ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2003, p. 2838–2843 Vol. 47, No. 9
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.9.2838–2843.2003

Molecular Characterization of a New Class 3 Integron in *Klebsiella pneumoniae*

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Received 3 March 2003/Returned for modification 21 April 2003/Accepted 10 June 2003

***Klebsiella pneumoniae* FFUL 22K was isolated in April 1999 from the urine of an intensive care unit patient in Portugal. The strain showed an extended-spectrum cephalosporin resistance profile. A typical synergistic effect between cefotaxime or cefepime and clavulanic acid was observed. An *Escherichia coli* transformant displayed a similar resistance phenotype and harbored a ca. 9.4-kb plasmid (p22K9). Cloning experiments revealed that the extended-spectrum β-lactamase was encoded by bla_{GES-1}, previously described in class 1 integrons from *K. pneumoniae* ORI-1 and *Pseudomonas aeruginosa* Pa695. Further sequence analysis demonstrated that the bla_{GES-1} gene cassette was located on a new class 3 integron. The integron was 2,863 bp long and consisted of an *intI3* integrase gene, an *attI3* recombination site, two promoter regions, and two gene cassettes. The IntI3 integrase was 98.8% identical to that of *Serratia marcescens* AK9373. The bla_{GES-1} gene cassette was inserted at the *attI3* site. The second gene cassette was the result of a fusion event between bla_{OXA-10}-type and *aac(6′)-Ib* gene cassettes and conferred resistance to kanamycin. This is the second class 3 integron reported and the first time that the bla_{GES-1} gene cassette has been found on an integron belonging to this class, highlighting the considerable heterogeneity of their genetic environment and the spread of gene cassettes among different classes of integrons.**

Many of the antibiotic resistance genes found in clinical isolates of gram-negative bacteria are contained in discrete mobile elements known as gene cassettes, located in integrons (19, 20, 21, 45). The integron encodes a site-specific recombinase (IntI) that belongs to a distinct family of the tyrosine recombinase superfamily (10), responsible for the insertion of gene cassettes at *attI*, and also provides the promoter responsible for expression of the cassette-encoded genes (5).

Ten classes of integrons have been identified, five of them associated with gene cassettes that codify antibiotic resistance (2, 7, 19, 21, 22, 23, 30, 37, 45; GenBank accession number AJ277063). However, the integrons most commonly isolated from resistant clinical isolates of members of the family *Enterobacteriaceae* belong to class 1. To date, only a single class 3 integron, isolated from a carbapenem-resistant *Serratia marcescens* strain, has been described (2). Characterization of this integron by Collis et al. (10) revealed that the integron module, consisting of the *intI3* gene, *attI3* site, and P_c promoter, is configured in the same way as the class 1 integron module.

Among the β-lactamase genes, bla_{VEB-1} was the first gene cassette identified encoding a class A enzyme that possesses extended-spectrum properties (34, 35, 44). Subsequently bla_{GES-1} and its homologous bla_{IBC-1}, bla_{GES-2} and bla_{IBC-2} gene cassettes were found in several class 1 integrons (15, 16, 29, 40, 41, 42).

In this work, we report the analysis of the *Klebsiella pneumoniae* FFUL 22K clinical isolate exhibiting extended-spectrum cephalosporin resistance. Cloning experiments revealed the presence of a new class 3 integron on a small plasmid.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this work are listed in Table 1.

Antibiotic susceptibility testing. The antibiotic susceptibilities of *K. pneumoniae* clinical isolate and *Escherichia coli* recombinant strains were determined by the disk diffusion method (36). The double-disk synergy test between clavulanic acid and extended-spectrum cephalosporins was used to detect production of extended-spectrum β-lactamases (4). The MICs of selected β-lactam antibiotics were determined by the E-test method (AB Biodisk, Solna, Sweden).

