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CTX-M-3 and CTX-M-15 Extended-Spectrum β-Lactamases in Isolates of *Escherichia coli* from a Hospital in Algiers, Algeria[▽]

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Sixteen strains of *Escherichia coli* isolated between January and June 2005 in a hospital in Algiers carry the IS*Ecp1* element and the TEM and either CTX-M-3 ($n = 3$) or CTX-M-15 ($n = 13$) β-lactamases. Fourteen of the isolates are multidrug resistant. Five isolates from the neonatal ward were indistinguishable by pulsed-field gel electrophoresis.

CTX-M-type enzymes are the extended-spectrum β-lactamases (ESBL) most commonly produced by *Enterobacteriaceae* (4), and more than 55 CTX-M-type β-lactamases have been described (http://www.lahey.org/studies/webt.htm). Despite the prevalence of ESBL in *Enterobacteriaceae*, data from Algeria are scarce (although the prevalence has been reported to be 20 to 45%) (18). We investigated the phenotypic and genetic profiles of clinical *Escherichia coli* ESBL producers isolated in an Algerian hospital.

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Between January and June 2005, 279 nonduplicate *E. coli* strains were recovered consecutively from patients at the

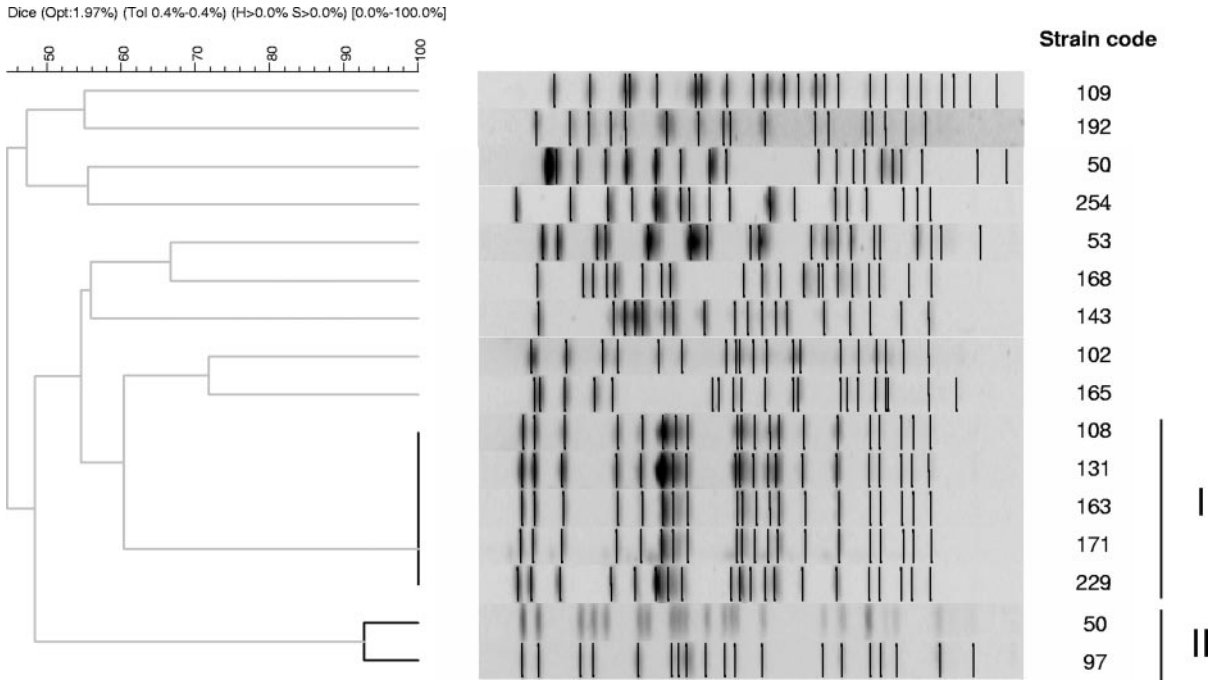

FIG. 1. Genetic relatedness of the 16 *E. coli* strains as assessed by PFGE. A band position tolerance of 0.4% was used in PFGE pattern analysis with the Dice band-based similarity coefficient. Strain code numbers are shown on the right. Clones in clusters I and II (indicated by vertical bands on the right) contain isolates of >80% similarity. Clones in cluster I were 100% identical, and those in cluster II had >90% similarity.

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Mustapha Pacha Hospital (1,800 beds) of Algiers, Algeria, and routinely analyzed in the hospital's microbiology laboratory. All strains were identified with an API 20E System (bioMérieux, Marcy l'Étoile, France). Antimicrobial susceptibility was determined by disk diffusion according to the CLSI guidelines (16), and 22 (7.9%) of the strains were resistant to extended-spectrum cephalosporins. Only 16 of these 22 were available for this study; related specimens, patient age, and ward of hospitalization are specified in Table 1. A double disk diffusion test (11) and Etest ESBL strips (AB Biodisk, Solna, Sweden), with cefotaxime and ceftazidime plus clavulanate, confirmed that all were ESBL producers.

