The importance of Escherichia coli clonal complex 10 and ST131 among Tanzanian patients on antimicrobial resistance surveillance programs

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Abstract

The objective of this study was to characterize antimicrobial resistance (AMR) of WHO priority 1 critical pathogen (extrapathogenic Escherichia coli (ExPEC), sequence types (STs), and ST131 clades from patients in Tanzania so as to guide specific antimicrobial therapies and preventive measures. A total of 143 ExPEC strains (128 from pregnant women with urinary tract infections and 15 from children with blood stream infections) were collected between March 2016 and October 2017. These were characterized into ST-fimH clones by a 7-single nucleotide polymorphism quantitative polymerase chain reaction (7-SNP qPCR) and gene sequencing, and to ST131 clades by multiplex PCR. The extended-spectrum beta-lactamases (ESBL) production was 16.1% (23/143), and was predominantly due to the $bla_{CTX-M-15}$ (91.3%, n=21). ESBL production was significantly more among strains from children (53.3%) than pregnant women (11.7%) (OR (95%CI): 8.61 (2.73-27.15); p-value <0.001)). Approximately 61.5% (n=88) ExPEC were typed into their respective STs/CCs (87 by the 7-SNP qPCR and by an additional of one or two genes sequencing). The commonest STs/CCs among typeable strains were CC10 (28.4%, n=25), ST131 (18.2%, n=16), and ST38 (10.2%, n=9). The ST131 clades (C1 (4, 25.0%) and C2 (6, 37.5%)) were predominantly associated with fluoroquinolone resistance and ESBL production, respectively. Approximately 60.8% of ExPEC strains and all dominant clones were typed by the 7-SNP qPCR by additional sequencing. The multiplex clade PCR allowed linkage of the global clone ST131 with AMR phenotypes. These feasible and user-friendly molecular tools can be routinely used for surveillance programs in resource-limited settings.

Keywords: Escherichia coli; Mwanza; ST131 clades; Tanzania.

Introduction

Extended-spectrum β-lactamases (ESBL) produced by Gram-negative bacteria are important causes of resistance to cephalosporins, leaving carbapenems and, to a less extent, piperacillin-tazobactam and fluoroquinolones as alternative antimicrobial therapeutic options for severe infections [1]. *Escherichia coli* and *Klebsiella pneumoniae* are the most common ESBL-producing Enterobacteriaceae, and are classified as priority 1 critical pathogens by the World Health Organization (WHO) [2]. *E. coli* can be broadly categorized into intestinal and extraintestinal strains based on their anatomical niches in humans [3]. Extraintestinal pathogenic *E. coli* (ExPEC) strains commonly cause urinary tract infections (UTI) and blood stream infections (BSI) [4]. Of note, the mortality attributed to ESBL-producing strains is alarmingly double compared to non-ESBL strains as previously shown in a review report across various countries (36% versus 18%, respectively) and in Tanzania (71% versus 39%, respectively) [5, 6]. The $bla_{\text{CTX-M-15}}$ accounts for over three-quarters of ESBL alleles among *Enterobacteriaceae* in Tanzania underscoring the need for its continuous monitoring [5, 7]. Pregnant women have increased vulnerability to UTI due to anatomical-structural relations and hormonal changes, and children under 5 years of age have also increased vulnerability to BSI due to immature immunity [8, 9]. Reported increased neonatal and maternal morbidities and mortalities in Tanzania are largely attributed to infections, and hence, a need to specifically focus on the two populations [10, 11].

Out of thousands of ExPEC sequence types (STs) described to date across the world, there are four predominant types namely ST131, ST95, ST73, and ST69 [12, 13]. Of note, the global dissemination of *E. coli* ST131 clone, and especially its clade C1 (*fim*H30R) and clade C2 (*fim*H30Rx), has been an area of scrutiny [13,14,15]. The predominance of ST131 has also been previously described in review across the world. However, variations across regions have been documented for other lineages like ST95 and ST10 [16]. Existing typing tools like multilocus sequence typing (MLST) and whole genome sequencing (WGS) are costly and laborious and require high-level technical know-how, limiting their universal utility [4]. The polymerase chain reaction (PCR) methods like a 7-single nucleotide polymorphism quantitative PCR (7-SNP qPCR) and ST131 clade multiplex PCR can offer alternative typing options [12, 17, 18]. The 7-SNP qPCR method can type ExPEC and infer the STs to their respective *fim*H clones [17], whereas the ST131 clade multiplex PCR can associate the clades with AMR profiles [19]. The current study was conducted among pregnant women with UTI and children less than 5 years of age with BSI to address the vulnerability of the two groups in line with the United Nations (UN) third strategic development goals (SDG) on good health and well-being (http://www.un.org/sustainabledevelopment/news/ communications-material/) [20, 21]. Despite the existence of robust phenotypic AMR data on UTI and BSI in tertiary hospitals in Tanzania, there is limited information on the molecular epidemiology of the dominant pathogens causing the two diseases which in turn limit the rational provision of antimicrobial therapies and evidence-based infection prevention and control (IPC) measures. Therefore, to address these critical gaps, we describe ExPEC AMR patterns, circulating STs, and the global clone ST131 clades isolated from pregnant women with UTI and children less than 5 years of age with BSI in Tanzania.

