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Extended-spectrum β-lactamases (ESBL) - producing *Escherichia coli* strains in blood cultures

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Abstract. Introduction: Extended-spectrum beta-lactamase (ESBL)–producing *Escherichia coli (E. coli)*, are major pathogens responsible for bacteraemia. The CTX-M types of ESBL are becoming dominant worldwide, particularly CTX-M-15. Bacteraemia caused by these strains is difficult to treat because the organisms are frequently resistant to the antimicrobials recommended for treatment of patients. Methods: We included in our study all consecutive episodes of bloodstream infection due to ESBL-producing *E. coli* during the period January 2007 - December 2010. Strains were isolated, and tested for antimicrobial resistance using Vitek2 system and API Biomerieux. ESBLs were characterized using polymerase chain reaction (PCR). Results: 127 episodes of *E. coli* bacteraemia, 30 ESBL-producing *E. coli* (24%) were included; 90% (19 strains) of the molecular characterizated isolates produced a CTX-M type of ESBL. The most frequent origins of infection were urinary tract (24%) and intra-abdominal infections (11%). Coresistance to other antimicrobials was important between ESBL-producing strains. The majority of isolates were resistant to ciprofloxacin 22 strains (73%) and gentamicin 21 strains (70%). Conclusions: ESBL-producing *E. coli* is a significant cause of bloodstream infection in the context of the emergence of CTX-M enzymes. Empirical treatment of sepsis potentially caused by *E. coli* may need special attention in areas where such ESBL-producing isolates are present.
Key words: *E. coli*, ESBL, CTX-M-15, bloodstream infections.

Introduction. Bloodstream infections remain a life-threatening occurrence and can be associated with a Gram-negative infection in other sites such as urinary tract, digestive tract or the lung. *Escherichia coli* is the most common Gram-negative pathogen associated with bacteraemia. *E. coli* is a facultative anaerobic Gram-negative bacillus that exists singly or in pairs. Resistance to beta-lactams antibiotics has become a particular problem in recent decades, as strains of *E. coli* that produce extended-spectrum beta-lactamases (ESBL) have become more common. ESBL–producing *E. coli* are emerging pathogens highly resistant to antibiotics, and infections by these strains are difficult to treat (Courpon-Claudinon et al 2010).

E. coli strains producing CTX-M beta-lactamases have become prevalent over the last years, especially in certain European and South American countries and infections caused by bacteria producing these enzymes are not limited to the hospital setting (Canton 2006). CTX-M producing *E. coli* strains often exhibit coresistance to trimethoprim-sulfamethoxazole, tetracycline, gentamicin, and ciprofloxacin (Pitout 2008).

The aim of our study is to identify bloodstream infections caused by ESBL-producing *E. coli* and to characterize the molecular mechanism of resistance.

Materials and Methods. Bacterial isolates and patients. Between January 2007 and December 2010, 127 strains of *E. coli* were identified in a series of cases of bacteraemia with 30 strains ESBL positive. Only no repeated isolates from true incident cases were included in this study. A case of *E. coli* bacteraemia was defined in a patient with a

systemic inflammatory response syndrome (e.g., fever, tachycardia, tachypnea and leucocytosis) documented by the growth of an isolate in at least one blood culture (Russell 2006). Clinical variables collected from patients with bacteraemia included age, gender, location of the patient in the hospital at the time of bacteraemia, underlying medical conditions. Blood culture samples were collected by peripheral venipuncture and processed in BacT/Alert Biomerieux.

Identification of strains was done with Vitek2 Compact (bioMerieux, Marcy l'Etoile, France) or API 10S (bioMerieux, France).

Antimicrobial susceptibility testing. Antibiotic susceptibility was tested by the Kirby-Bauer disc diffusion method using Bio-Rad discs (Marnes-la-Coquette, France). The MICs of the following drugs were determined with the Vitek2 system: piperacillin-tazobactam (TZP), imipenem (IPM), meropenem (MEM), gentamicin (GEN), amikacin (AMK), trimethoprim-sulfamethoxazole (SXT), ceftazidime (CAZ), amoxicillin/clavulanic acid (AMC),colistin (CO), tetracycline (TET) and ciprofloxacin (CIP). Throughout this study, the results were interpreted by using the criteria of the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2010). The quality control strains used for this part of the study were *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853.

