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Bloodstream infections caused by multidrug-resistant Enterobacteriaceae: report from two Portuguese hospitals

Madam,

We report bloodstream infections (BSIs) by Enterobacteriaceae, isolated from two different Portuguese hospitals during a two-year surveillance study (2004–2006). *Escherichia coli* and other members of the family Enterobacteriaceae are among the most frequent pathogens found in BSI.[1] In Portugal, there have been no studies related to BSI involving Enterobacteriaceae. Moreover, multidrug-resistant (MDR) enterobacteria are becoming a worldwide problem.[2] This study aimed to report BSI caused by Gram-negative bacilli of the Enterobacteriaceae family presenting MDR phenotypes in two separate hospitals. We considered strains presenting resistance phenotypes to at least three distinct antimicrobial chemical classes. The blood cultures were processed by the BacT/Alert microbial detection system (bioMérieux, Marcy l'Etoile, France) and the antimicrobial susceptibility was determined according to the Clinical Laboratory Standards Institute.[3] *E. coli* was the most frequent isolate from BSI ($N = 12$) followed by *Klebsiella pneumoniae* ($N = 8$), *Enterobacter cloacae* ($N = 3$), *Morganella morganii* ($N = 2$) and *Enterococcus faecalis* ($N = 1$). All the strains were resistant to ampicillin and to cefuroxime, 69% were resistant to cefotaxime, 62% to ceftazidime, 58% to ciprofloxacin, 58% to gentamicin, 38% to cefepime, 27% to cefoxin and 27% to the combination trimethoprim/sulfamethoxazole. All strains were susceptible to carbapenems (imipenem and meropenem). Extended-spectrum β-lactamase (ESBL) detection was performed by polymerase chain reaction (PCR) and the products were sequenced. Six *E. coli* producing CTX-M-15 type of ESBL were found, three from hospital A and three from hospital B. SHV-5, another ESBL type, was found in four *K. pneumoniae* strains from hospital A. The distance between hospitals is about 35 miles. All six *E. coli* producing CTX-M-15 strains presented the same antimicrobial pattern. They were resistant to ampicillin, amoxicillin/clavulanic acid, cefalotin, cefazolin, cefuroxime, ceftazidime, cefotaxime, cefepime, ciprofloxacin and gentamicin and susceptible to cefoxitin, to imipenem, to meropenem and to trimethoprim/sulfamethoxazole. In hospital A they were detected over a period of three months, from March to May 2005, and in hospital B they were detected over two months, from August to September 2005. With regard to SHV-5-producing *K. pneumoniae* found in hospital A, they present different phenotypes of antimicrobial susceptibility. Nevertheless, all were resistant to ampicillin, amoxicillin/clavulanic acid, cefalotin, cefazolin, cefuroxime, ceftazidime, cefotaxime and cefepime, and were susceptible to cefoxitin, imipenem and meropenem. Variations of susceptibility patterns were found for ciprofloxacin, gentamicin and trimethoprim/sulfamethoxazole. In order to understand the epidemiological origins of the

