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Letters to the Editor

Decreased Susceptibility to Cefepime in a Clinical Strain of *Escherichia coli* Related to Plasmid- and Integron-Encoded OXA-30 β-Lactamase

The most frequent extended-spectrum β-lactamases (ESBLs) in members of the family *Enterobacteriaceae* are derived from the clavulanic acid-inhibited Ambler class A enzymes TEM-1/TEM-2 and SHV-1, which may hydrolyze ceftazidime and cefepime (4, 5). The Ambler class B enzymes have the broadest hydrolysis profiles, including cefepime and ceftazidime (8), whereas several OXA-derived ESBLs (Ambler class D) usually hydrolyze more ceftazidime than cefepime (3). However, cefepime resistance combined with ceftazidime susceptibility has been detailed from *Pseudomonas aeruginosa* strains producing OXA-1-type enzyme (1). In this study, we describe a clinical isolate of *Escherichia coli* that was more resistant to cefepime than to ceftazidime due to another OXA-1 derivative, OXA-30.

Two strains of *E. coli*, Ec1484R and Ec1499S, were isolated from the urinary tract of a 70-year-old patient. This man was admitted to a neurological ward for a post-herpes zoster encephalopathy. He did not have any history of antimicrobial therapy. Randomly amplified polymorphic DNA (RAPD) with primer AP12h and enterobacterial repetitive intergenic consensus sequence (ERIC)-PCR with primers ERIC2 and ERIC1R (10) gave the same pattern for Ec1484R and Ec1499S, compared to distinct and different profiles obtained with two unrelated *E. coli* strains used as controls (data not shown). According to MIC results, Ec1484R exhibited resistance to aminopenicillins and carboxypenicillins, alone or combined with β-lactamase inhibitors, and showed a decreased susceptibility to cefepime, cefpirome, and cefotaxime, in contrast to susceptibility to ceftriaxone, cefoxitin, and ceftazidime (Table 1). This strain was also resistant to sulfonamides, streptomycin, and spectinomycin, which were cotransferred with β-lactam resistance by the filter-mating technique to a rifampin- and nalidixic acid-resistant *E. coli* K12 strain (conjugation rate, 4×10^{-5}). By isoelectric focusing analysis, Ec1484R and the transconjugant Tc1484 produced a single β-lactamase with pI value of 7.3, and plasmid DNA analysis identified a large plasmid (size of >70 kb). Amplification with primers specific for $bla_{\text{OXA-1}}$ (6) and subsequent sequencing revealed the presence of the $bla_{\text{OXA-30}}$ gene, which was recently described for *Shigella flexneri* from Chinese isolates (11). Since most of the bla_{OXA} genes are integron located, specific amplifications with integron-specific primers (9) and sequencing demonstrated that $bla_{\text{OXA-30}}$ was part of the first cassette of a class 1 integron, adjacent to a second cassette containing the adenyltransferase-encoding gene *aadA1* responsible for streptomycin and spectinomycin resistance (GenBank accession no. AY224185). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis did not show any evidence of outer membrane protein deficiency (data not shown). The $bla_{\text{OXA-30}}$ gene was cloned into the pBK-CMV vector, and the resulting recombinant plasmid, pCOI, was expressed in *E. coli* TOP10. After β-lactamase purification, performed as described previously (7), the specific activities of several β-lactams were determined, indicating that OXA-30 hydrolyzed cefepime and cefpirome more than ceftazidime and cefotaxime (Table 2).

TABLE 1. MICs of β-lactams for the *E. coli* strains used in this study

β-Lactam[a]	MIC (μg/ml)			
	Ec1499S	Ec1484R	Tc1484	K12
Ampicillin	8	>512	>512	8
Ampicillin + CLA	4	>512	>512	4
Ticarcillin	4	>512	>512	2
Ticarcillin + CLA	4	512	>512	2
Cephalothin	8	8	8	8
Cefoxitin	4	4	2	2
Ceftriaxone	0.05	0.1	0.05	0.05
Cefotaxime	0.05	0.5	0.2	0.02
Ceftazidime	0.2	0.2	0.1	0.1
Cefepime	0.05	4	2	0.02
Cefpirome	0.02	0.2	0.5	0.01

[a] CLA, clavulanic acid at a fixed concentration of 2 μg/ml.

TABLE 2. Specific activities of OXA-30 expressed from the recombinant plasmid pCOI in *E. coli* TOP10

Substrate (100 μM)	Sp act (mU/mg)[a]
Benzylpenicillin	7.61
Cephalothin	0.68
Amoxicillin	15.27
Cloxacillin	16.08
Ticarcillin	6.34
Aztreonam	0.21
Ceftazidime	0.03
Cefotaxime	0.20
Cefepime	3.58
Cefpirome	3.84

[a] Standard deviations were within 10%.

The present work is the first report of an *E. coli* clinical isolate in France that produces an OXA-1-like enzyme. Thus, this OXA-type enzyme may have also spread in members of the family *Enterobacteriaceae*, not only in *P. aeruginosa* (1, 2). Extensive use of cefepime may lead to the emergence of such an enzyme and subsequently select cefepim-resistant *Enterobacteriaceae*.

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