, particularly as some queries necessitated recollection extending

up to two months. This investigation focused on patients seeking treatment for UTI-like symptoms within a hospital setting. Consequently, the reported prevalence may not accurately represent the true prevalence within the broader community. Owing to the absence of contact details, as a significant proportion of patients did not provide phone numbers, we were unable to conduct follow-up assessments to investigate their clinical outcomes. Our analytical approach relied exclusively on whole-genome sequencing short-read data for inference. Unfortunately, the limitations of short-read data precluded an in-depth exploration of variations in plasmid population structures among isolates beyond the resolution of replicon types.

Furthermore, we did not collect environmental samples, limiting our ability to comprehensively investigate the role of the environment in the transmission and persistence of the investigated phenomena.

Abbreviations

AMR	Antimicrobial resistance
CARD	Comprehensive Antibiotic Resistance Database
CLED	Cystine Lactose Electrolyte Deficient agar
CFU/mL	Colony-Forming Units/mL
E. coli	*Escherichia coli*
ENA	European Nucleotide Archive
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ESBL	Extended Spectrum-ß-lactamases
fARGene	Fragmented Antibiotic Resistance Gene
gDNA	genomic DNA
HATUA	Holistic Approach to Unraveling Antibacterial Resistance
HOCH	Health Ostschweiz Cantonal Hospital St. Gallen
IDSA	Infectious Diseases Society of America
LMIC	Low and Middle-Income Countries
MDR	Multi-Drug Resistant
MLST	Multi-locus Sequence Type
NIMR	National Institute for Medical Research
PE	Paired-end reads
SFRRH	St. Francis Regional Referral Hospital
STG	NEMLIT-Standard Treatment Guidelines and National Essential Medicines List
SIM	Sulphur Indole and Motility
TSI	Triple Sugar Iron
TSB	Trypticase Soy Broth
UDI	Unique Dual Indices
UTIs	Urinary Tract Infections
VP	Voges-Proskauer
WHO	World Health Organization
WGS	Whole Genome Sequencing

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13756-025-01557-y.

Supplementary Table S1: Bacteria identification at SFRRH and re-identification in HOCH

Supplementary Table S2: The molecular patterns of ESBL and non-ESBL *Escherichia coli*

Supplementary Table S3: Antimicrobial resistance genes of 87 *Escherichia coli isolates*

Supplementary Table S4: Plasmid replicons of 87 clinical *Escherichia coli isolates*

Supplementary Table S5: Virulence factors of 87 *E. coli isolates*

Supplementary Table S6: Susceptibility results of the all-*Escherichia coli* (*n* = 87)

Supplementary Table S7: Univariable analysis of antibiotic resistance in ESBL and Non-ESBL *Escherichia coli*

Supplementary Table S8: Risk Factors for ESBL carriage

Supplementary Table S1: Multivariable analysis of ESBL risk factors

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Author contributions

MEM: conception and design of the work, search for ethical clearance certificate, data management, wrote the initial draft of the manuscript. PK critically reviewed and edited the draft manuscript. AB critically reviewed and edited the draft manuscript, HMU coordinated all research activities and data collection in Tanzania (SFRRH), WQ methodology, bioinformatics analysis, critically reviewed and edited the draft manuscript, SNS methodology, critically reviewed and edited the draft manuscript, SH critically reviewed and edited the draft manuscript, EW methodology, statistical analysis, critically reviewed and edited the draft manuscript, MWR co-supervision, critically reviewed and edited the draft manuscript, BBF conceptualization, data curation, funding acquisition, methodology, project administration, supervision, wrote the manuscript. All authors gave final approval of the version to be published and agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Data availability

De-identified participant data will be made available for related research and analysis upon reasonable written request to the corresponding author within 12 months of the date of publication and subject to appropriate ethics approvals. Additional documents are not available. The genomic sequences are deposited in the European Nucleotide Archive (ENA). The accession and secondary accession numbers of the study are PRJEB71751 and ERP156535, respectively.

Declarations

Conflict of interest

We declare no competing interests.

Ethical approval and consent to participate

Ethical clearance was obtained from the National Health Research Committee of the Tanzania National Institute for Medical Research with certificate number

NIMR/HQ/R.8a/Vol. IX/3759. Informed consent was obtained in Kiswahili. Clinicians or registered nurses offered detailed study explanations to illiterate individuals, with a chosen witness present; those willing to participate expressed consent through a thumbprint.

Consent for publication
Not available.

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