High prevalence of ESBL-producing Escherichia coli isolates among hemodialysis patients in Portugal: appearance of ST410 with the bla\textsubscript{CTX-M-14} gene

Susana Correia\textsuperscript{a,b,c,d}, Rui Pacheco\textsuperscript{a,b,c,d}, Hajer Radhouani\textsuperscript{a,b,c,d}, João Carlos Diniz\textsuperscript{e}, Pedro Ponce\textsuperscript{e}, Daniela Jones-Dias\textsuperscript{f}, Manuela Caniça\textsuperscript{f}, Gilberto Igrejas\textsuperscript{a,b}, Patrícia Poeta\textsuperscript{c,d,*}

\textsuperscript{a} Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal
\textsuperscript{b} Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal
\textsuperscript{c} Centre of Studies of Animal and Veterinary Sciences, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal
\textsuperscript{d} Veterinary Science Department, University of Trás-os-Montes and Alto Douro, 5001-801 Vila Real, Portugal
\textsuperscript{e} Nephrocare Lumiar, Rua José da Costa Pedreira, 1750-130 Lisbon, Portugal
\textsuperscript{f} Laboratory of Antimicrobial Resistance, National Institute of Health Dr Ricardo Jorge, 1649-016 Lisbon, Portugal

A R T I C L E   I N F O

Article history:
Received 15 February 2012
Accepted 19 August 2012
Available online 20 September 2012

Keywords:
Extended-spectrum \(\beta\)-lactamase
Escherichia coli
Hemodialysis
Portugal

A B S T R A C T

Ten extended-spectrum \(\beta\)-lactamase–producing Escherichia coli isolates were detected among 121 fecal samples (8.3%) recovered from hemodialysis patients in Portugal. The isolates harbored the \textit{bla}_{CTX-M-15}, \textit{bla}_{CTX-M-14}\textsubscript{a}, and/or \textit{bla}_{CTX-M-1} genes. A new sequence type, ST2229, was detected, and this study also reports, for the first time, ST410 CTX-M-14–producing isolates.

© 2012 Elsevier Inc. All rights reserved.

Among the various \textit{Escherichia coli}–associated extended-spectrum \(\beta\)-lactamases (ESBLs), the CTX-M family currently predominates, especially CTX-M-1, CTX-M-14, and CTX-M-15 (\textit{Johnson et al.}, 2010). The increasing spread of vancomycin-resistant enterococci among hemodialysis patients led to the replacement of vancomycin for cephalosporins as part of the primary empiric therapy for bacterial infections in hemodialysis units (\textit{Marchaim et al.}, 2005). To date, there is no report on the incidence of ESBL-producing \textit{E. coli} among hemodialysis patients. Hence, the aim of this study was to determine the prevalence of ESBL-producing \textit{E. coli} in fecal samples of hemodialysis patients from Portugal and to characterize these isolates in what concerns to antimicrobial resistance to other clinically important antimicrobials, virulence factors, and clonality.

Between September and December 2010, 1 fecal sample per patient was collected from a total of 121 hemodialysis patients from one of the largest dialysis clinics in Portugal (Nephrocare Lumiar, Lisbon). All isolates were tested for antimicrobial susceptibility against 17 antimicrobials by the agar disk diffusion method: ampicillin (10 µg), amoxicillin + clavulanic acid (20 + 10 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), imipenem (10 µg), ertapenem (10 µg), gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg), streptomycin (10 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), sulfamethoxazole–trimethoprim (1.25 + 23.75 µg), tetracycline (30 µg), and chloramphenicol (30 µg) following the CLSI (2010) criteria. ESBL production was tested by the double-disc test (CLSI, 2010). The presence of genes encoding \(\beta\)-lactamases and the genetic environment of the \textit{bla}_{CTX-M} genes were studied by polymerase chain reaction (PCR) and further sequencing. The presence of other antimicrobial resistance mechanisms, as well as the intI1 and intI2 genes and the variable region of class 1 and 2 integrons, was also studied by PCR (\textit{Poeta et al.}, 2008). The quinolone resistance–determining region (QRDR) of the \textit{gyr}A and \textit{parC} genes was amplified and further sequenced in all quinolone-resistant \textit{E. coli} isolates; the presence of genes associated with plasmid-mediated quinolone resistance (PMQR), \textit{qnr}A, \textit{qnr}B, \textit{qnr}C, \textit{qnr}D, and \textit{qnr}S, \textit{aac(6')-Ib-cr}, and \textit{qep}A, was also studied (\textit{Poirel et al.}, 2012). Genes encoding virulence factors (\textit{stx1-stx2}, \textit{fim}A, \textit{pap}GII, \textit{cnf}1, \textit{papC}, and \textit{aer}) were studied by PCR (\textit{Gonçalves et al.}, 2012). The CTX-resistant \textit{E. coli} isolates were characterized by multilocus sequence typing (MLST) (\textit{Wirth et al.}, 2006); phylogenetic groups and serotype O25 were studied as previously described (\textit{Clermont et al.}, 2000, 2007). Conjugation experiments were performed in brain heart infusion broth using rifampicin- and streptomycin-resistant \textit{E. coli} C600 and sodium azide–resistant \textit{E. coli} J53 as recipients.