Materials and methods

Study design, duration, and settings

This was a cross-sectional analytical study conducted from March 2016 to October 2017 in the northwestern part of Tanzania, involving seven health care facilities in the cascade of referral system in a catchment population of approximately 16 million people. These health care facilities were Bugando Medical Centre tertiary hospital (BMC) with a 950-bed capacity and Sekou Toure Regional Referral Hospital (SRRH) with a 375-bed capacity. Others were Nyamagana District Hospital (NDH) with an 88-bed capacity in an urban setting, Sengerema District Designated Hospital (SDDH) with a 320-bed capacity in a rural setting, and three lower level health care facilities (Makongoro and Buzuruga Health Centres in Mwanza city, and Sengerema Health Centre in Sengerema district), with bed capacities ranging from 4 to 10.

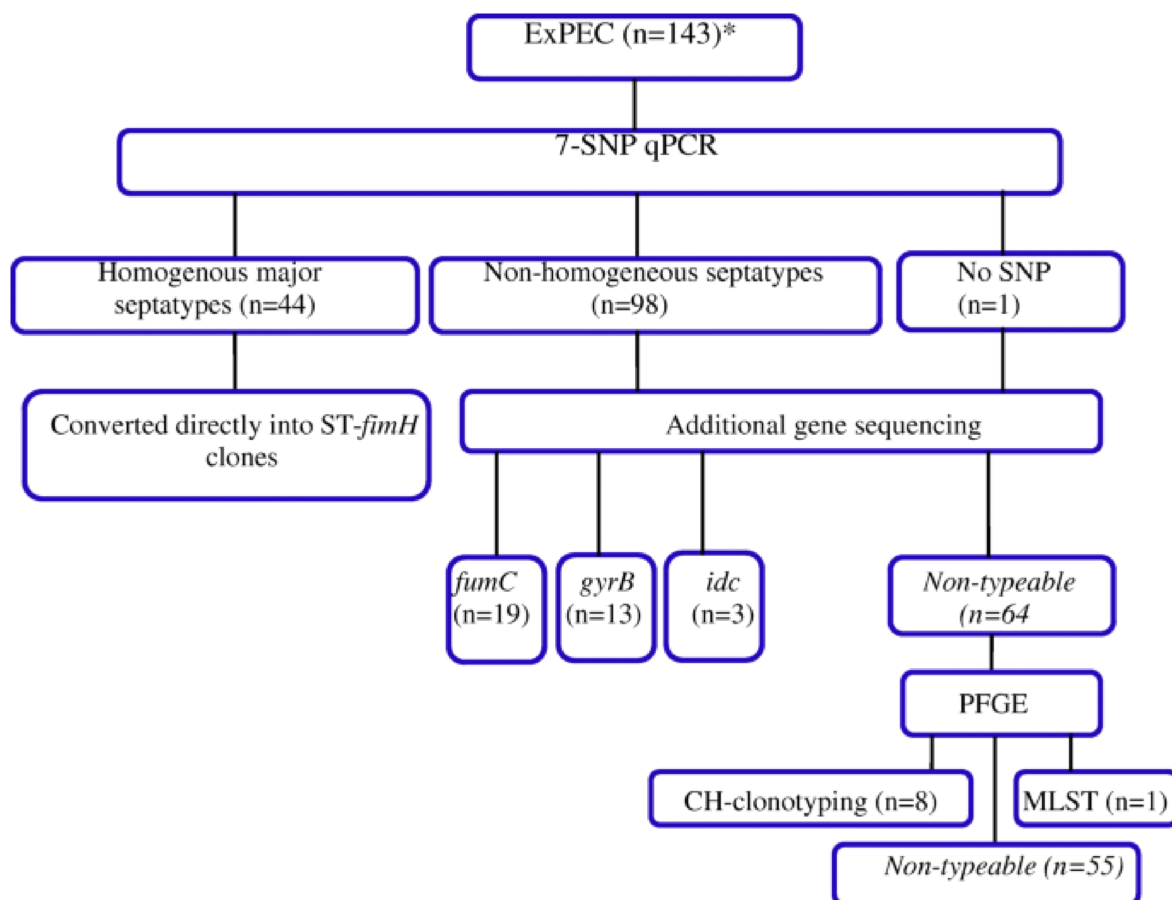

Fig. 1. Flow chart for enrollment of patients and laboratory procedures. AST, antimicrobial susceptibility testing; 7-SNP qPCR, 7-single nucleotide polymorphisms quantitative polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; MLST, multilocus sequence typing; the asterisk indicates the initial ExPEC isolates were 181 but 38 did not grow after sub-culture

Study population and sampling procedures

All socio-demographic and clinical data were collected using structured questionnaires and patients' clinical records, and their initial extensive analysis was published in two articles [20, 21]. A total of 181 E. coli strains were isolated (164 from pregnant women (out of 1828 pregnant women) and 17 from children less than 5 years of age (out of 950 children)). Of these, 38 ExPEC strains did not grow from frozen glycerol stocks on sub-culture. Therefore, 143 ExPEC were subjected to the final molecular assays (89.5% (n=128) from pregnant women and 10.5% (n=15) from children less than 5 years of age as summarized in Fig. 1). The distribution of 143 patients by health care facilities was 28.7% (n=41) from BMC, 18.2% (n=26) from SRRH, 38.5% (n=55) from NDH and SDDH, and 14.7% (n=21) from health centers.

