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# First Class A Carbapenemase Isolated from *Enterobacteriaceae* in Argentina

Although many β-lactamases have been described as conferring resistance to β-lactam antibiotics, including extended-spectrum cephalosporins, production of acquired carbapenemases remains infrequent in *Enterobacteriaceae*.

According to the National Survey for Antibiotic Resistance in Argentina (SIR Program) during 2000, carbapenem resistance was reported in only one enterobacterial isolate (out of 6,126 tested), identified as *Enterobacter cloacae* (1 of 128 strains). From 4,964 enterobacterial strains isolated during 2001, no carbapenem-resistant isolates were reported.

Acquired carbapenemase ocurrence is already rare, although some belonging to molecular classes A, B and D have been described. Sme-1 to Sme-3, NMC-A, IMI-1, and KPC-1 to KPC-3 are, to date, the only known class A carbapenemases (1, 4, 5) (T. Hong, E.S. Moland, B. Abdalhamid et al., K. Young, P. Tierno, Jr., L. Tysall et al., Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C1-665, 2003; K. Young, P. Tierno, Jr., L. Tysall et al., Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-50, 2003). All but KPC enzymes have greater activity against imipenem than meropenem, conferring resistance to penicillins, cephalosporins, and aztreonam; oxymino-cephalosporins are weak substrates (8). They are well inhibited by clavulanate and tazobactam.

*E. cloacae* D was isolated from blood cultures of a leukemic patient at the Hospital Israelita, Buenos Aires, Argentina, during 2000. Susceptibility tests were performed according to the recommendations of the National Committee for Clinical Laboratory Standards. The strain was resistant to imipenem and had intermediate resistance to meropenem (32 and 8 μg/ml, respectively), but was susceptible to ceftriaxone (0.125 μg/ml), ceftazidime (0.5 μg/ml), and cefepime (0.06 μg/ml). Clavulanate improved the activity of carbapenems by a factor of 4. Trimethroprim-sulfamethoxazole, aminoglycosides, polymyxin, and quinolone susceptibilities were conserved.

Double-disk diffusion tests were performed with EDTA and amoxicillin-clavulanate disks placed near carbapenem disks. The β-lactamase was inhibited by the latter, suggesting the presence of a serine β-lactamase.

The crude extract displayed two active bands after isoelectric focusing at apparent pIs of 5.4 (characterized as TEM-1) and 6.9 that were active on 500-μg/ml ampicillin; the latter was also active on 1,000-μg/ml imipenem, according to the iodometric overlay system (7).

A 2,058-bp fragment was amplified by PCR with specific primers for *nmcA* (NMC1, 5′-GCATTGATATACCTTTAGC AGAGA-3′; and NMC4, 5′-CGGTGATAAAATCACACTG AGCATA-3′) (3), with genomic DNA as a template. No positive reaction could be obtained on plasmid DNA preparations. The amplicon was cloned at *Sma*I site of a pK19 vector (kanamycin resistance) (6) and introduced by transformation in competent *Escherichia coli* Top10 cells (Invitrogen). Transformants were selected on Luria-Bertani agar plates containing isopropyl-β-D-thiogalactopyranoside (IPTG; 1 mM), 5-bromo--4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal; 40 μg/ml),and kanamycin (30 μg/ml).

The sequence of this fragment showed the presence of two genes and an intercistronic region (the putative binding site for NMC-R) identical to *nmcA* and its regulator, *nmcR* (AJ536087).

Transformant TKC-1, expressing these genes, was also moderately resistant to carbapenems (MICs of 16 and 4 μg/ml for imipenem and meropenem, respectively). No inducibility could be detected by double-disk tests with ampicillin-sulbactam, despite the fact that some induction could be detected in the original strain. Inhibition of the enzyme was observed when both inhibitors were used in the same test with the transformant.

We describe herein the detection of one imipenem-resistant *E. cloacae* isolate due to the production of a class A carbapenemase, NMC-A, corresponding to the first detection of this class of enzymes in South America.

It is interesting to point out that, except for plasmid KPCs, the other class A carbapenemases have been chromosomally encoded in microorganisms in which there is a functional regulatory system ending in AmpC-AmpR, reinforcing the general concept that some (or all) of the regulatory system components play a role in the expression of these enzymes reported before (2).

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