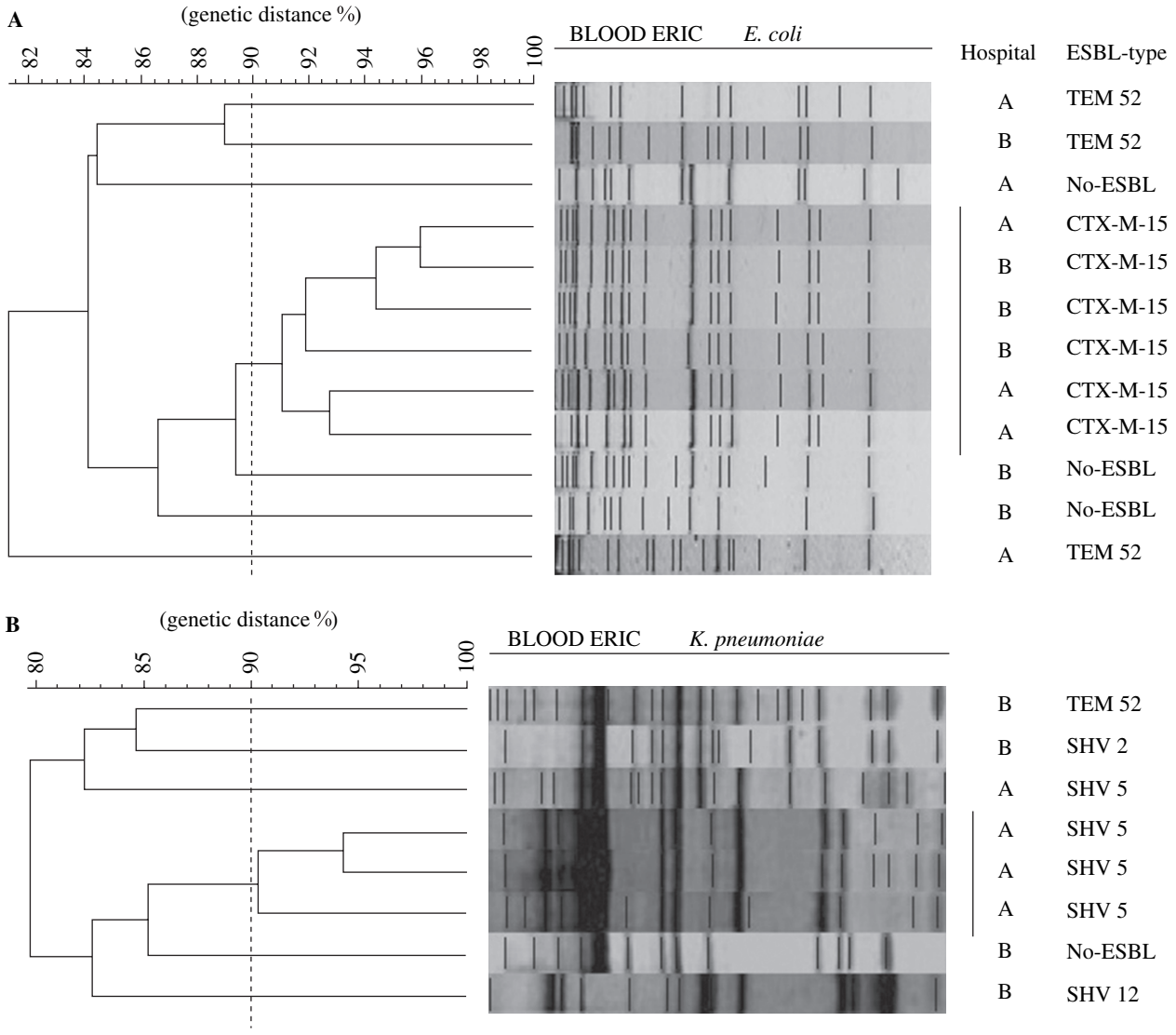

Figure 1 Enterobacterial repetitive intragenic consensus (ERIC) fingerprinting profiles of multidrug-resistant (MDR) *Escherichia coli* (A) and *Klebsiella pneumoniae* (B) isolated from bloodstream-infected patients from two distinct Portuguese hospitals A and B. Dendrograms were generated by UPGMA (unweighted pair group method with arithmetic averages) analysis of the agarose gels contrastained by rainbow algorithm with Pearson's correlation coefficient using the FPQuest version 4.1.5 (Bio-Rad, Hercules, CA, USA).

ESBL-producing strains, fingerprinting profiles were carried out based on enterobacterial repetitive intragenic consensus (ERIC) sequences.[4] The results for *E. coli* were surprising, i.e. consistent with the idea that it is the same clone (Figure 1A) in both hospitals (≥90% genetic similarity). Concerning *K. pneumoniae*, three of the strains are likely to be the same clone (Figure 1B).

