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Multiple ST clonal complexes, with a predominance of ST131, of *Escherichia coli* harbouring $bla_{CTX\text{-}M\text{-}15}$ in a tertiary hospital in Tanzania

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Abstract

The molecular epidemiology of 32 non-duplicate, CTX-M-15 extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* strains, isolated from clinical samples, was investigated. Multilocus sequence typing revealed multiple sequence type clonal complexes: ST131 (12), ST405 (4), ST638 (3), ST38 (2), ST827 (2), ST224 (1), ST648 (1), ST46 (1) and two new sequence type clonal complexes (1845 and 1848) in 22 pulsed field gel electrophoresis clusters. The $bla_{CTX\text{-}M\text{-}15}$ gene was located on conjugative IncF plasmids. This is the first report of the worldwide emerging clonal complex ST131 linked to $bla_{CTX\text{-}M\text{-}15}$ in Tanzania and demonstrates the need for constant surveillance in developing countries to prevent the spread of these multiresistant isolates.

Keywords: $bla_{CTX\text{-}M\text{-}15}$, *Escherichia coli*, ST131, Tanzania

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Extended-spectrum beta-lactamases (ESBLs) are the predominant resistance enzymes among enterobacteriaceae and most of the beta-lactamases described to date are plasmid-encoded enzymes [1,2]. CTX-M is a recently described family of ESBLs. These enzymes hydrolyze cefotaxime more effectively than ceftazidime [3] and are also able to hydrolyze cefepime with high efficiency. The $bla_{CTX\text{-}M\text{-}15}$ allele is considered to be the predominant allele worldwide [4,5]. Extensive studies investigating the association of the multilocus sequence typing (MLST) clonal complex ST131 and $bla_{CTX\text{-}M\text{-}15}$ have been reported from Canada, India, Kuwait, France, Switzerland, Portugal, Spain, Korea and Japan; worldwide dissemination of $bla_{CTX\text{-}M\text{-}15}$ seems to be linked to this clonal complex, which is situated in the phylogenetic group B2 [4,5].

Two studies revealed that CTX-M enzymes were present among *Enterobacteriaceae* in a few isolates in Tanzania [6,7]. However, no study has investigated molecular characteristics of *Escherichia coli* ESBL-producing isolates in detail from this region of the world. For the first time we report the existence of the ST131 and multiple other sequence type (ST) clones carrying $bla_{CTX\text{-}M\text{-}15}$ in a single tertiary hospital in Tanzania, East Africa.