Data collection and laboratory procedures

Socio-demographic and clinical characteristics of patients were collected from participants using structured questionnaires and patients' clinical records. The patients' enrollment process and laboratory procedures are summarized in Fig. 1.

Conventional phenotypic ExPEC identification and antimicrobial susceptibility testing

Urine and blood cultures for isolating ExPEC were done following the standard operating procedures at SDDH and CUHAS Multipurpose Laboratory at BMC in Mwanza, Tanzania [22,23,24]. ExPEC identification and the Kirby–Bauer disk diffusion method for AST were done by conventional methods [22, 25]. The phenotypic screening of ESBL was done in Muller Hinton agar, using a cutoff zone inhibition of ≤ 25mm for ceftriaxone and ≤ 22mm for ceftazidime as previously described in the Clinical Laboratory Standard Institute guidelines [25]. Confirmation of ESBL was done by the double-disk synergy method as previously described [26].

The *bla* $_{CTX\text{-}M\text{-}15}$ ESBL gene conventional PCR and ESBL allele gene sequencing

The presence of *bla*$_{\text{CTX-M-15}}$ gene was evaluated to all phenotypically ESBL producers and all ST131 strains (irrespective of the ESBL status) using the previously described conventional PCR method to identify 483 bp amplicon product in a GeneAmp® 9700 ThermoCycler instrument (Applied Biosystems, Norwalk, CT) [27]. ESBL strains that were negative for the *bla*$_{\text{CTX-M-15}}$ gene were sequenced to assess the presence of other *bla*$_{\text{CTX-M}}$ alleles [28].

The 7-single nucleotide polymorphisms quantitative PCR

The ExPEC strains were typed by the 7-SNP qPCR to identify septatypes and respective ST-*fim*H clones as shown in Fig. 1 based on the original method described by Tchesnokova and colleagues [17]. Briefly, this method uses 7 primer pairs to type ExPEC into different septatypes by assessing the presence or absence of 7 SNPs (four SNPs in the *fim*H gene and three SNPs in the *fum*C gene), and primers for *uidA* gene as an internal control for confirmation of *E. coli*. The assay was run in a ViiA7 Applied Biosystems qPCR machine, using the SYBR-Green PCR Kit (Invitrogen by Thermo Fisher Scientifica, CA, USA). Each SNP was assigned a number/score, and the presence of SNP in the first, second, and third positions in each gene was scored as 1, 2, and 4, respectively, whereas the absence of SNP was scored as 0. Then, the sum of the score assigned a strain to its respective septatype.

Finally, the respective ST-*fim*H clones were inferred from the database/table in the original publication [17]. These ExPEC strains which were directly typed into their respective ST-*fim*H clones were referred to as homogeneous major septatypes in Fig. 1.

Non-homogeneous septatypes were ExPEC strains which were not assigned directly to the ST-*fim*H clones, and therefore required an additional one gene sequencing. In these strains, conventional PCR was done to identify the presence of the respective gene/amplicon using the MLST scheme from the University of Warwick. Then, the respective DNA for each ExPEC strain was measured to a concentration of approximately 50ng/µL using *Nanodrop® 2000 spectrophotometer* (Thermo Scientific, USA), followed by sequencing of these genes by Functional Biosciences™, Madison, WI, USA. The *Sequence scanner®* software was used to assess the quality of the DNA chromatogram, and then *CLC Sequence Viewer®* software was used to check and sizing the allele sequence according to the MLST allele. Finally, the respective gene sequence was uploaded into the *E. coli* MLST database (https://enterobase.warwick.ac.uk/species/ecoli/allele_st_search) to obtain the allele number, and subsequent ST/CC.

The two-locus clonal typing of extraintestinal pathogenic *E. coli* strains

All non-homologous ExPEC strains by the 7-SNP qPCR which could not be typed by an additional gene sequence were regarded as non-typeable as demonstrated in Fig. 1. These strains were subjected to PFGE analysis to ascertain their heterogeneity or clustering patterns using the CDC protocol customized at the Calgary Laboratory Service (https://www.cdc.gov/pulsenet/ pdf/ecoli-shigella-salmonella-pfge-protocol-508c.pdf). The detailed information on PFGE analysis is beyond the scope of this article but can be accessed through the link: https://prism.ucalgary.ca/handle/1880/108935. Six ExPEC strains were selected based on PFGE cluster profiles (using a cluster cutoff similarity of ≥95%) and typed by the two-locus clonotyping method targeting the *fumC* and *fim*H genes as described before by Weissman and colleagues [29]. The PCR for the initial screening for the gene prior to gene sequencing is shown in Supplementary Fig. S1. This method assigned eight ExPEC strains into STs/CCs (six strains were directly typed and two strains were inferred based on ≥95% PFGE cluster similarity).

The *E. coli* ST131 clades multiplex PCR

The ExPEC ST131 were typed into clades A, B, C0, C1, and C2 by multiplex PCR method as described before using a GeneAmp® 9700 ThermoCycler instrument (Applied Biosystems, Norwalk, CT) [18, 19]. This assay identifies genomic region and SNP specific to clades and sub-clades. Specific genomic targets are ST131 specific (region 19, 580bp), clade A specific (region 4, 707bp), *prfC* SNPs specific for the clade B (442bp), *mgtA* SNPs specific for the C clade (103bp), clade C1 (337bp), and *nrdI* SNP specific for the C2 clade (164bp) [18, 19].