Isoelectric focusing. Analytical isoelectric focusing of crude cell extracts from *K. pneumoniae* FFUL 22K and *E. coli* transformants was performed in polyacrylamide gels containing ampholytes (pH 5.0 to 8.0; Amersham Biosciences Europe, Lisbon, Portugal) with a Multiphor II apparatus (Amersham Biosciences Europe, Lisbon, Portugal). The focused β-lactamases were detected after overlaying the gels with nitrocefin (Calbiochem EMD Biosciences, San Diego, Calif.), and the isoelectric points were determined and compared to those of known β-lactamases (4).

Plasmid content and transformation experiments. Plasmids were extracted from *K. pneumoniae* FFUL 22K and *E. coli* transformants by the alkaline lysis method (46). The plasmids isolated from *K. pneumoniae* FFUL 22K were used to transform *E. coli* DH5α competent cells with $CaCl_2$, with selection on Luria-Bertani (LB) agar plates containing ceftazidime (10 μg/ml) (GlaxoSmithKline, Lisbon, Portugal).

Cloning experiments. Plasmid DNA from *E. coli* DH5α(p22K9) was digested with *Hin*dIII (New England Biolabs Inc., Beverly, Mass.), and the fragments were purified from the agarose gel electrophoresis with the Concert rapid gel extraction system (Gibco-BRL Invitrogen Ltd., Paisley, United Kingdom). The purified restriction fragments were ligated to *Hin*dIII-linearized pBK-CMV phagemid (Stratagene Europe, Amsterdam, The Netherlands), and the ligation mixture was used to transform *E. coli* TOP10 One Shot chemically competent cells (Invitrogen Ltd., Paisley, United Kingdom). *E. coli* TOP10 cells harboring recombinant plasmids were selected on LB agar plates containing kanamycin (30 μg/ml) (Sigma Aldrich Química, Sintra, Portugal) and ampicillin (50 μg/ml) (Sigma Aldrich Química, Sintra, Portugal).

PCR experiments. Sets of primers were used for detection of bla_{TEM} genes (TEM-A and TEM-B) (4) and amplification of the class 1 integron variable

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype	Reference or source
Strains		
K. pneumoniae FFUL 22K	Extended-spectrum cephalosporin-resistant clinical isolate	This study
E. coli DH5α	*supE44* Δ*lacU169* (ϕ80*lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Gibco-BRL
E. coli TOP10	F^- *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*ΔM15 Δ*lacX74 recA1 deoR araD139* Δ(*ara-leu*)*7697 galU galK rpsL* (Str^r) *endA1 nupG*	Invitrogen
Plasmids		
p22K9	Natural plasmid from *K. pneumoniae* FFUL 22K	This study
pBK-CMV	Cloning vector, Kan^r Neo^r	Stratagene
pMFA-1	Recombinant pBK-CMV plasmid with a 1,284-bp *Hind*III insert from p22K9 containing part of the class 3 integron and the bla_{GES-1} gene	This study
pMFA-2	Recombinant pBK-CMV plasmid with a 1,388-bp amplicon containing the entire P_c promoter region from the integron and the bla_{GES-1} gene	This study

region (5′-CS and 3′-CS) (28) on plasmid p22K9, with Ready-to-go PCR beads (Amersham Biosciences Europe, Lisbon, Portugal). Confirmation of class 3 integrase was done with primers *intI3*F and *intI3*R (17) and that of the bla_{GES-1} gene with primers GES-1A and GES-1B (42). With the published sequences of the *intI3* (2, 10) and bla_{GES}/bla_{IBC} genes (15, 16, 29, 40, 41, 42), primers *intI3*P (5′-AATCCGCTTGCGTTCTGG-3′) and GES-1M (5′-CCAATTTCTCTCAG TAAGAGG-3′) were designed to amplify the promoter region of class 3 integron and the first half of the bla_{GES-1} gene cassette.

Cloning of PCR amplicons. For cloning and expression of bla_{GES-1} gene with the P_c promoter, primers *intI3*P and GES-1B were used to amplify a 1,388-bp fragment of the class 3 integron. The amplicon was cloned into the *Sma*I site of pBK-CMV (46), and the ligation mixture was used to transform *E. coli* TOP10 One Shot chemically competent cells. Transformants harboring recombinant plasmids were selected on LB agar plates containing kanamycin (30 μg/ml) and ceftazidime (50 μg/ml).

