CTX-M-15 extended-spectrum β-lactamases in Enterobacteriaceae in the intensive care unit of Tlemcen Hospital, Algeria

Z. Baba Ahmed, A. Ayad, E. Mesli, Y. Messai, R. Bakour and M. Drissi

ABSTRACT The aim of this study was to detect extended-spectrum β-lactamases (ESBL) in Enterobacteriaceae isolates in the intensive care unit (ICU) of Tlemcen hospital in north-western Algeria. Antimicrobial susceptibility testing, molecular typing, characterization of ESBL-encoding genes and the genetic environment, conjugation experiments and plasmid analysis were carried out. In all, 28 Enterobacteriaceae isolates were isolated from patients in the ICU and 2 from surfaces of the unit. Of these, 11 isolates (4 Escherichia coli, 5 Klebsiella pneumoniae and 2 Enterobacter cloacae) produced ESBL of the CTX-M-15 type. Molecular typing of the isolates showed the clonal nature of 4 K. pneumoniae isolates. The blaCTXM-15 gene was genetically linked to insertion sequence IS Ecp1B and was transferable by conjugation from 3 isolates. Regular monitoring of resistance mechanisms, the establishment of a prevention strategy, and more rational and appropriate use of antibiotics are needed.
Introduction

β-lactams are the most common drugs against bacterial infections and the emergence of resistance to expanded-spectrum cephalosporins is a major concern [1,2]. The production of extended-spectrum β-lactamas (ESBLs) is a significant mechanism of resistance to β-lactams in Enterobacteriaceae; it has been found in the community, mostly in hospitals and often in intensive care units (ICUs) [3]. ESBLs are plasmid-mediated clavulanate-susceptible enzymes of predominantly Bush class A, capable of hydrolyzing oxyimino-cephalosporins and monobactams but not cefamycins and carbapenems. Typically, they derive from the genes of old β-lactamases TEM-1, TEM-2 or SHV-1 by mutation. However, new classes of ESBL have emerged such as PER, VEB, TLA-1, GES/IBC, SFO-1, BES-1, CTX-M [3].

CTX-M type β-lactamas have been widely detected around the world [2,3]. They were named thus because of their high level of activity against cefotaxime. The prevalence of the different types ESBLs varies by the clinical context and region and is changing over time [2,3].

Algeria is a large country with over 1000 km of coastline and extending 2000 km to the south. All the studies that relate to ESBLs have only been conducted in the centre and east of the country. In this context, the objective of this qualitative study was to investigate the presence and the types of ESBLs in clinical isolates taken from the ICU of the university hospital of Tlemcen, a town located in the extreme north-west of the country, 600 km from the centre.

Methods

Bacterial isolates, antimicrobial susceptibility testing and molecular typing

From 19 October to 23 November 2008, 28 Enterobacteriaceae clinical isolates were isolated from various specimens recovered from patients in the ICU of Tlemcen university hospital; 2 cefotaxime-resistant isolates were also recovered from surfaces of the ICU. The patients had lung congestion, burning upon urination and fever.

Isolates were identified by using API 20E (BioMérieux, France). The antimicrobial susceptibility was determined by the disc diffusion and agar dilution methods according to CA-SFM (Comité de l’Antibiogramme de la Société Française de Microbiologie) guidelines [4]. Antibiotic disks were purchased from Bio-Rad (Marnes la Coquette, France). Escherichia coli ATCC 25922 was used as a control strain. ESBL production was screened by the double-disc synergy test (DDST) [5]. The clonal relationships between isolates were analysed by enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) using primer ERIC2 [6]. DNA was extracted by a boiling method as follows: 1.5 mL of an overnight broth culture were centrifuged at 15,000 × g for 3 min. The pellet was resuspended in 100 µL of sterile ultrapure water and was then boiled for 10 min. After a final centrifugation at 15,000 × g for 10 min, the supernatant was recovered. Thermal cycling was carried out as follows: an initial denaturation cycle at 95 °C for 3 min, 40 cycles of denaturation at 92 °C for 30 s, annealing at 40 °C for 1 min, extension cycle at 72 °C for 8 min, and a single final extension cycle at 72 °C for 16 min. PCR products were separated by gel electrophoresis on 0.7% (wt/vol.) agarose gels at 5 V/cm. Plasmid size was estimated by using reference plasmids RP4 (60 kb) and pLP113 (128 kb).