All 16 isolates were positive for bla_{TEM}- and bla_{CTX-M}-related genes and negative for bla_{OXA} and bla_{SHV} genes as assessed by PCR using previously described specific primers (13), and all isolates carried the ubiquitous *ampC* gene (13). Isoelectric focusing confirmed that all strains expressed both TEM-derived (pI of 5.4) and CTX-M-derived (pI of 8.0 and 8.9) enzymes (Table 1). The presence of an IS*Ecp1* element upstream from bla_{CTX-M} genes and the absence of IS*26* and IS*903* elements were shown by PCR experiments (8). ExoSAP IT (USB Corporation, Cleveland, Ohio) was used for purification of PCR products, which were sequenced with an ABI3100 automatic sequencer (Applied Biosystems, Warrington, United Kingdom). Thirteen isolates carried the $bla_{CTX-M-15}$ gene and three the $bla_{CTX-M-3}$ gene; 16 isolates carried a $bla_{TEM-1B-type}$ gene.

MICs of antibiotics were determined by broth microdilution (MicroScan panel Sólo 1S; Dade Behring, West Sacramento, California): 100% of strains were resistant to gentamicin, 31% to amikacin, 88% to cotrimoxazole, and 19% to ciprofloxacin; 88% of strains were multidrug resistant (Table 1). The 13 isolates carrying both TEM and CTX-M-15 enzymes were more resistant to ceftazidime (with MICs >16 μg/ml) than the 3 CTX-M-3-plus-TEM producers (with MICs ≤0.5 to 1 μg/ml). CTX-M-15, which harbors the Asp^{240}→Gly substitution, confers higher levels of resistance to ceftazidime than its parental enzyme CTX-M-3 (17).

The diversity of the isolates was investigated by a protocol for pulsed-field gel electrophoresis (PFGE) modified from that previously described (6), using XbaI-digested genomic DNA as suggested for *E. coli* (7) (Fig. 1). PFGE was performed on a CHEF MAPPER PFGE apparatus (Bio-Rad, Hercules, California) using a run time of 24 h, with initial and final switch times of 0.1 s and 36 s, respectively. Strain INSRA5754 and the Lambda ladder (Biolabs, Beverly, MA) were used as markers for intragel normalization and intergel comparison. The PFGE profiles of five isolates producing CTX-M-15 β-lactamase from the neonatal ward were indistinguishable (100% similarity; cluster I). This suggests the spread of an epidemic clone. Two other clones producing CTX-M-3 β-lactamase were closely related (with >90% similarity; cluster II). The PFGE profiles of the other isolates were heterogeneous.

ESBL-positive *Enterobacteriaceae* are frequently isolated in hospitals in Algeria, and the overall frequency of ESBL producers at the Mustapha Pacha hospital from January to June 2005 was 20.4% (n = 217 of the 1,066 *Enterobacteriaceae* isolates): 22 of 279 (7.9%) *E. coli* isolates, 131 of 259 (50.6%) *Klebsiella* sp. isolates, 8 of 131 (6.1%) *Proteus* sp. isolates, 35 of 90 (38.9%) *Enterobacter* sp. isolates, 13 of 48 (27.1%) *Serratia*

TABLE 1. Distribution, clinical features, and phenotypic and genotypic characteristics of 16 ESBL-producing *E. coli* strains[a]

