Epidemiology and molecular characterization of multidrug-resistant *Escherichia coli* isolates harboring bla<sub>CTX-M</sub> group 1 extended-spectrum β-lactamases causing bacteremia and urinary tract infection in Manhiça, Mozambique

**Background:** The emergence and spread of extended-spectrum β-lactamases (ESBLs), especially CTX-M, is an important public health problem with serious implications for low-income countries where second-line treatment is often unavailable. Knowledge of the local prevalence of ESBL is critical to define appropriate empirical therapeutic strategies for multidrug-resistant (MDR) organisms. This study aimed to assess and characterize the presence of ESBL and especially CTX-M-producing *Escherichia coli* MDR isolates from patients with urinary tract infections (UTIs) and bacteremia in a rural hospital in Mozambique.

**Materials and methods:** One hundred and fifty-one E. coli isolates from bacteremia and UTI in children were screened for CTX-M, TEM, SHV and OXA β-lactamases by polymerase chain reaction and sequencing. Isolates carrying CTX-M group 1 β-lactamases were further studied. The resistance to other antibiotic families was determined by phenotypic and genotypic methods, the location of the bla<sub>CTX-M</sub> gene and the epidemiology of the isolates were studied, and extensive plasmid characterization was performed.

**Results:** Approximately 11% (17/151) of *E. coli* isolates causing bacteremia and UTI were ESBL producers. CTX-M-15 was the most frequently detected ESBL, accounting for 75% of the total isolates characterized. The bla<sub>CTX-M</sub> gene is located in different plasmids belonging to different incompatibility groups and can be found in non-epidemiologically related isolates, indicating the high capacity of this resistance determinant to spread widely.

**Conclusion:** Our data suggest the presence of a co-selection of third-generation cephalosporin-resistant determinants in the study area despite limited access to these antibiotics. This highlights the importance of continuous surveillance of antimicrobial resistance of both genetic elements of resistance and resistant isolates in order to monitor the emergence and trends of ESBL-producing isolates to promote adequate therapeutic strategies for the management of MDR bacterial infections.

**Keywords:** CTX-M-15, multidrug-resistance, Enterobacteriaceae, resistance determinant location

**Introduction**

Infections caused by members of the Enterobacteriaceae family are among the major causes of hospital admission and associated morbidity and mortality in children,
particular in Africa. Infections caused by these microorganisms in low- and middle-income countries (LMIC) have been successfully treated with the inexpensive antibiotics available. Nevertheless, with the widespread development of multidrug-resistant (MDR) strains, the usefulness of the early effective antibiotics has greatly decreased, leading to the introduction of broad-spectrum antibiotics such as fluoroquinolones or third-generation cephalosporins (cefotaxime, ceftriaxone, or ceftazidime). Unfortunately, these agents are often unaffordable in most LMIC, especially in remote rural areas.

On the other hand, since their first description in 1983, extended-spectrum β-lactamases (ESBLs) produced by enteric pathogens have spread worldwide. The emergence and spread of ESBLs, especially those included in the CTX-M group, is an important public health problem. In fact, it has been considered that ESBL-carrying Enterobacteriaceae cause >1700 deaths yearly in the USA alone, and these pathogens have had a tremendous impact on the treatment of severe or MDR-associated infections, particularly in LMIC where second-line antibiotics are often unaffordable or unavaiable. In addition, few new antibiotics against Gram-negative bacteria have been marketed in the last decades which may favor the emergence of new resistances, further challenging the management of infectious diseases in this setting. This may play a role in the high morbidity and mortality observed in these countries, particularly in children <5 years of age.

Although different types of ESBLs have been reported among the Enterobacteriaceae family, CTX-M-15, a community-acquired ESBL that was originally described in India in the 1990s, is one of the most frequent type I CTX-M disseminated worldwide. The genes encoding ESBL enzymes are usually located in plasmids but can also be found in the chromosomal DNA as described elsewhere. It has been reported that the blaCTX-M-15 gene is usually found downstream from the insertion sequence ISεcp1 that may be involved in their dissemination and expression. Plasmid-mediated ESBL genes are of special interest due to their capability of getting transferred between strains or even species, favoring their dissemination among the bacterial population and from region to region. Moreover, these plasmids usually carry other antibiotic resistance determinants, resulting not only in the spread of ESBL but also in the dissemination of other resistance genes. The selection of one resistance gene due to environmental pressure harbored in the same genetic element as another resistance gene or genes is known as the co-selection of resistance genes phenomenon. Since ESBL-producing microorganisms are also often resistant to other commonly available antibiotics, including fluoroquinolones, especially in most LMIC, knowledge of their prevalence and characterization is important for defining local empirical stewardship programs for infections caused by MDR organisms.