DNA sequencing. DNA sequences were determined on both strands by the dideoxy chain termination method with the Big Dye terminator cycle sequencing kit (Applied Biosystems, Porto, Portugal) and analyzed with an ABI Prism 310 automatic sequencer (Applied Biosystems, Porto, Portugal). The sequences of the cloned DNA in pMFA-1 and of the natural plasmid p22K9 were determined with published or laboratory-designed sequencing primers (gene walking). The nucleotide and the deduced protein sequences were analyzed with the Blast (1) and Clustal W (51) programs, as previously described (12).

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper are available in the GenBank/EMBL/DDBJ sequence databases under accession number AY219651.

RESULTS

K. pneumoniae FFUL 22K was isolated in April 1999 at the Hospital Santa Maria (Lisbon, Portugal) from the urine of a patient hospitalized in an intensive care unit with respiratory problems.

K. pneumoniae was resistant to amino- and carboxy-penicillins and associations with clavulanic acid, cefoxitin, cefuroxime, ceftazidime, cefpirome, gentamicin, kanamicin, netilmicin, nalidixic acid, and fluoroquinolones. It showed intermediate susceptibility to cefotaxime, aztreonam, ceftriaxone and amikacin, and was susceptible to cefepime, and imipenem (13). Synergies were observed between clavulanic acid-amoxicillin and cefotaxime, aztreonam, and cefepime, suggesting the production of an extended-spectrum β-lactamase.

Localization and transfer of β-lactamase gene. Since acquired resistance determinants in *K. pneumoniae* are often carried on plasmids, the presence of plasmid DNA was investigated by the alkaline lysis method. Agarose gel electrophoresis revealed the presence of three plasmids, ca. 9.4, 6.3, and 3 kb.

Conjugation experiments were not attempted because *K. pneumoniae* FFUL 22K was resistant to the selective markers of the *E. coli* strains available at our laboratory. The plasmid preparation was used to transform *E. coli* DH5α. After selection on LB agar plates with ceftazidime (10 μg/ml), an *E. coli* transformant that harbored the natural 9.4-kb plasmid, named p22K9, was obtained.

E. coli DH5α(p22K9) displayed a resistance phenotype similar to that of the *K. pneumoniae* strain except that it was susceptible to ticarcillin-clavulanic acid, cefoxitin, cefotaxime, aztreonam, ceftriaxone, gentamicin, netilmicin, and amikacin (data not shown). Also, potentiation of cefotaxime and aztreonam activity by clavulanate was observed.

Isoelectric focusing showed production of a β-lactamase with an apparent isoelectric point of 5.9 in both *K. pneumoniae* FFUL 22K and the transformant strain. A second enzyme with a pI of 7.6 was also produced by *K. pneumoniae*, most probably representing a SHV-1 chromosomal β-lactamase.

Cloning and sequencing of β-lactamase gene. Preliminary PCR-based experiments failed to detect bla_{TEM} genes and class 1 integrons in the *E. coli* DH5α transformant.

Plasmid DNA from *E. coli* DH5α(p22K9) was digested with restriction endonuclease *Hin*dIII and ligated to pBK-CMV. The ligation product was transformed into *E. coli* TOP10, and recombinant clones obtained after selection on kanamycin- and ampicillin-containing plates were analyzed. Restriction analysis of the recombinant plasmid pMFA-1 revealed a 1.2-kb insert, and unlike the parental strain, *E. coli* TOP10(pMFA-1) showed resistance to aminopenicillins.

Analysis of the nucleotide sequence from the 1,212-bp insert in pMFA-1 revealed an 864-bp-long open reading frame encoding GES-1 β-lactamase. Also in this case, bla_{GES-1} appeared to be part of a mobile gene cassette inserted into a class 3 integron-like structure that was named In3-p22K9 (Fig. 1; see below for integron and cassette descriptions).

