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Intercontinental Dissemination of IMP-13-Producing *Pseudomonas aeruginosa* Belonging in Sequence Type 621[▽]

IMP-type metallo-β-lactamases (MBLs) were the first acquired MBLs detected in Gram-negative pathogens, in the early 1990s, and are among the most relevant due to their worldwide distribution (12, 18). Several IMP-type variants have been described (3). IMP-13 was first detected in clinical isolates of *Pseudomonas aeruginosa* from Italy (16), where IMP-13 has become a widespread carbapenem resistance determinant, even involved in relatively large outbreaks (11, 13, 17). IMP-13 was also occasionally detected in *P. aeruginosa* isolates from other European countries, including Austria (7), Romania (9), and France (6). Interestingly, the IMP-13-producing Italian isolates were found to belong in the same clonal lineage (13), and a clonal relationship with this lineage was also demonstrated for a Romanian isolate (9). In these isolates, the $bla_{\text{IMP-13}}$ gene was found to be carried in different class 1 integron structures (InPSG, In88, and In89), usually located on the chromosome or, more rarely, on plasmids (11, 13, 16).

IMP-13-producing *P. aeruginosa* isolates have also been detected in Argentina, where IMP-13 and IMP-16 represent the only IMP-type MBLs thus far reported (1, 4, 15). In this work, we investigated 20 IMP-13-producing *P. aeruginosa* isolates (19 clinical isolates and 1 environmental isolate) from an outbreak in Argentina (15) and compared them with *P. aeruginosa* AV65, as a representative of the IMP-13-producing clone spreading in Italy (13). The IMP-13-producing isolate from Romania (Pa247), previously described as clonally related to the Italian clone (9), was also included in the comparative analysis.

Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) were performed according to published protocols (5, 10). Sequence type (ST) numbers were assigned using the pubMLST database (http://pubmlst.org/paeruginosa/). Characterization of the variable region of class 1 integrons and their 5′ flanking sequences were performed by a PCR mapping and sequencing approach as previously reported (11, 16). The chromosomal or plasmid location of the β-lactamase gene was investigated by the I-CeuI and S1 nuclease-mapping techniques as described previously (2, 8). Under the assayed conditions, S1 digestion converts plasmids to linear DNA forms without cutting the genomic DNA (2), while I-CeuI only cuts into the genomic DNA (8).

Following digestion with SpeI, all the Argentinean isolates exhibited PFGE profiles that were identical or different by no more than three bands (Fig. 1), revealing clonal relatedness. A total of 8 clonal variants (A_1 to A_8) were identified (Fig. 1). The PFGE profiles of the Argentinean isolates appeared to be also related with that of AV65, with a difference of 2 to 6 bands (Fig. 1), suggesting a possible clonal relatedness between the Argentinean isolates and the Italian epidemic clone.

By MLST analysis, the AV65 and Pa247 isolates and all the Argentinean isolates were identified as belonging in ST621. Interestingly, the same ST was also reported for the IMP-13-producing isolate from Austria (7). Altogether, these results revealed a common genetic lineage for the IMP-13-producing *P. aeruginosa* isolates circulating worldwide.

Characterization of the variable regions of the class 1 integrons carrying the $bla_{\text{IMP-13}}$ cassette from the *P. aeruginosa* isolates from Argentina revealed structures identical to those previously found in European isolates. In particular, most Argentinean isolates harbored integron InPSG (11, 13), which was also the most common integron platform among Italian isolates (13) and that found in isolates from Romania (9) and Austria (7). Moreover, sequence analysis showed that InPSG from the Argentinean isolates was inserted into a Tn*5051*-like transposon at the same *res* insertion site already described for the InPSG-harboring Italian isolates (11), suggesting a common origin for these genetic structures. In the remaining Argentinean isolate, the $bla_{\text{IMP-13}}$ cassette was carried as a single gene cassette by In88, which was also described to occur in one of the IMP-13-producing Italian isolates (13) and could be derived from InPSG by excision of the *aacA4* cassette present in the second position.

Efforts aimed at purifying plasmids carrying $bla_{\text{IMP-13}}$ (14) or transferring the resistance determinant by conjugation (11) or transformation (14) were unsuccessful. Southern blot hybridization, using a $bla_{\text{IMP-13}}$ probe and a 16S rRNA probe after I-CeuI digestion and PFGE separation, showed that the bla_{IMP} gene was chromosomally located, since the bands that hybridized with the bla_{IMP} probe also hybridized with the 16S rRNA probe. Southern blot analysis of PFGE of SpeI-restricted genomic DNAs with the bla_{IMP} probe detected the resistance marker in the same fragment in all isolates, while for a single isolate the resistance marker was also detected in an additional fragment, suggesting the possibility of *in vivo* rearrangement events when the resistance determinant is carried on transposable genetic structures (data not shown).

The overall genomic relatedness of the IMP-13-producing isolates and the fact that the $bla_{\text{IMP-13}}$ gene was inserted in a conserved genetic context point to a common ancestry for the

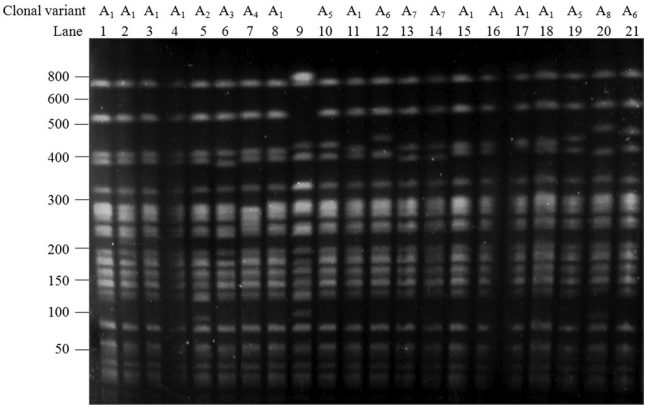

FIG. 1. PFGE patterns of IMP-13-producing *P. aeruginosa* isolates. Lane 9, Italian isolate AV65; lane 15, IMP-13-producing environmental isolate from Argentina; Lanes 1 to 8, 10 to 14, and 16 to 21, IMP-13-producing clinical isolates from Argentina. DNA size standards are indicated on the left.

IMP-13-producing strains disseminated in South America and Europe and underscore the role of the ST621 clonal lineage as a highly successful *P. aeruginosa* epidemic clone.

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