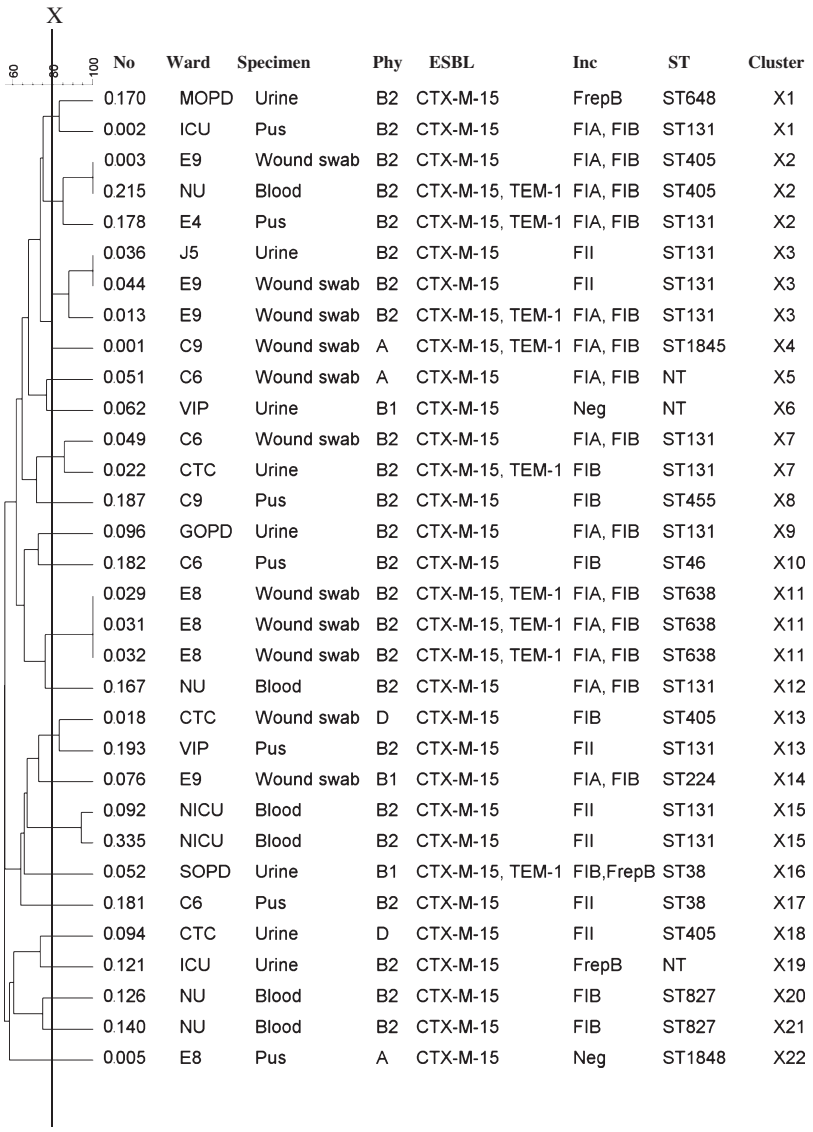
A total of 32 consecutive antimicrobial-resistant *Escherichia coli* clinical isolates were recovered from various wards and clinics in a tertiary hospital in Tanzania. Of these, 27 were from inpatients who had been on the ward for more than 72 h when the specimens were collected, whereas five strains were obtained from outpatients. Strains were identified as *E. coli* using in-house biochemical profiles [8]. Susceptibility patterns to ampicillin, amoxicillin/clavulanic acid, sulphamethaxazole/trimethoprim, tetracycline, gentamicin, ciprofloxacin, ceftriaxone, cefotaxime, cefepime and meropenem were determined by disk diffusion [9]; a disk synergy test was performed to detect ESBL production [8]. *Escherichia coli* ATCC 25922 was used as the quality control strain.

PCR amplification of *bla* genes (bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$) was performed using primers and methods described previously [10]. All PCR products were sequenced (LGC genomics GmbH, Berlin, Germany) and the resulting sequences were compared with known sequences using DNASTAR software (DNASTAR Inc, Madison, WI, USA) and the Basic Local Alignment Search Tool (BLAST, National Center for Biotechnology Information, Bethesda, MA, USA).

To determine the location of ESBL genes, conjugation experiments were carried out as described previously [11] with 15 isolates as donors and *Escherichia coli* CC118 (Rif^R, Str^R, Lac-, plasmid-free) as a recipient strain. In all transconjugants, plasmids were sized using S1-nuclease digestion as described previously [11,12] followed by Southern blotting and hybridization using digoxygenin (DIG)-labelled $bla_{CTX\text{-}M\text{-}15}$ amplicon probes (DIG High Prime DNA labelling and Detection Starter Kit II, Roche, Mannheim, Germany). PCR-based replicon typing was performed with all isolates and transconjugants using primers for the FIA, FIB, FII, FrepB, I1 and N replicons [13].

PFGE analysis was performed as previously described using *Xba*I (New England Biolabs, Ipswich, MA, USA) [14]. Multilocus sequence typing was carried out as recommended (http://mlst.ucc.ie/mlst/dbs/Ecoli). The phylogenetic groups of these isolates were determined by a previously described PCR-based method [15] and serotyping was performed on ST 131 strains.

Overall, 24.2% of all *Escherichia coli* isolates from this hospital were found to be ESBL producers [8]. In this study all