ESBL screening and confirmation testing. ESBLs were detected in clinical isolates of *E. coli* by using the CLSI criteria for screening for ESBLs and disk confirmation tests (CLSI 2010). Disks for ESBL confirmation tests were obtained from Bio-Rad. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls, respectively.

Beta-lactamase identification. For strains with a positive double-disk synergy test result, characterization of ESBL's was performed by specific PCR amplification of $bla_{\text{CTX-M-15}}$ with primers described as: CTATAATCCGATTGCGGAAAAG/GA and GGCTGGGTGAAGTAAGTGAC. The method used is called direct because the colonies grown on solid medium are suspended directly in ultra-pure water and then added to the reaction mixture. PCR products were visualized in 2% agarose gel and images were captured using the Bio-Profil gel documentation system (Vilbert Lourmat, Marne-La-Vallee Cedex 1, France). Using this technique we eliminated the step of DNA isolation and laboratory and personnel contamination is minimized (Jakab & Popescu 2005). Optimization of reaction conditions was made after McPherson and Moller respectively Roux (McPherson & Moller 2001; Roux 2003).

Statistical Analysis. Data were stored and analysed with Whonet 5.5.

Results. Patients. During the 4 years of study, a total of 127 patients with bloodstream infections due to *E. coli* isolates were identified. The mean age of the patients was 62.01 years, and 53 (42%) patients were males. The majority of patients (72 (57%) presented primary bacteremia or sepsis without a focus. About 30 (24%) patients presented clinical syndrome of urosepsis, followed by 14 (11%) with intra-abdominal infections. The remaining patients (11 (9%) presented respiratory infections. Regarding the origin, 72 (57%) patients belong to infectious diseases department, 21 (17%) were from internal medicine wards, 17 (13%) from intensive care unit, 12(9%) from surgical departments and 5 (4%) from pediatric wards.

About 30 patients (24%) had an ESBL-producing *E. coli* strain. In this group the mean age 63.31 and 19 patients (63%) were male. A large number 21 (70%) presented primary bacteremia or sepsis without a focus, 4 (13%) patients presented with the clinical syndrome of urosepsis, followed by 3 (10%) with intra-abdominal infections and the remaining 2 (7%) of patients presented with respiratory infections. These patients were admited on infectious diseases wards 11 (37%), intensive care unit 6 (20%), surgery 6 (20%), internal medicine wards 6 (20%) and pediatrics 1 (3%).

Table 1

Distribution of ESBL and non-ESBL producing *E. coli* isolated from different hospital departments

	ESBL+ strains	*ESBL- strains*	*p value*
male	19 (63%)	34 (35%)	0.003
nofocus	21 (70%)	51 (53%)	0.046
urosepsis	4 (13%)	26 (27%)	0.064
intraabdominal infection	3(10%)	11 (11%)	0.41
respiratory infection	2 (7%)	9 (9%)	0.32
infectious diseases wards	11 (37%)	61 (63%)	0.005
ICU	6 (20%)	11 (11%)	0.11
pediatrics	1 (3%)	4 (4%)	0.42
surgery	6 (20%)	6 (6%)	0.01
medical wards	6 (20%)	15 (15%)	0.27

There were more ESBL-producing strains with no precised origins isolated from surgical patients and ESBL non producing strains between patients admitted on infectious disease department.