In summary, the production of ESBLs by Enterobacteriaceae pathogens is an increasing worldwide problem with potentially grave consequences because they are genetically resistant to all β-lactams except carbapenems and cephamycins, and because of their genetic finger-print, which may contain resistance genes to other non-β-lactams. Several studies described a widely disseminated European clone of *E. coli* producing CTX-M-15, which may explain the molecular fingerprinting profile exhibited by the strains isolated in both hospitals in Portugal.[5,6]

Conflict of interest statement
None declared.

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A novel β-lactamase gene, LAP-2, produced by an *Enterobacter cloacae* clinical isolate in China

Madam,

Many β-lactamases classified by their amino acid sequences and functional substrate specificity profiles in various Gram-negative bacilli such as *Pseudomonas* spp. and members of the family Enterobacteriaceae have been documented.[1] Since the late 1980s, extended-spectrum β-lactamases (ESBLs) derived from TEM- and SHV-type penicillinases capable of hydrolysing the oxymino-cephalosporins, have been spreading globally, mainly in Enterobacteriaceae, including *Klebsiella pneumoniae* and *Escherichia coli*. Moreover, various non-TEM-, non-SHV-type class A β-lactamases exhibiting extended-spectrum activities, including β-lactamase types CTX-M, CARB, SFO, PER, TLA, VEB, GES, BEL, BES, KPC, IMI and SME, have also been reported in various Gram-negative bacilli.[2,3]

A novel Ambler class A β-lactamase, LAP-1, was identified in *Enterobacter cloacae* isolates from France and Vietnam.[4] It has a narrow-spectrum hydrolysis of β-lactams and is strongly inhibited by clavulanic acid and sulbactam and, to a lesser extent, by tazobactam. It shares 62 and 61% amino acid identity with the most closely related β-lactamases, TEM-1 and SHV-1, respectively. Recently, by analysing the prevalence of the resistance genes in a collection of *E. cloacae* clinical isolates recovered at the PLA 98th Hospital (China) from 2003 to 2004, a novel β-lactamase LAP-2 was identified in *E. cloacae* HZB9055.

E. cloacae HZB9055 was isolated in May 2004 from the venous catheter cusp of a 29-year-old patient undergoing treatment for burns. The isolate was identified by API 20 E (bioMérieux, Hazelwood, MO, USA) and the antimicrobial susceptibility profile was determined by ATB® G-5 system (bioMérieux). Minimum inhibitory concentration results were interpreted according to the guidelines of the CLSI.[5] *E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603 were used for quality control of the antimicrobial susceptibility testing.

Template DNA for polymerase chain reaction (PCR) was prepared by a rapid alkaline lysis procedure.[6] Forty-one kinds (or groups) of resistance gene oligonucleotide primers for PCR were designed based on the resistance genes published in GenBank, including 14 kinds (or groups) of β-lactamase genes (bla_{LAP}, bla_{DHA}, bla_{MIR}, bla_{LAT}, bla_{TEM}, bla_{SHV}, $bla_{CTx\text{-}M\text{-}1}$ cluster, $bla_{CTx\text{-}M\text{-}2}$ cluster, $bla_{CTx\text{-}M\text{-}9}$ cluster, $bla_{OxA\text{-}1}$ cluster, $bla_{OxA\text{-}10}$ cluster, bla_{PER}, bla_{VEB} and bla_{GES}), five kinds of 16S rRNA methylase genes (*arm*A, *rmt*A, *rmt*B, *rmt*C and *rmt*D), nine kinds of aminoglycoside-modifying enzyme genes [*aac*(3)-I, *aac*(3)-II, *aac*(3)-III, *aac*(3)-IV, *aac*(6′)-Ib, *aac*(6′)-II, *ant*(3″)-I, *ant*(2″)-I and *aph*(3′)-VI], *int*I1, *qacE*Δ1-*sul*1, *cat*B, *cml*1, *qnr*A, *qnrB*, *qnrS*, *qep*A, *dfrA*1, *dfrA*12, *dfrA*17, *mer*A and *tnp*A. The following primers for bla_{LAP}-specific amplification were used: 5′-CAATACAAAGCACA GAAGACC-3′ and 5′-CCGATCCCTGCAATATGCTC-3′,