In Africa, ESBLs have increasingly been reported. In Mozambique, the prevalence of these pathogens is extremely high, although the data available are limited to only a few studies. Herein, we report the prevalence of Escherichia coli harboring the ESBL gene blaCTX-M group I as well as its molecular characterization and epidemiology among isolates recovered from blood cultures and urine in a rural hospital in Southern Mozambique.

Materials and methods
Study population and clinical isolates
The study was conducted by the Centro de Investigação em Saúde de Manhiça (CISM) at the Manhiça District Hospital, a rural referral hospital of the Manhiça district, located 80 km north of Maputo, in Southern Mozambique. Invasive bacterial disease surveillance has been conducted in the pediatric population in this area since 1997. The full description and characteristics of the study area are detailed elsewhere. As described previously in standard clinical protocols, blood cultures are systematically collected upon admission of all children up to 14 years of age with an axillary temperature ≥37.5°C or meeting criteria of severe infection. We analyzed E. coli isolates recovered from children with community-acquired bacteremia between August 2004 and December 2009. Urine samples were also collected during the same study period from patients (adults and children) visited at the outpatient department or admitted to the hospital with clinical suspicion of urinary tract infection (UTI). All the isolates included in the present study were recovered from different patients.

Bacterial culture and identification
Blood culture tubes were incubated in an automated system (BACTEC® 9050; Becton Dickinson, Franklin Lakes, NJ, USA). Positive blood cultures were subcultured in solid media after Gram staining as appropriate. Urine samples were microscopically screened after centrifugation, and those with pathologic sediment (presence of leukocytes or bacteria) were cultured in MacConkey and blood agar media. Pathogens were identified according to conventional microbiology protocols. Among the Enterobacteriaceae isolates identified, ceftriaxone susceptibility was tested by disk diffusion in Mueller–Hinton agar (Oxoid®, Basingstoke, Hampshire, UK).
according to the Clinical and Laboratory Standard Institute (CLSI) 2013 guidelines.\textsuperscript{18} The selected ceftriaxone non-susceptible \textit{E. coli} isolates were screened for the ESBL enzyme CTX-M group 1 and the positive isolates were included in the study. The isolates were confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) prior to further analysis.\textsuperscript{19}

\textbf{Antimicrobial susceptibility testing}

The susceptibility phenotype for ampicillin, chloramphenicol, ceftriaxone, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, rifampicin, and amikacin was determined by a conventional disk diffusion method and for nalidixic acid and ciprofloxacin by minimum inhibitory concentration (MIC). The interpretative category of resistance for disk diffusion and MIC were done according to the CLSI 2013 guidelines.\textsuperscript{18} Antimicrobial susceptibility testing of the isolates was also performed by Siemens MicroScan panels NEG MIC TYPE 37. The \textit{E. coli} American Type Culture Collection 25922 strain was used as the quality control. Multidrug resistance was defined as resistance to 3 or more unrelated antibiotic families.\textsuperscript{20} Resistance genes to quinolones and resistance was defined as resistance to 3 or more unrelated antibiotic families.\textsuperscript{20} Resistance genes to quinolones and resistance was defined as resistance to 3 or more unrelated antibiotic families.\textsuperscript{20} Resistance genes to quinolones and resistance was defined as resistance to 3 or more unrelated antibiotic families.\textsuperscript{20} Resistance genes to quinolones and resistance was defined as resistance to 3 or more unrelated antibiotic families.\textsuperscript{20} Resistance genes to quinolones and resistance was defined as resistance to 3 or more unrelated antibiotic families.\textsuperscript{20} Resistance genes to quinolones and resistance was defined as resistance to 3 or more unrelated antibiotic families.\textsuperscript{20}