No	Ward	Specimen	Phy	ESBL	Inc	ST	Cluster
0.170	MOPD	Urine	B2	CTX-M-15	FrepB	ST648	X1
0.002	ICU	Pus	B2	CTX-M-15	FIA, FIB	ST131	X1
0.003	E9	Wound swab	B2	CTX-M-15	FIA, FIB	ST405	X2
0.215	NU	Blood	B2	CTX-M-15, TEM-1	FIA, FIB	ST405	X2
0.178	E4	Pus	B2	CTX-M-15, TEM-1	FIA, FIB	ST131	X2
0.036	J5	Urine	B2	CTX-M-15	FII	ST131	X3
0.044	E9	Wound swab	B2	CTX-M-15	FII	ST131	X3
0.013	E9	Wound swab	B2	CTX-M-15, TEM-1	FIA, FIB	ST131	X3
0.001	C9	Wound swab	A	CTX-M-15, TEM-1	FIA, FIB	ST1845	X4
0.051	C6	Wound swab	A	CTX-M-15	FIA, FIB	NT	X5
0.062	VIP	Urine	B1	CTX-M-15	Neg	NT	X6
0.049	C6	Wound swab	B2	CTX-M-15	FIA, FIB	ST131	X7
0.022	CTC	Urine	B2	CTX-M-15, TEM-1	FIB	ST131	X7
0.187	C9	Pus	B2	CTX-M-15	FIB	ST455	X8
0.096	GOPD	Urine	B2	CTX-M-15	FIA, FIB	ST131	X9
0.182	C6	Pus	B2	CTX-M-15	FIB	ST46	X10
0.029	E8	Wound swab	B2	CTX-M-15, TEM-1	FIA, FIB	ST638	X11
0.031	E8	Wound swab	B2	CTX-M-15, TEM-1	FIA, FIB	ST638	X11
0.032	E8	Wound swab	B2	CTX-M-15, TEM-1	FIA, FIB	ST638	X11
0.167	NU	Blood	B2	CTX-M-15	FIA, FIB	ST131	X12
0.018	CTC	Wound swab	D	CTX-M-15	FIB	ST405	X13
0.193	VIP	Pus	B2	CTX-M-15	FII	ST131	X13
0.076	E9	Wound swab	B1	CTX-M-15	FIA, FIB	ST224	X14
0.092	NICU	Blood	B2	CTX-M-15	FII	ST131	X15
0.335	NICU	Blood	B2	CTX-M-15	FII	ST131	X15
0.052	SOPD	Urine	B1	CTX-M-15, TEM-1	FIB,FrepB	ST38	X16
0.181	C6	Pus	B2	CTX-M-15	FII	ST38	X17
0.094	CTC	Urine	D	CTX-M-15	FII	ST405	X18
0.121	ICU	Urine	B2	CTX-M-15	FrepB	NT	X19
0.126	NU	Blood	B2	CTX-M-15	FIB	ST827	X20
0.140	NU	Blood	B2	CTX-M-15	FIB	ST827	X21
0.005	E8	Pus	A	CTX-M-15	Neg	ST1848	X22

FIG. 1. Pulsed field gel electrophoresis (PFGE) dendogram of CTX-M-15 producing *E. coli.* Heterogeneity of the 32 *E. coli* extended-spectrum beta-lactamase (ESBL) producers is seen on the dendogram. The diagram also shows the isolate number, wards, specimen, ESBL allele, incompatibility groups, sequence type, clonal complex and the PFGE cluster. The solid line X indicates a similarity level of 0.8, revealing 22 clusters (X1–X22). MOPD, medical outpatient department; NU, neonatal unit; CTC, care and treatment clinic; GOPD, gynaecological outpatient department; NICU, neonatal intensive care unit; (E4,E8,C9,E9,C6, J5), surgical wards; VIP, first class ward. Analysis was performed using GelCompar 11 software (Applied Maths, Gent, Belgium).

TABLE 1. Characteristics of *Escherichia coli* selected as donors from different pulsed field gel electrophoresis clusters

Serial no.	Isolate no.	Incompatability group	Sequence type	Conjugation frequency	Calculated plasmid size	Transferable resistance
1	02	FIA,FIB	ST131	10^{-7}	291 kb	GM
2	18	FIB	ST405	$2.7*10^{-4}$	50 kb	GM,SXT,TET
3	22	FIB	ST131	$2.8*10^{-4}$	291 kb	GM,SXT,TET
4	32	FIA,FIB	ST638	$2.2*10^{-4}$	50 kb	GM,SXT,TET
5	76	FIA,FIB	ST224	10^{-4}	194 kb	GM,SXT
6	140	FIB	ST827	10^{-4}	242 kb	SXT,TET
7	170	FrepB	ST648	10^{-5}	97 kb	GM
8	187	FIA,FIB	ST405	10^{-3}	200 kb	GM,SXT
9	178	FIB	ST131	10^{-4}	242 kb	Beta-lactams
10	181	FIB	ST38	10^{-6}	291 kb	GM,SXT,TET
11	51	FIA,FIB	NT	10^{-5}	291 kb	GM,SXT,TET
12	215	FIA,FIB	ST405	10^{-7}	145 kb	GM,SXT,TET
13	096	FIA,FIB	ST131	$4*10^{-4}$	242 kb	Beta-lactams
14	182	FIB	ST46	10^{-4}	194 kb	GM,SXT
15	092	FII	ST131	10^{-5}	145 kb	GM,SXT,TET

