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First Isolation of bla_{VIM-2} in *Klebsiella oxytoca* Clinical Isolates from Portugal

VIM-type carbapenemases were originally detected in Europe (2, 4, 7) and have been essentially found in *Pseudomonas aeruginosa*, as well as in *Enterobacteriaceae* (2, 6, 8). The bla_{VIM} genes are often carried by mobile gene cassettes inserted into class 1 integrons (7, 11). The *Klebsiella* genus is responsible for the most frequent human nosocomial infections of the respiratory and urinary tracts. Four *Klebsiella oxytoca* clinical isolates were recovered by blood culture from neonatal patients at a pediatric hospital. The clinical isolates showed resistance to amoxicillin and ticarcillin, which was restored by clavulanate, aminoglycosides, and fluoroquinolones, and intermediate susceptibility to imipenem (MIC, 4 μg/ml) and broad-spectrum cephalosporins and aztreonam (MICs, 8 and 16 μg/ml) and were susceptible to meropenem (MIC, 0.075 μg/ml). A deformation of ellipses between the two gradient sections with E-test MBL (metallo-β-lactamase) strips was indicative of MBL production (10).

Plasmid DNA was prepared by the alkaline lysis method (9), and the same 50-kb plasmid was detected in all of the isolates. Conjugation experiments were attempted with rifampin-resistant strain *Escherichia coli* C600 in liquid and solid media. *E. coli* DH10B was used as the bacterial host in electroporation experiments. No resistance phenotype transfer was observed in conjugation or transformation experiments.

M13 PCR fingerprinting, used as a typing method, was performed by using the core region of bacteriophage M13 as the primer (3). The four isolates have the same M13 profile.

Since several β-lactamase genes are part of gene cassettes included in class 1 integrons, PCR was performed for class 1 integrons with 5′-CS and 3′-CS primers (5) on plasmid DNA from *K. oxytoca* 17FFUL purified by a QIAGEN Plasmid Midi Kit, yielding an amplicon with a size of 3,500 bp. Consecutive PCR amplifications with specific primers for MBL and aminoglycoside acetyltransferase genes were performed. Analysis of sequence data revealed the presence of four gene cassettes in the class 1 integron. The first cassette contained the bla_{VIM-2} gene, coding for an MBL. The second and the third cassettes contained genes coding for 6′-*N*-aminoglycoside acetyltransferase (*aacA4*) and an aminoglycoside adenylyltransferase (*aadA1*), respectively. The last gene cassette contains a bla_{OXA-2} gene coding for an oxacillinase.

As in *P. aeruginosa* COL-1 (7), the bla_{VIM-2} gene was found to be carried on a plasmid-borne integron. However, the VIM-2-encoding plasmids and the structure of the bla_{VIM-2} gene cassette were different in the two bacteria. Key signatures of class 1 integrons were identified, such as a 5′-CS *attI1* recombination site, a core site (GTTATGC), and an inverse core site (GCATAAC). The 59-base element was 72 bp long as the 59-base element of the bla_{VIM-2} gene cassette in COL-1 but differed by three nucleotides, showing 95.8% homology.

A chromosomal bla_{VIM-2} gene in a *P. aeruginosa* clinical isolate was already detected in Portugal (1), and the detection of a plasmid-borne bla_{VIM-2} gene in *K. oxytoca* demonstrates that the problem of MBL-producing pathogens no longer involves gram-negative nonfermenters alone but also involves enterobacteria.

This study found that a clonal VIM-2 spread occurred in the pediatric ward during the study.

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