\textbf{ESBL phenotype detection}

All ceftriaxone non-susceptible \textit{E. coli} isolates were phenotypically screened for the presence of ESBL. Phenotypic confirmation of ESBL expression was carried out using the ESBL disk synergy test with disks containing cefotaxime, amoxicillin with clavulanate, and ceftazidime on Mueller–Hinton agar (Oxoid) as described elsewhere.\textsuperscript{30} The presence of \textit{bla} \textit{CTX-M} and \textit{bla} \textit{SHV}, \textit{bla} \textit{TEM}, \textit{bla} \textit{OXA-1-like}, \textit{bla} \textit{OXA-2-like}, \textit{bla} \textit{OXA-5-like}, and \textit{rpoB} already described.\textsuperscript{21-29}

\textbf{β-lactamases analysis}

The presence of \textit{bla} \textit{CTX-M} was detected by PCR using universal primers, while the \textit{bla} \textit{CTX-M} groups 1, 2, 8, 9, and \textit{bla} \textit{CTX-M-15} were determined using specific CTX-M group primers and subsequently sequenced.\textsuperscript{31} Moreover, the presence of \textit{bla} \textit{SHV}, \textit{bla} \textit{TEM}, \textit{bla} \textit{OXA-1-like}, \textit{bla} \textit{OXA-2-like}, and \textit{bla} \textit{OXA-5-like} genes was also determined by PCR and sequencing as described elsewhere.\textsuperscript{32} The presence of the insertion sequence IS\textit{Ecp1} upstream from the \textit{bla} \textit{CTX-M} genes was determined by PCR and sequencing as previously described.\textsuperscript{11} Sequencing of the genes was performed by the Macrogen\textsuperscript{®} DNA Sequencing Service (Macrogen, Amsterdam, the Netherlands) using sets of consecutive primers specific for each gene type.

\textbf{Class I integron analysis}

The presence of class I integrons was analyzed in all the \textit{E. coli} isolates. A PCR was carried out with primers 3\textsuperscript{′}CS and 5\textsuperscript{′}CS as described by Lévesque et al\textsuperscript{31} and the amplicons obtained were sequenced by Beckman Coulter Sequencing Genomics\textsuperscript{®} sequencing facilities (Takeley, UK).

\textbf{Typing}

Pulsed-field gel electrophoresis (PFGE) was performed with the \textit{XbaI} restriction enzyme (New England Biolabs, Beverly, MA, USA) as described previously.\textsuperscript{34} PFGE profiles were analyzed with InfoQuest FP software version 4.5 (Bio-Rad Laboratories Inc., Hercules, CA, USA). In order to establish the epidemiological relationship among the isolates from the electrophoretic patterns, the Dice coefficient was used and clustering was based on the unweighted pair group method with arithmetic mean with a 1% tolerance in band position differences. The isolates were considered to belong to the same epidemiological group when the PFGE-\textit{XbaI} profiles showed \(\geq 80\%\) of homology, adapting the criteria described by Tenover et al.\textsuperscript{35} Multi-locus sequence typing (MLST) was carried out by amplification and sequencing of the 7 \textit{E. coli} housekeeping genes as described previously.\textsuperscript{18} The database available at http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/ was used for assigning sequence types (STs) and clonal complexes (CCs). Classification of isolates into \textit{E. coli} phylogenetic groups was done using a previously described triplex PCR-based protocol.\textsuperscript{37}

\textbf{Plasmid transferability analysis}

Conjugation assays were carried out with all the isolates in order to determine if the \textit{bla} \textit{CTX-M} group 1 gene was located in a conjugative plasmid adapting the protocol described elsewhere.\textsuperscript{34} The \textit{E. coli} K7 759 lac- kanamycin-resistant isolate was used as the recipient strain. Both parental and recipient strains were cultured over-night (ON) with Luria Bertani (LB) broth (Laboratorios Conda, Barcelona, Spain). The parental isolates were grown in LB medium supplemented with 32 µg/mL of cefotaxime in order to force resistance determinant replication. An aliquot of 500 \textmu L of a parental isolate subcultured for 2 hours was mixed with the same volume of the recipient strain and cultured ON at 37°C. Transconjugant strains were finally grown in MacConkey agar plates supplemented with 256 µg/mL of kanamycin and 32 µg/mL of cefotaxime. Repetitive extragenic palindromic
PCRs and a \( \text{bla}_{\text{CTX-M}} \) group I PCR were performed to ensure the correct selection of the transconjugants.\(^{39} \)