GM, gentamicin; SXT, sulphamethaxazole/trimethoprim; TET, tetracycline.

32 isolates were found to be resistant to cefotaxime (MIC > 30 mg/L) and showed the classic ESBL phenomenon on disk synergy test. The rate of resistance to non-beta-lactam antibiotics tested was 100% to tetracycline, 93% to sulphamethaxazole/trimethoprim and 84% resistance to gentamicin and ciprofloxacin. This poses a serious challenge for the treatment of such infections due to the limited choice of antibiotics in developing countries. All isolates were found to carry $bla_{CTX\text{-}M\text{-}15}$, while eight isolates (25%) also carried $bla_{TEM\text{-}1}$. In all isolates tested, $bla_{CTX\text{-}M\text{-}15}$ was linked to an ISEcp1 element. [16]. As described in other studies that involve clinical isolates, 24 (75%) of the isolates were assigned to the phylogenetic group B2 [5]. As demonstrated in previous studies [4,5,11], multiple clones were seen on PFGE and using a similarity level (SAB) of 0.8, 22 clusters (X1–X22) could be created among 32 isolates (Fig. 1). With a similarity index of >0.99, three small outbreaks were observed (clusters X2, X3 and X11), occurring on paediatric wards and a surgical ward. MLST revealed an atypical high diversity of ST carrying $bla_{CTX\text{-}M\text{-}15}$ in this single hospital in Tanzania, which contrasts with findings from previous reports [17–19]. Many of the *Escherichia coli* carrying $bla_{CTX\text{-}M\text{-}15}$ from different countries in Europe and North America are homogenously grouped into the *E. coli* O25:H4-ST131 [4,5,18], which was also the commonest group in this study (40%). Most of the STs (ST131, ST405, ST638, ST648, ST827 and ST224) in our study have been reported to carry $bla_{CTX\text{-}M\text{-}15}$. ST1845, ST1848, ST46 and ST455 are reported here for the first time to be associated with $bla_{CTX\text{-}M\text{-}15}$. ST 648 has been found to be common in birds and close proximity to birds can be a risk factor for infection [20]. Only two isolates were grouped in the phylogenetic group D and these were found to be associated with ST405, thus supporting previous observations [5].

PCR-based replicon typing revealed that FIA, FIB, FII and FrepB were present in 30 clinical isolates and transconjugants in various combinations. The commonest combination was FIA- FIB, which was demonstrated in 14 (47%) cases. IncFII was found in eight (26%) cases. The $bla_{CTX\text{-}M\text{-}15}$ gene in this study is carried on multiple, conjugative IncF plasmids, with estimated plasmid sizes ranging from 50kb to 291kb (Table 1). The commonest plasmid was 291 kb in size and was found in six (40%) transconjugants. We demonstrated the presence of multiple conjugative IncF plasmids carrying this allele, with conjugation frequencies ranging from 10^{-3} to 10^{-7} per donor cell (Table 1). The majority of isolates displayed multidrug resistance, being also resistant to gentamicin, tetracycline, ciprofloxacin and sulphamethaxazole/trimethoprim, whereby the gentamicin resistance was transferrable in most cases. These results provide first evidence for the existence of the ST131 strains harbouring the CTX-M-15 allele in East Africa, and indicate a need for constant surveillance to study the epidemiology and diversity of these ESBL-producing *Escherichia coli* isolates in developing countries, as the scope of the problem seems to be wider than previously suspected.

Authors' Contributions

SEM, EFL, CI, ED and TC designed the study; SEM and CI performed the experiments; SEM, CI and TC analyzed the data; TH analyzed sequences; and SEM, CI and TC wrote the manuscript, which was corrected and approved by all the other coauthors.

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Transparency Declaration

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