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Characteristics of infections caused by extended-spectrum β-lactamase–producing *Escherichia coli* from community hospitals in South Africa☆,☆☆

Gisele Peirano[a,c], Cornelius H.J. van Greune[b], Johann D.D. Pitout[a,c,d,*]

[a]*Division of Microbiology, Calgary Laboratory Services, Calgary, AB T2L 2K8, Canada*
[b]*Ampath Pathology Laboratories, Cape Town, South Africa*
[c]*Department of Pathology & Laboratory Medicine, University of Calgary, Calgary, AB, Canada*
[d]*Microbiology and Infectious Diseases, University of Calgary, Calgary, AB, Canada*

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Abstract

A study was designed to characterize 22 nonrepeat extended-spectrum β-lactamase (ESBL)–producing *Escherichia coli* clinical isolates recovered from specimens originating from doctor's consultation rooms and several private and a state hospital in the Cape Town metropolitan area during 2008–2009. Characterization was done by using isoelectric focusing, PCR, sequencing of $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, and bla_{OXA} as well as PCR for plasmid-mediated quinolone resistance determinants, ST131, phylogenetic groups, and plasmid replicon typing. Genetic relatedness was determined with pulsed-field gel electrophoresis using *Xba*I and multilocus sequencing typing. The majority of patients (17/22 [77%]) presented with urinary tract infections (UTIs) originating from the hospital setting. Thirteen (59%) of the isolates produced CTX-M-15, 7 produced CTX-M-14, and 1 isolate each produced CTX-M-3 and SHV-2, respectively. Sixteen (73%) isolates were nonsusceptible to ciprofloxacin and 8 (36%) were positive for *aac(6′)-Ib-cr*. Overall, 10/22 (45%) of ESBL producers belonged to clonal complex ST131 that produced CTX-M-15 or CTX-M-14. Molecular characteristics of ST131 showed that this clone belonged to phylogenetic group B2. Our study illustrated that clonal complex ST131 isolates producing CTX-M-15 and CTX-M-14 had emerged as an important cause of UTIs due to ESBL-producing *E. coli* in the Cape Town area. This is the first report to identify ST131 in ESBL-producing *E. coli* from Southern Africa.

Keywords: CTX-M; Urinary tract infections; ST131; *E. coli*

1. Introduction

CTX-M enzymes are currently the most prevalent extended-spectrum β-lactamase (ESBL) type detected on a worldwide basis and are identified mainly in *Escherichia coli*. Currently, the most widely distributed CTX-M enzyme is CTX-M-15, and bacteria that produce this enzyme have been isolated from all corners of the world (Pitout & Laupland, 2008). Recent studies using multilocus sequence typing (MLST) identified a single clone of CTX-M-15–producing *E. coli*, named *ST131*, in isolates from several countries, including Spain, France, Canada, Portugal, Switzerland, Lebanon, India, Kuwait, and Korea (Coque et al., 2008; Nicolas-Chanoine et al., 2008). This clone is characterized by co-resistance to several classes of antibiotics; it belongs to highly virulent phylogenetic type B2, serotype O25:H4, and harbors the multidrug-resistant narrow host range type IncFII plasmids. Clone ST131 that produces CTX-M-15 often co-produce OXA-1 and TEM-1 β-lactamases as well as *aac(6′)-Ib-cr*, a variant of an aminoglycoside modifying enzyme *aac(6′)-Ib*, which is also responsible for reduced susceptibility to certain fluoroquinolones

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* Corresponding author. Tel.: +1-(403)-770-3309; fax: +1-(403)-770-3347.
E-mail address: johann.pitout@cls.ab.ca (J.D.D. Pitout).

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such as ciprofloxacin. The molecular epidemiology of ST131 CTX-M-15–producing *E. coli* has since been described in several countries across different continents (Peirano & Pitout, 2010).

In South Africa, the cefotaximases CTX-M-2 and CTX-M-3 were first detected in *Klebsiella pneumoniae* clinical isolates, as part of a worldwide study (Paterson et al., 2003), and CTX-M-15 and CTX-M-37 were identified among *Salmonella enterica* serotypes (Govinden et al., 2006; Usha et al., 2008). Reports about the prevalence of CTX-M–producing *E. coli*, especially in the community settings from South Africa, are still rare.

This study was undertaken to characterize infections caused by ESBL-producing *E. coli*, collected in the Cape Town metropolitan area, and to investigate the occurrence of clone ST131 in this collection.