Strain	Patient age	Specimen source	Hospital ward	pIs	β-Lactamases produced	MIC (μg/ml) of[b]:						Other resistance marker(s)
						AMC	AZT	CTX	CAZ	CAZ/CA	FOX	IMP	
50	23 yr	Wound	Orthopedic surgery	5.4, 8.0	TEM-1, AmpC, CTX-M-3	≤4/2	16	>32	1	≤0.5	≤4	≤0.5	GM, TOB, AN, CHL, SXT
53	47 yr	Ascitic fluid	Gastroenterology	5.4, 8.9	TEM-1, AmpC, CTX-M-15	8/4	>16	>32	>16	≤0.5	≤4	≤0.5	GM, TOB, AN, OFX, SXT
95	4 yr	Urine	Pediatric	5.4, 8.0	TEM-1, AmpC, CTX-M-3	≤4/2	16	>32	≤0.5	≤0.5	≤4	≤0.5	GM, TOB, AN, SXT
97	32 yr	Wound	Digestive surgery	5.4, 8.0	TEM-1, AmpC, CTX-M-3	8/4	>16	>32	1	≤0.5	≤4	≤0.5	GM, TOB, SXT
102	3 yr	Urine	Pediatric	5.4, 8.9	TEM-1, AmpC, CTX-M-15	>16/8	>16	>32	>16	≤0.5	≤4	≤0.5	GM, TOB, AN, CHL, SXT
108	20 days	Urine	Pediatric	5.4, 8.9	TEM-1, AmpC, CTX-M-15	≤4/2	>16	>32	>16	≤0.5	≤4	≤0.5	GM, TOB, SXT
109	24 yr	Wound	Gastroenterology	5.4, 8.9	TEM-1, AmpC, CTX-M-15	≤4/2	>16	>32	16	≤0.5	≤4	≤0.5	GM, TOB
131	2 days	CSF	Neonatal	5.4, 8.9	TEM-1, AmpC, CTX-M-15	8/4	>16	>32	>16	≤0.5	≤4	≤0.5	GM, TOB, SXT
143	86 yr	Urine	Outpatient	5.4, 8.9	TEM-1, AmpC, CTX-M-15	8/4	>16	>32	>16	≤0.5	≤4	≤0.5	GM, TOB, AN, CHL, OFX, SXT
163	8 days	Blood	Neonatal	5.4, 8.9	TEM-1, AmpC, CTX-M-15	8/4	>16	>32	>16	≤0.5	≤4	≤0.5	GM, TOB, SXT
165	12 yr	Urine	Pediatric	5.4, 8.9	TEM-1, AmpC, CTX-M-15	>16/8	>16	>32	>16	>2	>16	≤0.5	GM, TOB, SXT
168	14 yr	Blood	Pediatric	5.4, 8.9	TEM-1, AmpC, CTX-M-15	≤4/2	>16	>32	>16	≤0.5	≤4	≤0.5	GM, TOB, SXT
171	16 days	CSF	Neonatal	5.4, 8.9	TEM-1, AmpC, CTX-M-15	≤4/2	>16	>32	>16	≤0.5	≤4	≤0.5	GM, SXT
192	9 yr	Blood	Pediatric	5.4, 8.9	TEM-1, AmpC, CTX-M-15	≤4/2	>16	>32	>16	≤0.5	≤4	≤0.5	GM, TOB, SXT
229	3 days	Blood	Neonatal	5.4, 8.9	TEM-1, AmpC, CTX-M-15	≤4/2	>16	>32	>16	≤0.5	≤4	≤0.5	GM
254	8 yr	Wound	Pediatric surgery	5.4, 8.9	TEM-1, AmpC, CTX-M-15	≤4/2	>16	>32	>16	≤0.5	≤4	≤0.5	GM, TOB, AN, OFX, SXT

[a] CSF, cerebrospinal fluid; AMC, amoxicillin-clavulanic acid; AZT, aztreonam; CTX, cefotaxime; CAZ, ceftazidime; CAZ/CA, ceftazidime-clavulanic acid; FOX, cefoxitin; IMP, imipenem; GM, gentamicin; TOB, tobramycin; AN, amikacin; CHL, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; and OFX, ofloxacin.

[b] When two values separated by a slash are given for the MIC, the first value is of the antibiotic alone and the second value is of the antibiotic in the presence of clavulanate.

sp. isolates, 2 of 19 (10.5%) *Morganella morganii* isolates, 4 of 18 (22.2%) *Citrobacter* sp. isolates, and 2 of 14 (14.3%) *Salmonella* sp. isolates. CTX-M-15 has been described in Asia, Europe, and recently Africa (2, 9, 10, 12, 19) in both nosocomial and community-acquired *E. coli* isolates (14, 21). Several studies in African countries report a high prevalence of ESBL-producing *Enterobacteriaceae* (3, 10, 18, 19). There have been reports of ESBL producers in North Africa: TEM-3 in *S. enterica* serovar Typhimurium in Morocco (1), CTX-M-27 in *S. enterica* serovar Livingstone in Tunisia (5), and CTX-M-3 in *S. enterica* serovar Senftenberg in Algeria (15). The frequency of *Enterobacteriaceae* producing ESBL in Algeria has not been reported.

The production of similar TEM and CTX-M-type enzymes in various genetically related strains and in isolates from different wards of the hospital suggests horizontal transfer of the corresponding genes. Five CTX-M-15-producing isolates were genetically indistinguishable; they were isolated from patients in the neonatal ward, except for isolate 108, which was from a patient hospitalized elsewhere for 20 days but who had previously been in this ward. Three of the patients in the neonatal ward were preterm (with 31 to 34 weeks): the patient infected with strain 171 had nosocomial meningitis, and the patients with isolates 131 and 229 had probably acquired the infection by transmission from the mother. The two cases of meningitis were cured, but the case of bacteremia was fatal.

Invasive infections due to *E. coli* isolates that produce ESBL are a major problem in neonates, because the choice of drug is restricted. The widespread use of cefotaxime and ceftriaxone has been suggested to have favored the emergence of CTX-M enzymes (20). However, treatment of infections with ESBL-producing strains in this hospital usually does not involve those antibiotics for meningitis. Therefore, this hospital may have experienced the spread of an epidemic clone not directly due to antibiotic selection pressure but with IS*Ecp1* insertion sequences, involved in the mobilization of CTX-M-enzymes, contributing to the process. Dissemination of community clones in the hospital environment is also a possibility.

To our knowledge, this is the first report of CTX-M enzymes in *E. coli* from Algeria. We show that CTX-M-15 is widespread among *E. coli* isolates which are multidrug resistant, substantially restricting therapeutic alternatives. Implementation of a strict hospital infection control policy associated with efforts to promote judicious use of antibiotics is needed. Continuous monitoring of ESBL-producing *Enterobacteriaceae* in the community and the hospital setting is also required.

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