Molecular characterization of class 3 integron. To further analyze the structure of In3-p22K9, gene walking was performed on the natural plasmid p22K9. The class 3 integron was 2,863 bp long and consisted of an *intI3* integrase gene, two (P_c and P_{int}) promoter regions, an *attI3* recombination site, a bla_{GES-1} gene cassette, and a fused bla_{OXA-10}-type/*aac(6′)-Ib* gene cassette (Fig. 1).

The *intI3* gene encodes an integrase that is 98.8% identical to the IntI3 from *S. marcescens* AK9373 (2, 10). Four substi-

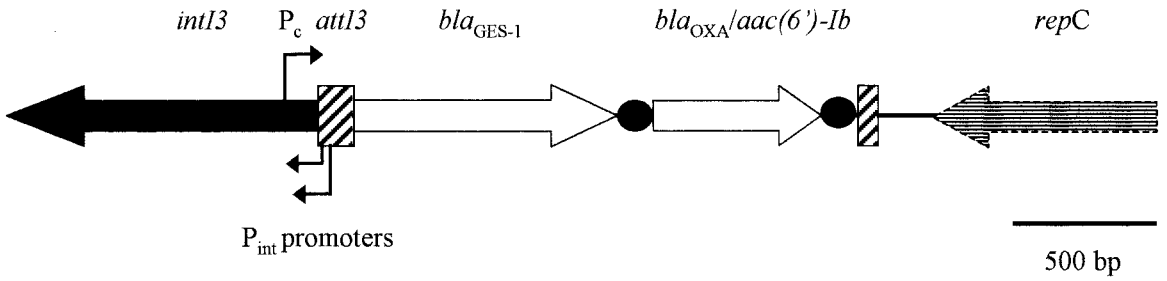

FIG. 1. Schematic representation of class 3 integron In3-p22K9 and its flanking region from p22K9. Open reading frames are indicated by arrows, and the 59-base elements are indicated by black solid circles.

tutions in the deduced amino acid sequences were observed: Gly-6→Arg, Ser-7→Asn, Ala-8→Asp, and Ser-34→Ile. None of them falls in the residues that are highly conserved among the tyrosine family of recombinases neither in the IntI patch (31, 37).

The sequence of the putative P_{int} promoter nearest the *intI3* gene (regions $^{-35}$[TTGAAA] and $^{-10}$[CTTACT]) differed in the −10 region by an A/T transversion from the *S. marcescens* AK9373 integron ($^{-10}$[CATACT]) (10). The putative P_c sequence (regions $^{-35}$[TAGACA] and $^{-10}$[TAGGAT]) differed by a C/A transversion in the −10 region from the reported sequence ($^{-10}$[TAGGCT]).

The 131-bp region where the 5′ region of *attI3* site was located (10) differed by five nucleotides, including two A/T transversions (one of them on P_{int}) and three transitions (one G/A and two C/T, one of these on *intI3* and the other on the simple-site region). None of the substitutions falls in the five GTTRRRY motifs (8, 38).

The gene cassettes were inserted in tandem in the variable region of In3-p22K9. The first one was the *bla*$_{GES-1}$ gene cassette, with 1,020 bp and 100% homologous to the gene cassette from *P. aeruginosa* Pa695 (15). The sequence of the *bla*$_{GES-1}$ gene differed by a single silent mutation from that described in *K. pneumoniae* ORI-1 (42). The amino acid sequence of GES-1 differed by two substitutions from IBC-1, reported in *Enterobacter cloacae* strains (16, 26), and by one substitution from GES-2 and IBC-2, reported in *P. aeruginosa* strains (29, 40, 41).

The second gene cassette was 635 bp long and revealed homology with sections of *bla*$_{OXA-10}$-type and *aac(6′)-Ib* gene cassettes. Thus, this cassette was the result of a fusion event between two gene cassettes (Fig. 2). The first 32 bp were 100% homologous to the initial region of *bla*$_{OXA-10}$-type gene cassettes (3, 18, 24, 32, 33, 39, 48). The remaining 603 bp, which included the 59-base element, showed 100% homology with previously described *aac(6′)-Ib* cassettes (27, 32, 39, 42, 43) and differed by a C/A transversion from the *aac(6′)-Ib* (also known as *aacA4*) gene cassette present in the integron from *S. marcescens* AK9373 (10).