**The \( \text{bla}_{\text{CTX-M}} \) gene location**

Considering the previously described large size of the plasmids carrying \( \text{bla}_{\text{CTX-M}} \) group 1 ESBL genes,\(^{40} \) a S1 nuclease (Promega, Madison, WI, USA) digestion followed by PFGE analysis were performed in the 12 \( E. \text{coli} \) isolates and the transconjugants obtained as described elsewhere.\(^{41} \) To determine the plasmid or chromosomal location of the ESBL-encoding gene, a Southern blot of the PFGE gel followed by hybridization with a \( \text{bla}_{\text{CTX-M}} \) group 1 probe was carried out.

**PCR-based Replicon Typing (PBRT)**

Plasmids from both parental and transconjugant isolates were assigned to incompatibility groups depending on the presence of specific replicon sequences identified by PCR using the primers designed by Carattoli et al in 2005 but employing the adapted amplification protocols for commensal and pathogenic \( E. \text{coli} \) isolates described by Johnson et al.\(^{42} \)

**Ethical clearance**

The strains characterized here were isolated from the ongoing invasive bacterial surveillance system that included several research protocols reviewed and approved by the Mozambican National Bioethics Committee for Health (IR00002657) and by Institutional Review Boards of Hospital Clinic of Barcelona, Spain; the US Centers for Disease Control and Prevention; and the School of Medicine, University of Maryland. Written informed consent was obtained from parents or caretakers of the eligible children.

**Results**

**Study population and clinical isolates**

During the study period, a total of 15,057 blood cultures were collected and 1325 (8.8%) were found to be positive for any pathogen evaluable. Of these, 27.7% were identified as belonging to the Enterobacteriaceae family, with \( E. \text{coli} \) being the second most frequent after non-typhoidal \( \text{Salmonella} \), accounting for 29% (106/368). Among these, 8 out of 12 \( E. \text{coli} \) isolates non-susceptible to ceftriaxone and positive for the ESBL disk synergy test (11.3% of total \( E. \text{coli} \) isolates) were found to carry a \( \text{bla}_{\text{CTX-M}} \) group I gene.

Of the 298 urine samples cultured for bacterial isolation, 35% (n=103) were positive for pathogenic bacteria. Among these, 81 (78.6%) corresponded to Enterobacteriaceae with \( E. \text{coli} \) being the most prevalent species with 45 isolates of which 5 (11.1%) were non-susceptible to ceftriaxone and positive for the ESBL double-disk synergy test. Four were found to carry a \( \text{bla}_{\text{CTX-M}} \) group I gene.

A total of 12 \( E. \text{coli} \) isolates from bacteremia and UTI carrying a \( \text{bla}_{\text{CTX-M}} \) group I gene were selected for further characterization.

**\( \beta \)-lactamases analysis**

Gene amplification sequencing revealed that 92% (n=11) of the isolates harbored \( \text{bla}_{\text{CTX-M-15}} \), while the remaining strain presented the ESBL gene \( \text{bla}_{\text{CTX-M-37}} \). The non-ESBL resistance genes also detected, \( \text{bla}_{\text{TEM-1}} \) and \( \text{bla}_{\text{OXA-1}} \) were found in 100% and 58.3% of the isolates, respectively. Another ESBL-encoding gene detected was \( \text{bla}_{\text{SHV-12}} \) which was found in 2 of the isolates also presenting \( \text{bla}_{\text{CTX-M-15}} \). In all cases, the insertion sequence ISEcpI was found upstream from the \( \text{bla}_{\text{CTX-M}} \) group 1 gene. The overall results are summarized in Table 1.

