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Plasmid-Mediated Rifampin Resistance Encoded by an *arr-2*-Like Gene Cassette in *Klebsiella pneumoniae* Producing an ACC-1 Class C β-Lactamase

For *Pseudomonas* spp., two plasmid-mediated mechanisms of resistance to rifampin have been reported: one involves an efflux pump (1), and the other involves an ADP-ribosylating transferase (3) encoded by a gene (*arr-2*) that is integrated as a gene cassette in a class 1 integron (10). Recently, plasmid-mediated rifampin resistance (Arr-2) was described for various *Enterobacteriaceae* from Southeast Asia producing the extended-spectrum β-lactamase VEB-1 (4, 6). The genes encoding these mechanisms of resistance are integron located as gene cassettes (4, 6).

We recently described an outbreak in France of *Klebsiella pneumoniae* isolates that produce the ACC-1 β-lactamase and that are resistant to rifampin, the first of which (strain SLK54) was isolated from a patient previously hospitalized in Tunisia (7). Resistance to ceftazidime and rifampin was cotransferred by conjugation to *Escherichia coli* K-12 strain HB101 for all strains (rifampin MICs, >256 μg ml^{-1}) except one (KP SLK55) (rifampin MIC, 16 μg ml^{-1}). Plasmid DNA from the transconjugant *E. coli* HB101 of the first isolate in the outbreak (*K. pneumoniae* SLK54) was partially digested with *Sau*3A (Roche Molecular Biochemicals, Meylan, France) and ligated (T4 DNA ligase; Amersham Pharmacia Biotech, Saclay, France) into the *Bam*HI site of the cloning vector pBK-CMV Kan^r (Stratagene, La Jolla, Calif.) (8). The recombinant plasmid, pRIF-1, which harbored the smallest insert (2 kb) was sequenced (9). Analysis of the DNA sequence revealed an open reading frame of 453 nucleotides (Fig. 1) predicting a 150-amino-acid sequence. This protein was 99% identical to the rifampin ADP-ribosylating transferase Arr-2. The predicted rifampin ADP-ribosylating transferase differed from Arr-2 by a Lys98Arg substitution. In *Pseudomonas aeruginosa* the location of the gene encoding this protein is unclear (10), although in *Enterobacteriaceae* it has been found to be plasmid located (4, 6). However, in both cases, the *arr-2* gene was a gene cassette located in a class 1 integron. In *K. pneumoniae* SLK54, the plasmid-mediated *arr-2*-like gene was also a gene cassette, located immediately downstream of the integrase and the promoter region (Fig. 1). The expression of the *arr-2*-like gene is probably driven by the P_{ant} promoter and not the P2 promoter because the insertion of three G residues increasing the spac-

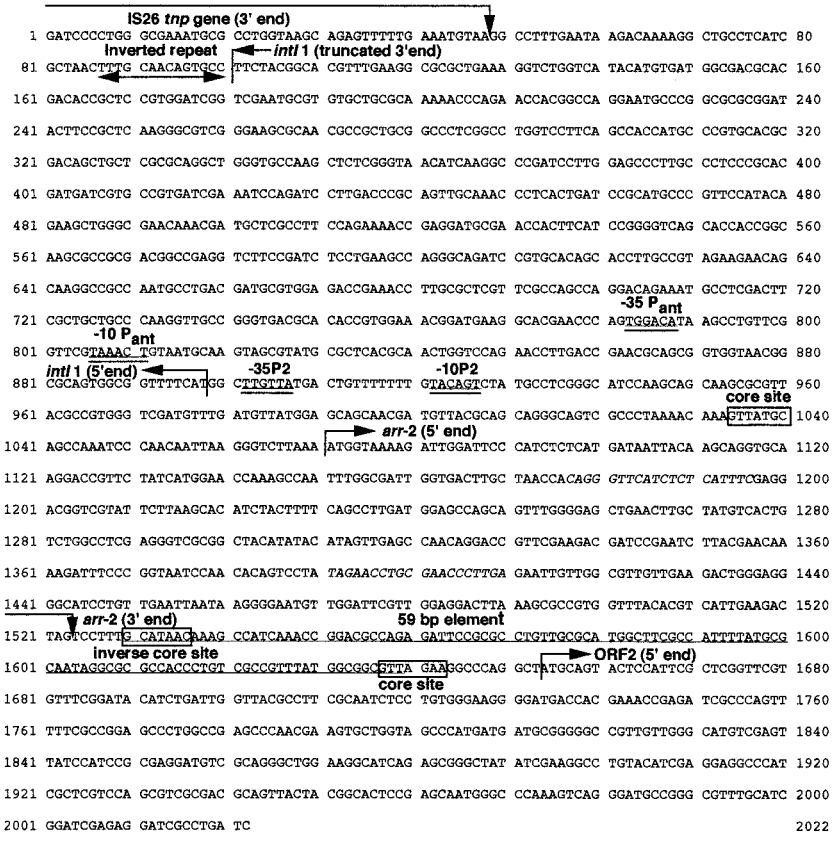

FIG. 1. Nucleotide sequence of the *arr-2* gene and its genetic environment. The 5′ and 3′ ends, the −35 and −10 promoter regions, the core sites and inverse core sites, and the 59-bp element are indicated.

ing between the −35 and −10 boxes to 17 was absent in the P2 promoter (Fig. 1) (2). The configuration of the P_{ant} promoter was a hybrid combining the −35 box (TGGACA), found in weak P_{ant} promoters, and the −10 box (TAAACT), found in strong P_{ant} promoters, and has been shown to have intermediate strength (5). The 5′ coding sequence of the integron, corresponding to the last 214 bp (71 amino acids) of the integrase gene, was truncated by the insertion of the IS26 sequence and resulted in a nonfunctional integrase. A similar observation was made for In53, which carries the *arr-2* gene cassette in *E. coli* (6), in which the IS26 truncated the integrase gene and especially the P_{ant} promoter.

The rifampin ADP-ribosylating transferase Arr-2 was previously detected in strains of *P. aeruginosa* and *Enterobacteriaceae* isolated from patients in Southeast Asia. These strains also produce the VEB-1 extended-spectrum β-lactamase (4, 6, 10). We now report the plasmid-mediated rifampin resistance encoded by the *arr-2*-like gene cassette in *K. pneumoniae* which also produces the plasmid-mediated cephalosporinase ACC-1 and which was isolated in France from a patient previously hospitalized in Tunisia (7).

The EMBL accession number for the nucleotide sequence reported in this paper is AJ277027.

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