Bacterial isolates and susceptibilities. During the 4-year study period, 127 *E. coli* isolates (1 isolate per patient) were isolated from blood at SCBI Laboratory, and 30 (24%) tested positive for ESBL production. Of the 97 isolates ESBL negative included in this study, 38.1% (95% C.I. 28.6-48.6) were resistant to SXT, 17.5% (95% C.I. 10.8-26.8) were resistant to CIP, 4.2% (95% C.I. 1.4-11) were resistant to GEN, 12.4% (95% C.I. 6.9-21) were resistant to AMC, 4.2% (95% C.I. 1.4-11) were resistant to TZP, 38.1% (95% C.I. 28.6-48.6) were resistant to TET. No isolate with resistance to IPM, MEM and CO was detected.

About 30 (24%) *E. coli* strains tested positive to ESBL. Results of antimicrobial susceptibility test for ESBL strains were: 73% (95 C.I. 53.8-87) were resistant to SXT, 73% (95% C.I. 53.8-87) were resistant to CIP, 70% (95% C.I. 50.4-80.6) were resistant to GEN, 37% (95% C.I. 20.6-60.1) were resistant to AMC, 17% (95% C.I. 6.5-36.4) were resistant to TZP, 43.6% (95% C.I. 33.5-54.2) were resistant to TET and 3.3% (95% C.I. 0.2-19%) were resistant to AMK. No isolate with resistance to IPM, MEM and CO was detected.

Table 2

Resistance of ESBL and non-ESBL producing *E. coli* to the selected antibiotics

Antibiotic	*ESBL+ Number of strains (%)*	*ESBL- Number of strains (%)*	*p Value*
trimethoprim/ sulfamethoxazol	22 (73%)	37 (38.1%)	0.000
ciprofloxacin	22 (73%)	17 (17.5%)	0.000
gentamicin	21 (70%)	4 (4.2%)	0.000
amoxicillin/ clavulanic acid	11 (37%)	12 (12.4%)	0.001
tetracicline	13 (43.6%)	37 (38.1%)	0.30
piperacillin/ tazobactam	5 (17%)	4 (4.2%)	0.009

ESBL-producing *E. coli* strains differ significant from those ESBL negative regarding resistance to other antimicrobials.

Beta-Lactamases determinants. Of the 21 ESBL-producing *E. coli* isolates recovered from blood and tested by PCR, 19 (90%) were positive for $bla_{CTX\text{-}M\text{-}15}$ genes. A $bla_{CTX\text{-}M\text{-}15}$ genotype was confirmed in eight strains that were coresistant to gentamicin, trimethoprim-sulfamethoxazole and ciprofloxacin.

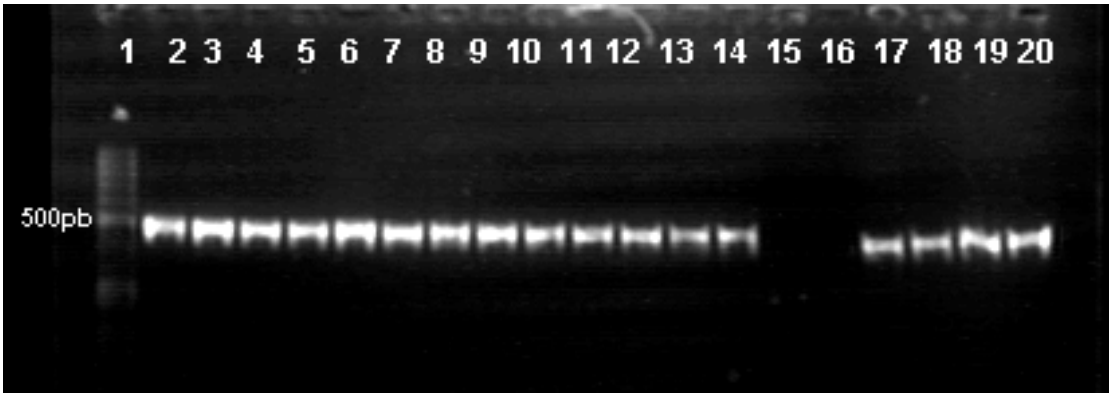

Figure 1. Agarose gel electrophoresis for $bla_{CTX\text{-}M\text{-}15}$ PCR products (wells 2-20) for *E. coli* (10µL/well); well 1 - molecular marker (O'Range Ruler 100 DNA Ladder, SM1133-Fermentas); band size 500 base pairs (bp) was marked.