**Antimicrobial susceptibility testing**

All the isolates were MDR, presenting not only resistance to third-generation \( \beta \)-lactams (ceftriaxone) but also to other classes of antimicrobial agents. All the isolates were resistant to rifampicin, gentamicin, chloramphenicol, and trimethoprim-sulfamethoxazole, while 66.7% were resistant to quinolones (Table 1). Regarding the resistance genotype of the \( E. \text{coli} \) isolates to quinolones, among the isolates with a MIC=1 µg/mL of ciprofloxacin, 4 showed the presence of the \( qnrB \) gene without mutations in the \( \text{gyrA} \) and \( \text{parC} \) genes; 1 isolate showed only a mutation in amino acid codon Ser83 of \( \text{gyrA} \) and 1 did not show any of the resistance determinants studied. The isolate with a MIC=64 µg/mL of ciprofloxacin showed 3 mutations (2 in \( \text{gyrA} \) and 1 in \( \text{parC} \)) and the other isolate with a MIC >256 µg/mL has the same mutations plus the presence of the \( qnrB \) gene (Table 2).

The resistance genotype to rifampicin was not well elucidated as \( \text{arr} \) genes were not detected and any significant mutations in \( \text{rpoB} \) gene were observed in any isolate.

All the isolates were susceptible to fosfomycin, nitrofurantoin, and carbapenems.

**Class 1 integron analysis**

Seven isolates were found to carry class 1 integrons. Two isolates harbored an integron of ~1000 bp carrying the resistance gene \( \text{aadA1} \), conferring resistance to streptomycin and spectinomycin. One isolate had a 2000 bp class 1 integron carrying 2 resistance genes: \( \text{dfrA12} \) and \( \text{aadA2} \) that confer resistance to trimethoprim and streptomycin-spectinomycin, respectively. Four isolates presented 2 integrons of ~800 and...
Characterization of multidrug-resistant E. coli harboring bla\textsubscript{CTX-M} group 1

Table 1

| Isolate | Source | Isolation date | ESBL enzymes | Other β-lactamases | Non-β-lactam resistance profile | Integron class I | Resistance genes in Integron class I |
|---------|--------|---------------|--------------|--------------------|------------------------------|-----------------|-------------------------------------|
| E12     | Blood  | 09/10/2008    | TEM-1        | CHL-GM-TET-SXT-RIF | −                            | −               | −                                   |
| E15     | Blood  | 15/04/2009    | TEM-1        | CHL-GM-TET-SXT-RIF | −                            | −               | −                                   |
| E11     | Blood  | 15/09/2008    | TEM-1/OXA-1  | CHL-GM-CIP-TET-SXT-RIF | 1000 bp | aadA1                             |
| E4      | Blood  | 04/05/2007    | TEM-1        | CHL-GM-NAL-TET-SXT-RIF | −                            | −               | −                                   |
| E3      | Blood  | 14/12/2006    | TEM-1        | CHL-GM-TET-SXT-RIF | −                            | −               | −                                   |
| E16     | Blood  | 29/03/2009    | TEM-1/OXA-1  | CHL-GM-NAL-CIP-TET-SXT-RIF | 1000 bp | aadA1                             |
| E18     | Blood  | 11/10/2008    | TEM-1        | CHL-GM-SXT-RIF     | −                            | −               | −                                   |
| E4      | Blood  | 04/05/2007    | TEM-1        | CHL-GM-NAL-TET-SXT-RIF | −                            | −               | −                                   |
| E3      | Blood  | 14/12/2006    | TEM-1        | CHL-GM-TET-SXT-RIF | −                            | −               | −                                   |

Abbreviations: CHL, chloramphenicol; CIP, ciprofloxacin; ESBL, extended-spectrum β-lactamases; GM, gentamicin; NAL, nalidixic acid; RIF, rifampicin; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.