2. Materials and methods

2.1. Patients, bacterial isolates, and antimicrobial susceptibility

Twenty-two nonrepeat ESBL-producing *E. coli* clinical isolates recovered from specimens originating from doctor's consultation rooms and several private and a state hospital in the Cape Town metropolitan area during 2008 and 2009 were included in the study. These hospitals provide emergency and several speciality care services. The majority of isolates (n = 17) were recovered from urines and 5 from pus swab cultures. ESBL production was confirmed phenotypically by using the Clinical and Laboratory Standards Institute (CLSI, 2009) criteria for ESBL screening and disk confirmation tests.

Antimicrobial susceptibility was determined with the VITEK 2 instrument (Vitek AMS; bioMerieux Vitek Systems, Hazelwood, MO). The MICs of the following drugs were determined: amoxycillin/clavulanic acid (AMC), piperacillin–tazobactam (TZP), cefoxitin (FOX), ertapenem (ERT), amikacin (AMK), gentamicin (GEN), tobramycin (TOB), ciprofloxacin (CIP), tigecycline (TIG), and trimethoprim–sulfamethoxazole (SXT). Throughout this study, results were interpreted using CLSI (2009) criteria for broth dilution.

2.2. β-Lactamase identification

Isoelectric focusing, which included cefotaxime hydrolysis and determination of inhibitor profiles on polyacrylamide gels, was performed on freeze–thaw extracts as previously described (Pitout et al., 2007). PCR amplification and sequencing for $bla_{\text{CTX-Ms}}$, bla_{OXAs}, bla_{TEMs}, and bla_{SHV} was carried out on the isolates with a GeneAmp 9700 ThermoCycler instrument (Applied Biosystems, Norwalk, CT) using PCR conditions and primers as previously described (Pitout et al., 2007).

2.3. Plasmid-mediated quinolone resistance determinants

The amplification of the *qnrA*, *qnrS*, and *qnrB* genes was undertaken in all ESBL-positive isolates with multiplex PCR (Robicsek et al., 2006b). *aac(6′)-Ib* and *qepA* were amplified in a separate PCR using primers and conditions as previously described (Robicsek et al., 2006a; Yamane et al., 2008). The variant *aac(6′)-Ib-cr* was further identified by digestion with BstF5I (Park et al., 2006) (New England Biolabs, Ipswich, MA).

2.4. Pulsed-field gel electrophoresis

Genetic relatedness of the ESBL-producing isolates was examined by pulsed-field gel electrophoresis (PFGE) following the extraction of genomic DNA and digestion with *Xba*I using the standardized *E. coli* (O157:H7) protocol established by the Centers for Disease Control and Prevention, Atlanta, GA (Hunter et al., 2005). Cluster designation was based on isolates showing approximately 80% or greater relatedness, which corresponds to the "possibly related (4–6 bands difference)" criteria of Tenover et al. (1995).

2.5. Identification of clone ST131

All the ESBL-producing isolates were screened for ST131 using a PCR for the *pabB* allele, recently described by Clermont et al. (2009). MLST was performed on those isolates that tested positive for ST131 using 7 conserved housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, *recA*). A detailed protocol of the MLST procedure, including allelic type and sequence type assignment methods, is available at MLST Databases at the ERI, University College Cork website (http://www.mlst.ucc.ie/mlst/dbs/Ecoli).

2.6. Phylogenetic grouping

The ESBL-positive isolates were assigned to 1 of the 4 main *E. coli* phylogenetic groups (A, B1, B2, and D) by the use of a multiplex PCR-based method (Clermont et al., 2000).

3. Results

3.1. Patients, bacterial isolates, and susceptibilities

The majority of patients (17/22 [77%]) presented with urinary tract infections (UTIs), 4 patients presented with intra-abdominal abscesses (including perforated diverticle [n = 1], post intra-abdominal surgery [n = 3]), while 1 patient had an infected burn wound. The majority of patients (12 [55%]) were inpatients. The prevalence of ESBL-producing *E. coli* isolated in the community hospital included in this study was less than 7%. Of the 22 isolates, 20 (91%) were nonsusceptible (i.e., intermediate or resistant) to SXT, 17 (77%) to AMC, 15 (68%) to TZP, 16 (73%) to CIP, 13 (59%) to TOB, 12 (55%) to GEN, and 9 (41%) to AMK. No resistance to ERT or TIG was detected.