Results

The results are summarized in Table 1. Nine isolates (32.1%) (E. coli, n = 4; Klebsiella pneumoniae, n = 4 and Enterobacter cloacae, n = 1) of the 28 Enterobacteriaceae clinical isolates and the 2 selected isolates from surfaces (K. pneumoniae and Ent. cloacae) showed a resistance to ESBL marker antibiotics (cefotaxime, ceftazidime, cefepime...
<table>
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<th>Isolate</th>
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<td>Escherichia coli (Ec61)</td>
<td>Tracheal secretion</td>
<td>CTX, CAZ, FEP, ATM, GM, TM, AN, NET, CIP, SXT</td>
<td>512 128 CTX-M-15 + +</td>
<td>A1</td>
<td>GM, TM, AN, SXT</td>
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<td>E. coli (Ec68)</td>
<td>Urine</td>
<td>CTX, CAZ, FEP, ATM, GM, TM, NET, CIP, SXT</td>
<td>64 8 CTX-M-15 – +</td>
<td>A2</td>
<td>NT NT</td>
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<td>E. coli (Ec69)</td>
<td>Urine</td>
<td>CTX, CAZ, FEP, ATM, GM, TM, NET, CIP, SXT</td>
<td>64 16 CTX-M-15 – +</td>
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<td>+ GM, TM</td>
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<td>E. coli (Ec79)</td>
<td>Urine</td>
<td>CTX, CAZ, FEP, ATM, GM, TM, NET, CIP, SXT</td>
<td>64 8 CTX-M-15 – +</td>
<td>A4</td>
<td>NT NT</td>
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<td>Klebsiella pneumoniae (Kp62)</td>
<td>Urine</td>
<td>CTX, CAZ, FEP, ATM, GM, TM, NET, CIP, SXT</td>
<td>128 16 CTX-M-15 + +</td>
<td>B1</td>
<td>NT NT</td>
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<td>K. pneumoniae (Kp66)</td>
<td>Tracheal secretion</td>
<td>CTX, CAZ, FEP, ATM, GM, TM, NET, CIP, SXT</td>
<td>256 32 CTX-M-15 + +</td>
<td>B2</td>
<td>NT NT</td>
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<td>K. pneumoniae (Kp74)</td>
<td>Surfaces</td>
<td>CTX, CAZ, FEP, ATM, GM, TM, NET, CIP, SXT</td>
<td>256 32 CTX-M-15 + +</td>
<td>B2</td>
<td>NT NT</td>
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<td>K. pneumoniae (Kp82)</td>
<td>Urine</td>
<td>CTX, CAZ, FEP, ATM, GM, TM, NET, CIP, SXT</td>
<td>256 32 CTX-M-15 + +</td>
<td>B2</td>
<td>NT NT</td>
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<td>Enterobacter cloacae (Et17)</td>
<td>Urine</td>
<td>CTX, CAZ, FEP, ATM, GM, TM, NET, CIP, SXT</td>
<td>64 4 CTX-M-15 – +</td>
<td>C1</td>
<td>NT NT</td>
</tr>
<tr>
<td>Ent. cloacae (Et19)</td>
<td>Surfaces</td>
<td>CTX, CAZ, FEP, ATM, GM, TM, NET, CIP, SXT</td>
<td>64 16 CTX-M-15 + +</td>
<td>C2</td>
<td>NT NT</td>
</tr>
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MIC = minimum inhibitory concentration; PCR = polymerase chain reaction.
CTX = cefotaxime; CAZ = ceftazidime; ESBL = extended spectrum beta-lactamases; ERIC = enterobacterial repetitive intergenic consensus.
FEP = cefepime; ATM = aztreonam; GM = gentamicin; TM = tobramycin; NET = netilmicin; CIP = ciprofloxacin; SXT = trimethoprim/sulfamethoxazole; NT = no transfer.
+ = present, – = absent.
and aztreonam), a susceptibility to imipenem and a marked synergistic effect between clavulanic acid and the marker antibiotics, characteristic features of ESBL-producing bacteria. Agar dilution minimum inhibitory concentrations (MIC) of cefotaxime and ceftazidime were from 64 to 512 mg/L and 4 to 128 mg/L respectively. These ESBL producers showed resistance to aminoglycosides (gentamicin, tobramycin and netilmicin), ciprofloxacin and trimethoprim–sulfamethoxazole. ERIC-PCR genotyping gave different electrophoresis patterns for E. coli and Ent. cloacae isolates, whereas 4 K. pneumoniae isolates, one of which was from the surfaces, had identical profiles.