0732-8893/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.diagmicrobio.2012.08.017
Transconjugants were selected on MacConkey agar plates containing cefotaxime (2 μg/mL) plus rifampicin (250 μg/mL), streptomycin (200 μg/mL), or sodium azide (150 μg/mL) and then subjected to the detection and identification of \( \text{bla}\) genes as mentioned above. Then, plasmids from both clinical and transconjugant strains were assigned to incompatibility groups by PCR-based replicon typing (Carattoli et al., 2005).

All parental plasmids were typable, and the coexistence of more than 1 replicon in the same isolate was observed; the classic multi-replicon Inc plasmid was the most prevalent (Table 1). The presence of ESBL-producing Enterobacteriaceae among hemodialysis patients was only reported once, in Israel (Marchaim et al., 2005), and, to our knowledge, our study is the first to evaluate the prevalence of ESBL-producing \( E.\ coli\) in hemodialysis patients. CTX-resistant \( E.\ coli\) isolates were detected in 10 of the 121 studied fecal samples (from patients without story of previous use of third-generation cephalosporins), representing a prevalence of 8.3%, which is similar to the one obtained in the referred study from Israel (8.6 %). In our study, the majority of the isolates harbored genes encoding group 1 CTX-M enzymes, \( \text{bla}_{\text{CTX-M-1}a} (n = 3) \) and \( \text{bla}_{\text{CTX-M-15}} (n = 3) \); these genes are disseminated in different continents through epidemic plasmids and/or particular epidemic strains (Canton and Coque, 2006). As expected, the PMQR gene \( \text{aac}(6')-\text{Ib-cr} \) was linked to CTX-M-15. The dissemination of \( \text{bla}_{\text{CTX-M-15}} \) associated with strains and plasmids harboring the \( \text{bla}_{\text{OXA-1}}, \text{bla}_{\text{TEM-1}}, \) and \( \text{aac}(6')-\text{Ib-cr} \) genes was described in different Portuguese regions (Canton and Coque, 2006) and was verified in this study as well for isolate H64. However, although the CTX-M-15–carrying plasmids were not capable of conjugative plasmid transfer, the 7 remaining isolates were conjugative. The CTX-M-14 enzyme is, besides CTX-M-9, the most widespread enzyme of the CTX-M-9 group; it was initially identified in Korea in 1995 and is now globally disseminated, being endemic in numerous countries, including Portugal (Canton and Coque, 2006).

In this study, the 2 isolates assigned to the B2 phylogenetic group (H03 and H64) belonged to ST131, a recently emerged and disseminated lineage of virulent \( E.\ coli\) that is usually fluoroquinolone resistant and associated with CTX-M-15 (Johnson et al., 2010). In these 2 isolates, 4 amino acid changes were detected in the QRDR that were also previously identified in fluoroquinolone-resistant B2 ST131 isolates (Johnson et al., 2010). Moreover, the correlation of the B2-ST131 \( E.\ coli\) isolate with serotype O25 was not verified. Two of the ST410 isolates carried the \( \text{bla}_{\text{CTX-M-14a}} \) gene, and, to our knowledge, this is the first report of ST410-CTX-M-14-producing isolates. In the MLST analysis of isolate H57, we identified a new allele for the \( \text{purA} \) gene that originated a new sequence type, registered as ST2229 (http://www.mlst.net), which most likely belongs to the CC101 clonal complex.

In summary, this study revealed a high prevalence of CTX-M–producing \( E.\ coli\) isolates among hemodialysis patients in Portugal associated with successful bacterial lineages, particularly the recently emerged virulent \( E.\ coli\) ST131. We also identified a new sequence type, ST2229, and CTX-M–producing ST410 isolates that are unique to this study.

Acknowledgments

The authors thank Gabriela Filipe and Jesus Salgueiro from Nephrocare Lumiar, Lisbon, for their contribution in the collection of samples and all the patients who agreed to participate in this study, allowing the fecal sample recovery.

References