Clinical evaluation suggests that the CTX-M-producing *E. coli* strains contributed to death in one patient due to delayed efficient antimicrobial therapy.

Discussion. There has been a dramatic increase in the number of *E.coli* reported in the literature that produces CTX-M-beta-lactamases (Bonnet 2004). Phenotypic differentiation of organisms producing CTX-M-beta-lactamases from organisms producing other types of ESBLs can be difficult. The PCR amplification of *bla*CTX-M genes have been used to characterize organisms producing CTX-M-beta-lactamases (Bou et al 2002).

Recent reports from Europe revealed that CTX-M-producing *E. coli* is emerging as an important cause of bloodstream infections. A report from Italy has shown that these organisms are also important causes of nosocomially acquired bloodstream infections (Tumbarello et al 2008). A study from Israel investigated patients with bacteraemia and found that 14% of the cases were due to multiresistant ESBL-producing organisms (most often *E. coli* producing CTX-M-2) (Ben-Ami et al 2006). Another study from Spain, on cases of ESBL-producing *E. coli* bloodstream infections over a 4-year period in Seville, demonstrated that 51% cases were community-onset infections most often caused by CTX-M-9- and CTX-M-14-producing isolates (Rodriguez-Bano et al 2006). These bacteria were also multiresistant, and the most frequent origin of infection was the urinary and the biliary tracts.

Starting with 2000 an identical clone has been identified among CTX-M-15-producing *E. coli* isolates recovered from several countries, including Spain, France, Canada, Portugal, Switzerland, Croatia, India, Kuwait, South Korea, Italy, United Kingdom, Turkey and United States (Cagnacci et al 2008; Johnson et al 2008; Lau et al 2008; Yumuk et al 2008; Literacka et al 2009).

The presence of CTX-M-15 variants was expected since the most common CTX-M enzyme in Europe has beenCTX-M-15, confirming the observation of the worldwide dissemination of this type of ESBL since 2000.

The number of strains with ESBL was higher than in other studies. This high ESBL frequency may have been caused by the use of broad-spectrum antibiotics and possible a higher level in our community setting, or due to the spread of a clone and/or plasmid.
In our study, bacteraemia caused by ESBL-producing *E. coli* isolates predominantly occurred in males ($p=0.003$) presenting with primary bacteremia or sepsis without a focus and urosepsis, admited on surgical wardst. Our results are consistent with previous published data (Melzer & Petersen 2007). In addition, these organisms were

multiresistant and showed high levels of resistance to CIP, GEN, AMC and SXT. The efficacy of beta-lactamase inhibitor combination is unclear, with some patients responding and other failing. Carbapenems and colistin remain the choice for therapy in cases of blood stream infections caused by ESBL-producing *E. coli*. Separately from the rise of ESBL-related multiresistance, fluoroquinolone, aminoglicoside but especially tetracycline and trimethoprim resistance exist also in ESBL-negative *E. coli* isolates.

For patients with urinary sepsis treatment with aminoglicoside associated to a cephalosporin or a fluoroquinolone may be an option but with caution.

The ESBL-producing strains compare with no-ESBL strains were more frequent isolate from intensive care units and surgery wards.

Conclusions. ESBL-producing *E. coli* is a significant cause of bloodstream infection in hospitalized patients in the context of the emergence of CTX-M enzymes. Local knowledge of the epidemiology and characterization of resistance in *E. coli* has become even more important when considering empirical therapy of sepsis. Microbiology laboratories need to be alert to the correct identification and control of infections caused by such microorganisms. Extra efforts, such as molecular test to precise identification of resistance mechanisms, can provide optimal patient treatment. Due to extended use of carbapenems the development of carbapenem resistance would be a serious problem. As the treatment options are limited, infection control measures and implementation of antibiotic control policies are very important.

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