Quality control

E. coli ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive ESBL controls for antimicrobial tests, respectively. Four control strains (*E. coli* 85.01, *E. coli* CFT 07, *E. coli* BUT 3.1.4, and *E. coli* UHN. 026) validated previously at Calgary Laboratory Services by MLST were used as positive controls for the major *E. coli* STs (ST69, ST73, ST95, and ST131, respectively). The reliability of the 7-SNP qPCR method was done by re-running 5% of all ExPEC strains using the same machine and same

PCR conditions, and the results were 100% reproducible. In addition, two strains were purposefully selected and typed by full MLST scheme (one strain with PFGE cluster similarity of >95% and another strain with PFGE cluster similarity of <85% with other ExPEC strains). The former was also typed by the *gyr*B gene sequencing and both methods revealed CC155, whereas the latter was found to be ST196, and was unrelated to all other ExPEC strains.

Statistical analysis

Patients' clinical data and ExPEC strains' laboratory data were analyzed by the STATA version 13.0 software (College Station, TX, USA). The proportions of ESBL-producing ExPEC among pregnant women with UTI and children less than 5 years of age with BSI were calculated and compared using the Pearson chi-squared test, OR, and 95% CI. ExPEC septatypes, ST/CC-*fimH*-based clones, and ST131 clades were presented as proportions/percentages and analysis of their occurrence in various hospitals and clinical conditions were computed. The association between *E. coli* ST131 and non-ST131 attributable infections for various risk factors was compared using the Pearson chi-squared test (or Fisher's exact test where appropriate) using a p-value of less than 0.05 as a cutoff value.

Results

Baseline characteristics of 143 patients with *E. coli* attributable infections

The majority of enrolled patients were female (93.7%), residing in urban areas (77.6%, n=111) and their median age (IQR) was 24 (19–28) years, ranging from 1 month to 38 years. Approximately 37.1% (n=53) of the patients were admitted and 35.0% (n=50) had a previous history of antibiotic use. A molecular marker for *E. coli* (*uidA* gene) was found to be present in 140 (97.9%) strains. The remaining three *uidA* gene negative strains were confirmed to be *E. coli* by the MS VITEK 2 system (BioMerieux, Marcy, I'Etoile, France) with a precision of 99.9%. The majority of ExPEC strains were uropathogens (89.5%, n=128).

***E. coli* sequence types and their occurrence in various health care facilities**

A total of 88 (61.5%) ExPEC were typed into their respective STs/CCs, resulting into 24 different STs/CCs. Out of these, 44 (50.0%) were typed directly by the 7-SNP qPCR into their respective ST-*fimH* clones, 35 (39.8%) by an additional one gene sequencing, 8 (9.1%) by CH-clonotyping, and one strain (1.1%) by MLST. The most common STs/CCs among typeable ExPEC strains were CC10 (28.4%, n=25), ST131 (18.2%, n=16), ST38 (10.2%, n=9), and ST69 (4.5%, n=4). Other STs/CCs were ST394 (3.4%, n=3), ST297 (3.4%, n=3), ST95 (3.4%, n=3), CC88 (3.4%, n=3), and other STs/CCs (25.0%, n=22). The 7-SNP qPCR correctly typed all dominant clones (i.e., ST131-*fimH30/41* (n=16), ST38-*fimH5* (n=8), ST69-*fimH27* (n=4), ST95-*fimH41* (n=3), and ST73-*fimH9/10*) (n=2) within this Tanzanian ExPEC collection. The CH-typing correctly typed the remaining ST38-*fim54* strain. The ST131 strains were predominantly found at BMC (9/16). Non-dominant STs/CCs and non-typeable STs/CCs were commonly found in the district hospitals as demonstrated in Fig. 2.