Table 2

| Isolate | Minimum inhibitory concentration (µg/mL) | Resistance genes | Aminoacidic mutations |
|---------|-----------------------------------------|------------------|-----------------------|
|         | NAL | CIP | qnrA | qnrB | qnrC | qnrD | qnrS | qepA | aac (6)’ Ib-cr | gyrA | parC |
|         |     |     |     |     |     |     |     |     | 83 (wt Ser) | 87 (wt Ser) | 80 (wt Ser) | 84 (wt Glu) |
| E2      | >256 | R   | 64   | R    | −   | −   | −   | −   | −   | −   | −   | −   |
| E3      | 4    | S   | 0.03 | S    | −   | −   | −   | −   | −   | −   | −   | −   |
| E4      | 128  | R   | I    | I    | −   | −   | −   | −   | −   | −   | −   | −   |
| E7      | 16   | I   | I    | I    | −   | +   | −   | −   | −   | −   | −   | −   |
| E8      | 16   | I   | I    | I    | −   | −   | −   | −   | −   | −   | −   | −   |
| E11     | >256 | R   | >256 | R    | −   | +   | −   | −   | −   | −   | −   | −   |
| E12     | 1    | S   | 0.007| S    | −   | −   | −   | −   | −   | −   | −   | −   |
| E14     | 16   | I   | I    | I    | −   | +   | −   | −   | −   | −   | −   | −   |
| E15     | 4    | S   | 0.015| S    | −   | −   | −   | −   | −   | −   | −   | −   |
| E16     | 16   | I   | I    | I    | −   | +   | −   | −   | −   | −   | −   | −   |
| E17     | 16   | I   | I    | I    | −   | +   | −   | −   | −   | −   | −   | −   |
| E18     | 8    | S   | 0.5  | S    | −   | −   | −   | −   | −   | −   | −   | −   |

Abbreviations: CIP, ciprofloxacin; NAL, nalidixic acid; R, resistant; I, intermediate; S, susceptible; Leu, leucine; Ser, serine; Asn, asparagine; Asp, aspartic acid; Ile, isoleucine; Glu, glutamic acid.

Molecular typing

According to the PFGE analysis constructed from the electrophoresis patterns of the XbaI restriction and considering the same profile of ≥80% of similarity, there were 8 different epidemiological groups among the 12 isolates studied. The analysis showed 4 and 2 other isolates to be in the same epidemiological group, thereby being epidemiologically related isolates. This association, however, involved grouping isolates harboring different bla\textsubscript{CTX-M} group 1 genes. The MLST analysis also showed the same number of ST groups as epidemiologically unrelated isolates (singletons).

This data correlates 100% with the epidemiological grouping established by the PFGE analysis and with the genetic characterization of non-β-lactam resistance genes and the antimicrobial susceptibility profiles. Only 2 out of the 8 STs described belonged to the same CC (ST10). Four E. coli phylogenetic groups were represented in the collection of isolates (A, B1, B2, and D), with none having a statistically significant prevalence taking into account the epidemiological associations. All the isolates causing UTI belonged to phylogenetic group A (Table 3).

Plasmid characterization

Transconjugants were obtained from 10 parental isolates as shown in Table 4. S1 endonuclease digestion allowed visualizing the plasmid profile of each isolate. The parental isolates 1000 bp containing the dfrA16 and aadA1 genes, respectively, conferring the same resistances as those mentioned earlier (Table 1).
Table 3 Typing and epidemiological relationship between the Escherichia coli isolates

| Isolate | Phylogenetic group | Sequence type | Clonal complex | PFGE profile | Dendrogram |
|---------|--------------------|---------------|----------------|--------------|------------|
| E12     | B1                 | ST3           | ST3            |              |            |
| E15     | B1                 | ST3           | ST3            |              |            |
| E11     | D                  | ST405         | ST405          |              |            |
| E4      | D                  | ST38          | ST38           |              |            |
| E3      | B2                 | ST2451        | Singleton      |              |            |
| E16     | A                  | ST10          | ST10           |              |            |
| E18     | B1                 | ST453         | ST86           |              |            |
| E2      | A                  | ST617         | ST10           |              |            |
| E7      | A                  | ST216         | Singleton      |              |            |
| E14     | A                  | ST216         | Singleton      |              |            |
| E8      | A                  | ST216         | Singleton      |              |            |
| E17     | A                  | ST216         | Singleton      |              |            |

Abbreviations: PFGE, pulsed-field gel electrophoresis; ST, sequence type.