The fused *bla*$_{OXA-10}$-type/*aac(6′)-Ib* gene cassette showed a 555-bp open reading frame encoding a 184-amino-acid fusion protein that contained the four conserved motifs of the AAC(6′)-Ib enzymes (50). The deduced amino acid sequence showed three alterations when compared with AAC(6′)-Ib enzymes (27, 39, 43): Thr-2→Lys, Asn-3→Thr, and Ser-4→Phe. These enzymes conferred resistance to gentamicin, while the new enzyme conferred resistance to kanamycin.

The core site (GTTAGCC) of the fused cassette was the same identified in the *bla*$_{OXA-10}$-type cassettes (3, 18, 24, 32, 33, 39, 48). The inverse core site (GCCTAAC), which showed a 1-bp mismatch, was 100% homologous to that found in the *aac(6′)-Ib* cassettes as well the 59-base element (10, 27, 32, 39, 43).

The sequence immediately beyond the second gene cassette was related to the remainder of the original *attI3* site. Thereafter, only a region of about 40 bp was clearly related to the end of the integron from *S. marcescens* AK9373.

Downstream the integron In3-p22K9 and separated by approximately 150 bp, there was a putative open reading frame whose product was 76% identical and 86% similar to the replication protein C of plasmid RSF1010 (47).

Cloning of β-lactamase gene with P_c region and antibiotic susceptibility. As the insert in pMFA-1 contains only the −10 region of the P_c promoter, since the *Hin*dIII restriction endonuclease recognizes and cleaves between the −35 and −10 regions of this promoter, a 1,388-bp fragment of the class 3 integron containing the P_c promoter and the *bla*$_{GES-1}$ gene was amplified for cloning and expression. Sequence analysis of the recombinant plasmid pMFA-2 revealed 100% homology with the same sequence determined in the integron.

