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Letter to the Editor

Characterization of an Inhibitor-Resistant TEM (IRT) β-Lactamase in a Novel Strain of *Klebsiella pneumoniae*

Since 1991, inhibitor-resistant TEM (IRT) β-lactamases have been described to be present in *Escherichia coli* clinical strains (4, 6, 8). The IRT-producing strains are resistant to penicillins and to their combinations with β-lactamase inhibitors but remain susceptible in vitro to cephalosporins. Twelve IRT-type β-lactamases have been described so far: TEM-30 to TEM-41 (IRT-1 to IRT-12) (7).

In 1994, we isolated a clinical strain of *Klebsiella pneumoniae* (Kp1018) which was highly resistant to amoxicillin (MIC, >1,024 mg/liter) and ticarcillin (MIC, 1,024 mg/liter) and also to their combinations with clavulanic acid (respective MICs, 1,024 and 128 mg/liter); however, it was susceptible to cephalothin (MIC of 8 mg/liter compared with a MIC susceptibility breakpoint of ≤8 mg/liter) and cefuroxime (MIC, 2 mg/liter). This resistance was transferred to *E. coli* C600 (azide resistant). The clinical isolate Kp1018 and its transconjugant synthesized a β-lactamase with a pI of 5.2, similar to that of the TEM-30, -31, -35, -36, -37, -38, and -41 enzymes (3, 7). Hybridization experiments with a $bla_{\text{TEM-1}}$ probe demonstrated that this β-lactamase was a TEM-derived enzyme. The plasmid DNA of Kp1018 carried a single resistance gene encoding the inhibitor-resistant β-lactamase, which may explain its relatively small size of about 42 kb. Plasmids encoding the IRT β-lactamases previously described to be present in two *K. pneumoniae* isolates were larger (>180 kb) (5). After PCR amplification, the sequence analysis showed that the Kp1018 IRT-type gene differed from the TEM-1 sequence by a single substitution, Arg→Ser-244, as described for the TEM-30 (IRT-2) β-lactamases (1, 5). The residue Arg-244 has been demonstrated to play a role in maintaining the integrity of the active site, and also in the catalytic apparatus of the TEM-1 enzyme (9). Its substitution by a cysteine (in the TEM-31 or IRT-1 β-lactamase) or a serine (in the IRT-2 β-lactamase) leads to the production of mutant β-lactamases which exhibit reduced affinities for clavulanic acid and other β-lactams and reduced efficiency of their enzymatic activity and inhibition (9). In addition, the nucleotide sequence of the Kp1018 IRT gene revealed two silent mutations in comparison with the TEM-1A gene, at positions 436 (C→T) and 604 (G→C). The same mutations have been recently found in the IRT-2b gene of a strain of *K. pneumoniae* (5). We report here the third clinical isolate of *K. pneumoniae* producing an IRT β-lactamase, exhibiting characteristics closely related to those of the IRT-2 enzymes. So far, the IRT β-lactamases have been found almost exclusively in strains of *E. coli*. However, our study confirms the diffusion of this mechanism of resistance to *K. pneumoniae*, a species frequently implicated in nosocomial infections and known to easily develop new resistances to antimicrobial agents, such as the production of extended-spectrum β-lactamases. Very recently, IRT-2-producing strains of *Proteus mirabilis* have been described (2). As expected for self-transferable plasmid-borne resistances, IRT β-lactamases appear to disseminate among enterobacteria. The diffusion to other types of bacteria known to frequently produce TEM-1 β-lactamases, such as *Haemophilus influenzae* or *Neisseria gonorrhoeae*, might be considered.

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