PCR amplification and sequencing revealed that all isolates produced ESBL of the CTX-M-15 type belonging to CTX-M-1 group. Insertion sequence IScep1B was found upstream of the blaCTX-M-15 gene in 7 isolates including the 5 K. pneumoniae, 1 E. coli and 1 Ent. cloacae; the genetic linkage investigation between this sequence and blaCTX-M-15 gene was positive for all isolates with PCR product of 1000 bp being yielded.

Mating assays allowed the transfer of ESBL phenotype (oximinocephalosporin resistance) from 3 isolates including 2 E. coli and 1 K. pneumoniae in association with blaCTX-M-15 gene and plasmids of about 90 kb. Resistance determinants against the following non-lactam antibiotics were co-transferred: gentamicin; gentamicin–tobramycin and gentamicin–tobramycin–amikacin–trimethoprim/sulfamethoxazole.

**Discussion**

In total, 11 Enterobacteriaceae isolates resistant to 3rd generation cephalosporins were ESBL producers; they were positive for the DDST and blaCTX-M-15 gene. The presence of ESBL bacteria in the ICU of Tlemcen Hospital may be related to the fact that cefotaxime is the first-line treatment; in fact, the heavy use of cefotaxime and ceftriaxone is considered a factor supporting the emergence of CTX-M enzymes [11]. CTX-M-15 with CTX-M-3 enzymes have been reported as prevalent ESBLs in the east [12] and centre [6,13,14] of Algeria, while only CTX-M-15 was found in our isolates. The fact is that CTX-M enzymes have recently and sharply accumulated in Enterobacteriaceae [1]. The presence of CTX-M-15 enzyme in the west of Algeria indicates a countrywide spread of the CTX-M β-lactamasases. This situation is comparable to those reported in numerous countries, such as Argentina, Poland and Lebanon where CTX-M-producing Enterobacteriaceae have been described to be endemic [15–18].

CTX-M-15 enzyme, like CTX-M-16 and CTX-M-19, confers a higher resistance to ceftazidime than other types of CTX-M ESBLs. However, some of our isolates were moderately resistant to ceftazidime; this is in agreement with studies that described CTX-M-15-producing isolates susceptible or moderately resistant to ceftazidime [19,20]. Resistance to aminoglycosides (gentamicin, tobramycin and netilmicin), ciprofloxacin and trimethoprim/sulfamethoxazole was observed in all the isolates and transconjugant analysis showed the co-transfer of these resistances with CTX-M-15. The close association of ESBL production with these resistances has been previously reported [7,19,21].

Genetic environment analysis of the CTX-M gene showed the presence of the sequence IScep1B in 7 isolates, while the genetic association between them was positive for all strains. The fact that IScep1B was not detected in 3 E. coli and E. cloacae isolates may be related to the modification in its transposase gene, possible by insertion of IS26 [22]. IScep1B can enhance the expression of the blaCTX-M-15 gene and its presence could explain the ease with which this gene is spreading among bacteria in a clinical setting [8].

ERIC-PCR genotyping demonstrated the clonal diffusion of K. pneumoniae isolates. Epidemiological investigation has revealed that patients infected by these clones underwent invasive procedures (artificial ventilation and installation of probes). The detection of CTX-M ESBL in the remaining non-clonal E. coli and E. cloacae isolates is probably due to horizontal transmission via plasmids. Several nosocomial outbreaks caused by endemic or epidemic ESBL (CTX-M)-producing K. pneumoniae have been described, particularly in ICUs [23]. According to previous work, this is related to the misuse of broad spectrum antibiotics, invasive procedures and the immune status of patients in these care units [24].

**Conclusion**

This study demonstrated the presence of CTX-M-15 allele within Enterobacteriaceae in a hospital in north-western Algeria and suggests that its dissemination is associated with the spread of clonal isolates, plasmids and IScep1B. These data complement those of studies conducted in other areas of the country. There is a need for regular monitoring of resistance mechanisms and the establishment of a prevention strategy combining strict compliance to hygiene rules and a more rational and appropriate use of antibiotics.

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References