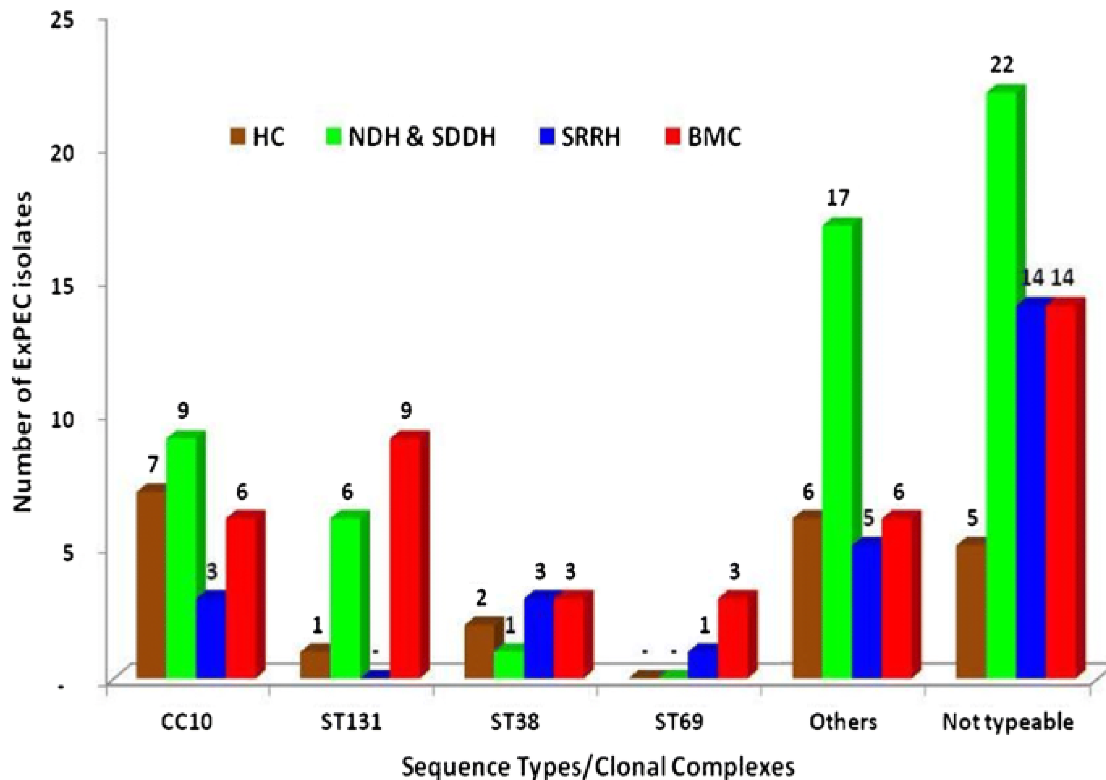

Fig. 2. Occurrence of *E. coli* sequence types in different health care facilities (n=143). BMC, Bugando Medical Center; SRRH, Sekou Toure Regional Referral Hospital; NDH, Nyamagana District Hospital; SDDH, Sengerema District Designated Hospital; HC, health centers; ST, sequence type; CC, clonal complex

Molecular characterization of *E. coli* strains from pregnant women and children

The overall ESBL production among 143 ExPEC was 16.1%, 95% CI: 10.9–23.1%, with a predominance of *bla*$_{CTX\text{-}M\text{-}15}$ gene (21 (91.3%)). Other ESBL two alleles were *bla*$_{CTX\text{-}M\text{-}14}$ gene and the *bla*$_{CTX\text{-}M\text{-}27}$ gene (both from two ST131 strains). The ESBL production was significantly more among ExPEC strains from children, 53.3% (8/15), than pregnant women, 11.7% (15/128) (OR (95% CI): 8.61 (2.73–27.15); *p*-value <0.001)), as presented in Table 1 and Table 2. Fortunately, all 143 ExPEC strains were sensitive to meropenem.

Table 1. The population structure of 128 *E. coli* from pregnant women

ExPEC n (%)	Septatypes (n)	Additional gene sequenced	ST/CC	FimH	CTM-M-15[#] (*n*)	Resistance patterns (%)
24 (18.8)	771 (14)	*fum*C	CC10	NA		STX (83.3); NITRO (4.2); GENT (8.3); CIP (16.7); CRO (0.0)
	751 (6)	NI	ST10	*fim*H54	NI	
	761 (4)	*fum*C	CC10	NA		
13 (10.2)	561 (11)	NI	ST131	*fim*H30	4	STX (100.0); NITRO (23.1); GENT (53.9); CIP (61.5); CRO (46.2)
	560 (2)	NI	ST131	*fim*H41	0	
7 (5.5)	231 (7)	NI	ST38	fimH5	1	STX (85.7); NITRO (0.0); GENT (14.3); CIP (14.3); CRO (14.3)
3 (2.3)	261 (3)	*icd*	CC394	NA	NA	STX (100.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
3(2.3)	760 (3)	NI	ST95	*fim*H41	NA	STX (66.7); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
2 (1.6)	271 (2)	NI	ST69	*fim*H27	0	STX (0.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
2 (1.6)	361 (2)	*gyr*B	CC155	NA	NA	STX (0.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
2 (1.6)	371 (2)	*gyr*B	ST648	NA	2	STX (100.0); NITRO (0.0); GENT (100.0); CIP (100.0); CRO (100.0)
2 (1.6)	371 (2)	CH-typing	ST641	*fim*H21	NA	STX (100.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
2 (1.6)	361 (2)	CH-typing	ST210	*fim*H31	NA	STX (100.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
2 (1.6)	530 (2)	NI	ST127	fimH2	NA	STX (50.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
2 (1.6)	371 (2)	CH-typing	ST88	*fim*H24	NA	STX (100.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
2 (1.6)	620 (2)	NI	ST73	*fim*H9/10	NA	STX (100.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
1 (0.8)	371 (1)	CH-typing	ST607	*fim*H305	NA	STX (0.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
1 (0.8)	771 (1)	*fum*C	ST1457	NA	NA	STX (100.0); NITRO (0.0); GENT (0.0); CIP (100.0); CRO (0.0)
1 (0.8)	360 (1)	*gyr*B	ST718	NA	NA	STX (100.0); NITRO (100.0); GENT (100.0); CIP (0.0); CRO (0.0)
1 (0.8)	371 (1)	*gyr*B	ST405	NA	NA	STX (100.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
1 (0.8)	371 (1)	*gyr*B	ST297	NA	NA	STX (100.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
1 (0.8)	360 (1)	*gyr*B	ST224	NA	NA	STX (100.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
1 (0.8)	361 (1)	MLST	ST196	NA	NA	STX (0.0); NITRO (100.0); GENT (0.0); CIP (0.0); CRO (0.0)
1 (0.8)	360 (1)	*gyr*B	ST101	NA	NA	STX (0.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
1 (0.8)	371 (1)	*gyr*B	CC58	NA	NA	STX (0.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
1 (0.8)	571 (1)	NI	ST14	*fim*H27/64	NA	STX (100.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
1 (0.8)	131 (1)	NI	ST12	*fim*H5	NA	STX (100.0); NITRO (0.0); GENT (0.0); CIP (0.0); CRO (0.0)
51 (39.8)	Variable *	ND	-	-	6	STX (86.3); NITRO (5.9); GENT (15.7), CIP (15.7); CRO (11.8)
Total = 128					13	