Table 4 Plasmid transferability assay and characterization

| Donor isolates | Transconjugants |
|----------------|-----------------|
| Isoate | No of plasmids | Plasmid replicon types | Transconjugant | Plasmid replicon types |
|---------|----------------|------------------------|----------------|------------------------|
| E12     | 3              | FIIA                   | E12T           | FIIA                   |
| E15     | 3              | FIIA                   | E15T           | FIIA                   |
| E11     | 3              | HI2/FIB                | E11T           | HI2                    |
| E4      | 2              | HI2                    | Not obtained   | –                      |
| E3      | 3              | FIB                    | E3T            | HI2                    |
| E16     | 1              | HI2                    | E16T           | HI2                    |
| E18     | 1              | FIB                    | E18T           | None                   |
| E2      | 1              | FIIA                   | Not obtained   | –                      |
| E7      | 2              | HI2                    | E7T            | HI2                    |
| E14     | 2              | HI2                    | E14T           | HI2                    |
| E8      | 2              | HI2                    | E8T            | None                   |
| E17     | 2              | HI2                    | E17T           | HI2                    |

The plasmid incompatibility groups amplified in the PBRT analysis of the parental isolates were IncFIIA, IncFIA, IncHI2, and IncFIB, but only the incompatibility groups IncFIIA and IncHI2 were found in the plasmids carrying the bla<sub>CTX-M</sub> group 1 gene (such as those amplified in the transconjugants; Table 4).

Discussion

This is one of the few studies on the prevalence of CTX-M group 1 ESBLs in E. coli causing both bacteremia and UTIs among children in Mozambique. The prevalence of ESBLs reported here is a matter of concern as MDR pathogens causing infectious diseases are common in this area, limiting the therapeutic options for treating severe infections often associated with a poor outcome. The rates of ESBLs and other antibiotic resistances observed in this study may be associated with the high prevalences of other infectious diseases, such as tuberculosis, respiratory infections, malaria, and human immunodeficiency virus, which requires the frequent use of antibacterial agents. Despite the high prevalence of ESBLs reported in this study, it is lower compared with the prevalence of those causing UTIs in children admitted to the malnutrition and pediatric wards described in the central region of the country (Beira City).

Regarding the resistance mechanisms to non-β-lactamic antibiotic families found among the E. coli isolates studied, the resistance determinants to quinolones correspond to those described so far, whereas the lack of detection of resistance determinants to rifampicin suggests other mechanisms of resistance, such as the effect of efflux pumps, as described elsewhere.
Figure 1 S1 endonuclease pulsed-field gel electrophoresis (PFGE).
Abbreviation: MWM, molecular weight marker.

Figure 2 Hybridization of S1 endonuclease pulsed-field gel electrophoresis (PFGE) with bla<sub>CTX-M</sub> group 1 probe.
The most prevalent resistance mechanism to third-generation cephalosporins found in the collection of MDR isolates studied is the bla\textsubscript{CTX-M} gene belonging to a sublineage or group I (accounting for 70.6% of the ESBL-carrying isolates), which is consistent with other reports\textsuperscript{8} showing isolates harboring a bla\textsubscript{CTX-M} Group I gene with almost 92% being bla\textsubscript{CTX-M-15}. Within the CTX-M-1 group, bla\textsubscript{CTX-M-15} is the most frequently described resistance gene in isolates causing both community-acquired bacteremia and UTI.\textsuperscript{6,16} In fact, bla\textsubscript{CTX-M-15} is currently the most common variant detected worldwide in clinically important Gram-negative bacteria together with bla\textsubscript{CTX-M-14}.\textsuperscript{9} According to the current data and the widely reported bla\textsubscript{CTX-M-15} dissemination, this is not the first description of this gene in the area since it has previously been described in ESBL-carrying Klebsiella pneumoniae isolates.\textsuperscript{16}

Regarding the other bla\textsubscript{CTX-M} Group I gene detected, this is the first description of bla\textsubscript{CTX-M-37} in Mozambique. This infrequently detected CTX-M was first described in an Enterobacter cloacae isolated in Mongolia in 2002, and to our knowledge, it has only been previously reported in 1 other African country, the neighboring South Africa, as well as in the chromosome of an isolate of Kluivera cryocrescens from Argentina (GenBank access No: FN813246.1).\textsuperscript{48}