ST, sequence type; CC, clonal complex; NA, not applicable; NI, not indicated; ND, not done; SXT, trimethoprim-sulfamethoxazole; NITRO, nitrofurantoin; GENT, gentamicin; CIP, ciprofloxacin; CRO, ceftriaxone

*Non-homogeneous septatype except one strain which had no SNP (these were regarded as non-typeable); [#] Done to all ST131 and to only other STs/CCs which were phenotypically ESBL producers; all MLST genes = *fumC*, *gyrB*, *icd*, *mdh*, *purA*, *recA*, and *adk*

Table 2. The population structure of 15 *E. coli* from children with blood stream infections

ExPEC n (%)	Septatypes (n)	Additional gene sequenced	ST/ CC	fimH	CTM-M-15# (*n*)	Resistance patterns (%)
3 (20.0)	561 (2)	NI	ST131	*fim*H30	1	STX (66.7); GENT (0.0); CIP (66.7); CRO (33.3)
	560 (1)	NI	ST131	*fim*H41	0	
2 (13.3)	231 (1)	NI	ST38	fimH5	1	STX (100.0); GENT (50.0); CIP (0.0); CRO (50.0)
	253 (1)	CH-typing		*fim*H54	NI	
2 (13.3)	271 (2)	NI	ST69	*fim*H27	1	STX (100); GENT (50.0); CIP (0.0); CRO (50.0)
2 (13.3)	360 (2)	*gyr*B	ST297	NA	2	STX (100.0); GENT (100.0); CIP (100.0); CRO (100.0)
1 (6.7)	751 (1)	NI	ST10	*fim*H54	NI	STX (100.0); GENT (0.0); CIP (0.0); CRO (0.0)
1 (6.7)	371 (1)	*gyr*B	CC88	NA	1	STX (100.0); GENT (100.0); CIP (100.0); CRO (100.0)
4 (26.7)*	251 (1)	ND	-	-	2	STX (100.0); GENT (50.0); CIP (50.0); CRO (50.0)
	511 (1)					
	700 (1)					
	720 (1)					
n = 15					8	

ST, sequence type; CC, clonal complex; NA, not applicable; NI, not indicated; SXT, trimethoprim-sulfamethoxazole; GENT, gentamicin; CIP, ciprofloxacin; CRO, ceftriaxone

Done to all ST131 and to only other STs/CCs which were phenotypically ESBL producers; *non-homogeneous septatypes (these were regarded as non-typeable)

Of the 128 ExPEC isolated from pregnant women with UTIs, 77 (60.2%) were typeable and the predominant clones were CC10 (24, 18.8%) and ST131 (13, 10.2%), as presented in Table 1. Of the 15 ExPEC isolated from children less than 5 years of age with BSIs, 11 were typeable (73.3%) and the predominant clone was *E. coli* ST131 (3, 20.0%), as presented in Table 2.

Multiplex PCR characterization of *E. coli* ST131 into clades

The most common clades were clade C2 (n=6, 37.5%) and clade C1 (n=4, 25.0%), as demonstrated in Fig. 3. Two out of three clade A strains were sensitive to ciprofloxacin and ceftriaxone. All clade B strains were sensitive to ciprofloxacin and ceftriaxone. All clade C1 strains were resistant to ciprofloxacin and three were sensitive to ceftriaxone. On the other hand, all clade C2 strains were resistant to ciprofloxacin and ceftriaxone, except for one strain. Therefore, the prediction of phenotypic fluoroquinolone resistance (FQ-R) and ESBL production by the ST131 clade multiple PCR method was correct in 87.5% (14/16) of strains.