Whereas the mechanism of resistance to third-generation cephalosporins in the collection of isolates of our study was the same, the isolates showed low relatedness at an epidemiological level, being distributed in 4 phylogenetic groups and 8 epidemiological groups. Although the range of phylogenetic groups represented is wide, it is important to highlight that all the isolates from UTIs were phylogenetic group A. This phylogenetic group has been associated with MDR strains causing UTI.\textsuperscript{49} Based on PFGE and MLST results, 4 isolates within the same epidemiological group belonged to ST ST216. However, these isolates showed some divergent evolution concerning the resistant determinants, as one harbored CTX-M-37 (isolate E8) instead of CTX-M-15 and another (isolate E17) also showed SHV-12. Furthermore, isolates E12 and E15 belonged to the same epidemiological group although they were isolated at a different period. The non-related isolates belonged to different STs and even different CCs, indicating that it was not a clonal dissemination.

Regarding the plasmid analysis to determine the location of bla\textsubscript{CTX-M} Group 1, the collection presented a wide range of different plasmid incompatibility groups. Furthermore, 2 epidemiologically unrelated isolates harbored the resistance determinant in the chromosome and, therefore, no transconjugants were obtained. The transconjugant E11 showed a hybridization signal in a larger plasmid from its donor isolate, but its size corresponded to the recombination of the 2 plasmids harbored by the parental isolate, a common phenomenon in conjugation assays. Moreover, together with E16, which belongs to ST ST10, this isolate harbored the same plasmid (in terms of size and incompatibility group) as the isolates belonging to the clone ST216, suggesting a potential dissemination of the same plasmid among E. coli strains belonging to different STs.

The location of the bla\textsubscript{CTX-M} gene upstream from the insertion sequence ISEcp1 in all the isolates and in a conjugative plasmid in most of the isolates implies a high potential of dissemination of this ESBL, suggesting that this is not a result of the dissemination of particular clones but rather is due to the spread of multiple specific clones and/or mobile genetic elements. However, it is interesting that these strains presented such resistance to third-generation cephalosporins, as these antimicrobial agents are little used in Mozambique.

With regard to the resistance phenotype, 100% of the isolates were resistant to gentamicin, chloramphenicol, and trimethoprim-sulfamethoxazole. The first 2 antibiotics are used in the empirical treatment of bacteremia (chloramphenicol or penicillin plus gentamicin) whereas ceftriaxone is reserved for MDR cases,\textsuperscript{2} which would not be effective in the isolates studied. The transconjugant isolates showed not only β-lactam resistance but also the same resistance profile to other antibiotic families, which may have an impact on the clinical management of the patients in this setting. There are 3 main non-exclusive explanations for the finding of CTX-M group 1 ESBL resistance genes in these isolates: 1) the use of third-generation cephalosporins as second-line treatment may play a role in the emergence and subsequent dissemination of β-lactamase resistance; 2) the resistance to β-lactam antibiotics is the result of a co-selection from another family of antibiotic resistance mechanisms located in the same genetic mobile element; 3) globalization may influence the dissemination of ESBLs-carrying isolates in the community similar to what has been shown with New Dehli metallo-β-lactamase.\textsuperscript{50} The historical movement of the population between Manhiça and neighboring South Africa,\textsuperscript{17} as well as the increasingly more frequent presence of international travelers to the Manhiça District may support the international dissemination of these strains, as third-generation cephalosporins are not commonly used in this community.
Characterization of multidrug-resistant E. coli harboring bla<sub>CTX-M</sub> group 1

Conclusion
As observed in this study, continuously increasing resistance among Gram-negative bacteria associated with the emergence and spread of MDR isolates, including ESBL producers, is an important public health problem with serious implications in low-income countries. Taking into account that the availability of effective antibiotics is a challenge worldwide and is of special concern in LMIC due to limited resources, clinical microbiology services in Mozambique – and in all the Sub-Saharan African countries – need to be reinforced in order to perform coordinated antimicrobial resistance surveillance and establish national policies to control this public health problem.

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Disclosure
The authors report no conflicts of interest in this work.

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