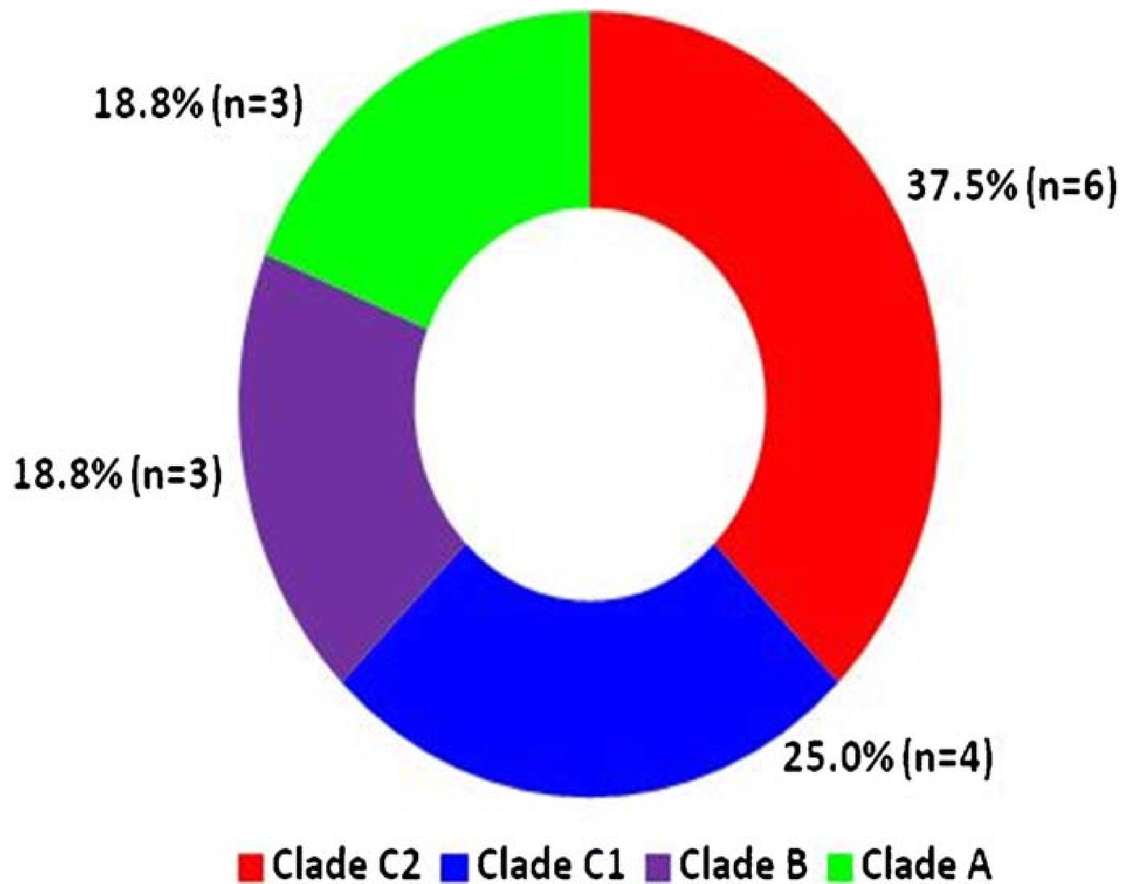

Fig. 3. Distribution of *E. coli* ST131 strains into clades (n=16). Pregnant women (n=13) and children under 5 years of age (n= 3)

Risk factors associated with *E. coli* ST131 and non-ST131 infections in pregnant women and children combined

There was predominance of ExPEC ST131 among patients attending BMC in approximately 22.0% (9/16) compared to other health care facilities, i.e., SRRH (0.0%), NDH and SDDH (10.9% (6/55)), and health centers (4.8% (1/21); (p-value=0.029)). ExPEC ST131 strains exhibited higher resistance to ciprofloxacin than non-ST131 strains (62.5% vs. 16.5%; Pearson chi^2=17.682, p-value<0.001) and gentamicin (43.8% vs. 15.8%; Pearson chi^2=7.275, p-value<0.001). ESBL production was found also to be significantly higher among ST131 than non-ST131 strains (43.8% vs. 12.6%; Pearson chi^2=10.217, p-value=0.001). There were no statistical differences between ExPEC ST-131 infections compared to non-ST131 with regard to age, sex, residence, admission status (in-patients versus out-patients), and previous history of antibiotic use.

Discussion

Polymerase chain reaction–based molecular typing methods

In the present study, all global dominant clones were typed directly by the 7-SNP qPCR to their respective ST-*fimH* clones [12, 13, 30,31,32]. The most common clones in this Tanzanian ExPEC collection were CC10 (28.4%), the global clone ST131 (18.2%), and ST38

(10.2%). Similarly, *E. coli* ST131 has been previously reported in the global review [16]; in USA, 23 to 28% [30, 31]; in Canada, 46% [33]; in Nigeria, 23% [18], and in Tanzania (21 to 37.5%) [34, 35]. Interestingly, the 7-SNP qPCR correctly typed all the dominant ExPEC ST-*fimH* clones (i.e., ST131-*fimH30/41*, ST38-*fimH5*, ST69-*fimH27*, ST95-*fimH41*, and ST73-*fimH9/10*) within this Tanzanian ExPEC collection. In the current study, ST131 strains predominated in the two groups of patients among typeable strains (10.2% in pregnant women with UTI and 20.0% in children with BSIs) reiterating the predilection of this clone with urosepsis as previously reported in an extensive global review [36]. A recent global review and three recent studies in Africa have also reported the presence of *E. coli* CC10 in Tanzania (10 out of 32 strains), Mozambique (two out of 12 strains), and Nigeria (two out of 60 strains) [16, 18, 37, 38]. Similarly, ST131, ST10, and ST38 have recently been reported in Tanzania among *E. coli* fecal strains from health community members in Mwanza and also among patients in Kilimanjaro, connoting a potential niche and/or transmission beyond hospital settings [37, 39]. The findings call for the strengthening of hospital-based and community-based surveillance systems in Tanzania.

The multiplex PCR was able to type all ExPEC ST131 strains into their respective clades (A, B, C1, and C2), and also linked clade C1 and clade C2 strains with FQ-R and ESBL production, respectively, as previously described from global and Japanese ExPEC isolates [19], and in our recent paper from a non-biased Nigerian ExPEC collection [18]. Therefore, the combination of the 7-SNP qPCR and the ST131 clade multiple PCR provides an alternative feasible molecular typing platform for routine characterization of the *E. coli* ST131 global clone in low- and middle-income countries (LMICs) like Tanzania.

Heterogeneity of non-typeable *E. coli* strains

The non-typeability of nearly one-third of the strains connotes heterogeneity in the ExPEC strains and has been previously shown to be remarkable on PFGE analysis (https://prism.ucalgary.ca/handle/1880/108935). This heterogeneity may connote the fact that bacteria causing community-associated infections (like the majority of pregnant women with UTI in our study) usually display non-clonal occurrence and spread, whereas strains involved in hospital-associated infections usually display clonality.

Risk factors for *E. coli* ST131 attributable infections

The two dominant clones (ST131 and CC10) were found to circulate among patients in all health care facilities, but the majority of these cases occurred at BMC, among patients with UTIs and BSIs. The reason for this may be related to a larger number of patients with underlying clinical conditions requiring tertiary health care to this hospital.

In this study, the odds of ESBL production among ExPEC strains from children less than 5 years of age with BSI was 8.6 times than that in pregnant women with UTI. This may be accounted for by the preponderance of hospital-associated BSI in the formed group compared to community-associated UTI in the latter group. Over three-quarters of ESBL-producing ExPEC in this study had the *bla*$_{CTX-M-15}$ gene, similar to previous reports in Tanzania [5, 7], East African hospitals [35], Mozambique [38], and South Africa [40]. In these studies, the majority of strains had also ciprofloxacin co-resistance. Strikingly, ExPEC ST131 with the *bla*$_{CTX-M-14}$ and *bla*$_{CTX-M-27}$ genes previously reported in Japan among patients in acute care hospitals over the period of 10 years [41] and the *bla*$_{CTX-M-14}$ reported in South Africa [40] were also reported in the current study. But in contrast to these findings in Tanzania, Japan,

and South African, *bla*$_{CTX-M-37}$ was reported in one out of 12 ExPEC strains typed in a study in Mozambique [38]. The current study has shown that ST131 strains exhibited higher resistance to gentamicin and ciprofloxacin than non-ST131 strains. The reason for ExPEC ST131's success remains a matter of further studies, but sufficing to say that it may be related to the sequential acquisition of several virulence determinants over the past two decades, including AMR genes. Similar to previous reports, our study showed that ExPEC ST131 clade C predominantly displays FQ-R and ESBL production, as opposed to its preceding clades (A and B), which most often remain susceptible to fluoroquinolones and cephalosporins [14].

Our study had some limitations. Firstly, the molecular analysis of *E. coli* strains was based on the 7-SNP qPCR and additional gene sequencing. These methods target the pre-determined gene locus/loci for the ExPEC strains, and therefore could not ascertain the presence of novel ExPEC clones. Secondly, the existence of multiple ESBL alleles/genes in one ExPEC strain was not assessed based on the sequential nature of molecular assays, i.e., only strains that were negative for the *bla*$_{CTX-M-15}$ gene were sequenced to assess the presence of other *bla*$_{CTX-M}$ alleles.

In conclusion, approximately 60.8% of ExPEC strains were typeable by the 7-SNP qPCR and an additional one or two genes sequencing, with the predominance of CC10, ST131-*fimH30/41*, and ST38-*fimH5*. These dominant clones were found in all four levels of health care facilities in Northwestern Tanzania. All the dominant ExPEC ST-*fimH* clones (i.e., ST131-*fimH30/41*, ST38-*fimH5*, ST69-*fimH27*, ST95-*fimH41*, and ST73-*fimH9/10*) were precisely typed by the 7-SNP qPCR. The multiplex PCR associated the commonest ST131 clades C1 and C2 with FQ-R and ESBL production, respectively. These feasible and user-friendly molecular tools can be used routinely for AMR surveillance programs in resource-limited settings.

Data availability

The dataset used and/or analyzed in this study are available from the corresponding author on reasonable request.

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Code availability

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Contributions

JS, JDDP, SEM, and RD conceived and designed the study; JDDP, SEM, and RD supervised the execution of the study; JS collected data and samples; JS and JP analyzed samples and data. JS wrote the initial draft of the manuscript which was critically reviewed by all authors. All authors have read and approved the final version of the manuscript.

Ethics declarations

Ethics approval and consent to participate

This study was approved by the joint CUHAS/BMC Research and Ethics Committee (CREC 123/2016) in Tanzania. Permission to conduct the study in various health care facilities was sought and provided by relevant authorities in Tanzania. All patient information were kept confidential and results on antimicrobial susceptibility testing (AST) were timely reported to the attending doctors for specific management based on the respective health care facility treatment guidelines. Voluntary written informed consent to participate was obtained from every pregnant woman (or from children's parent/guardian) prior to being involved in the study.

Consent for publication

All pregnant women (or children's parent/guardian) gave voluntary consent to publish study findings anonymously.

Conflict of interest

The authors declare no competing interests.

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