The MICs of selected β-lactams for *K. pneumoniae* FFUL 22K and the transformant strains harboring either the natural plasmid p22K9 or the recombinant plasmid pMFA-2 are

```
bla OXA-10    ggcagtcgccctaaaacaaagTTAGCCACCAAGAAGGTGCCATGAAAACATTTgccgcatatgtaattatcgcgtgtctttcgagtacgg
                                   ::::::::::::::::::::::::::::::::
p22K9         caccgcggcttaactcaggcgTTAGCCACCAAGAAGGTGCCATGAAAACATTTAACGATTCCGTCACACTGCGCCTCATGACTGAGCATG
                                                                    ::::::::::::::::::::::::::::::::::::::
aac(6')-Ib    gtcgccctaaaacaaagttaggcatcacaaagtacagcatcgtgaccaacagcAACGATTCCGTCACACTGCGCCTCATGACTGAGCATG
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FIG. 2. Sequence alignment and comparison of fusion point of *bla*$_{OXA-10}$-type/*aac(6′)-Ib* fused gene cassette (shown in capital letters) in p22K9 with the initial regions of the *bla*$_{OXA-10}$ (24) and *aac(6′)-Ib* (39) gene cassettes. The nucleotide sequences of the *bla*$_{OXA-10}$ and *aac(6′)-Ib* gene cassettes presenting 100% homology with the fused cassette are also shown in capital letters, where colons indicate identical nucleotides. The putative ribosome-binding sites are underlined, and the translational start codons are double underlined.

TABLE 2. β-Lactam susceptibilities of *K. pneumoniae* FFUL 22K, *E. coli* DH5α(p 22K9), and *E. coli* TOP10(pMFA-2)

β-Lactam	MIC (μg/ml)				
	K. pneumoniae FFUL 22K	*E. coli* DH5α(p22K9)[a]	*E. coli* DH5α[b]	*E. coli* TOP10(pMFA-2)[c]	*E. coli* TOP10(pBK-CMV)[d]
Amoxicillin	>256	>256	4	>256	2
Amoxicillin/clavulanic acid	12	12	4	16	2
Cefuroxime	256	>256	3	>256	4
Cefotaxime	6	2	0.064	8	0.125
Cefotaxime/clavulanic acid	>1	0.19	ND[e]	>1	ND
Ceftazidime	256	96	0.25	>256	0.75
Ceftazidime/clavulanic acid	>4	1	ND	>4	ND
Aztreonam	3	0.75	0.125	1.5	0.094
Cefepime	2	0.5	0.047	ND	0.047
Imipenem	0.25	0.19	0.19	0.38	0.25

[a] *E. coli* DH5α (p22K9) expressed β-lactamase GES-1.
[b] The susceptibility of *E. coli* DH5α is shown for comparison.
[c] *E. coli* TOP10 (pMFA-2) expressed β-lactamase GES-1.
[d] The susceptibility of *E. coli* TOP10 carrying the empty vector is also shown for comparison.
[e] ND, not determined.

shown in Table 2. In all cases, the cefuroxime and ceftazidime MICs were higher than those of cefotaxime and aztreonam. All strains were susceptible to imipenem, with similar MICs.

DISCUSSION

This work was initiated with the observation that *K. pneumoniae* FFUL 22K exhibited resistance to cefuroxime and ceftazidime and had a positive double-disk synergy test. The apparent pI of 5.9 of the β-lactamase differed from that previously described (pI of 5.6) in *K. pneumoniae* strains producing the TEM-10 β-lactamase isolated in the same hospital since 1991 (4, 14). Also, the *K. pneumoniae* strain showed an aztreonam MIC (3.0 μg/ml) lower than those previously determined for the *K. pneumoniae* strains producing TEM-10, whose aztreonam MICs ranged from 16 to >256 μg/ml (4).

bla_{GES-1} has been found previously in *K. pneumoniae* and *P. aeruginosa* strains isolated at two French hospitals (15, 42) and is located on a gene cassette inserted in class 1 integrons. The integron from *K. pneumoniae* ORI-1 was located on a 140-kb nontransferable plasmid (42), while that of *P. aeruginosa* Pa695 was chromosomally located (15). The other bla_{GES}/bla_{IBC} gene cassettes are also inserted in class 1 integrons, located either on large transferable plasmids (bla_{IBC-1} and bla_{GES-2}) (16, 40, 41) or in the chromosome (bla_{IBC-2}) (29). In the present study, the bla_{GES-1} gene cassette was inserted at the *attI3* site of a class 3 integron, located on a 9.4-kb plasmid. This fact suggests a higher heterogeneity of the genetic environment where bla_{GES} genes can be found.

To date, only a single representative class 3 integron has been reported and characterized (2, 10), although *intI3*-specific fragments had previously been amplified in bla_{IMP}-positive strains (49) and were detected by DNA-DNA hybridization in several *Enterobacteriaceae* isolates (17). The integron from *S. marcescens* AK9373 was located on a ca. 120-kb transferable plasmid, contained bla_{IMP-1} and *aac(6′)-Ib* gene cassettes, and was probably part of a transposable element related to Tn*402* (2, 10, 25).

The integron In3-p22K9 from *K. pneumoniae* FFUL 22K was located on a 9.4-kb plasmid and contained a bla_{GES-1} gene cassette and a fused bla_{OXA-10}-type/*aac(6′)-Ib* gene cassette. The integron module, consisting of an *intI3* allele, an *attI3* site and a P_c promoter region, was configured in the same way as the class 3 integron previously characterized (10). Despite the fact that a 21-bp sequence in the terminal region of the integron was 80.9% identical to the sequence of Tn*402* and 90.4% to that of *S. marcescens* AK9373, it differed from both by the absence of IRi, suggesting that probably In3-p22K9 is not part of a transposon backbone.

The IntI3 enzyme from In3-p22K9 differed by four substitutions from the deduced amino acid sequence of the previously characterized IntI3. Since none of these alterations occurred in the conserved residues of the integrases (31, 37) and the IntI3 and IntI1 show stringent specificity for recombination involving the cognate *attI* site (9), we can assume that this *intI3* allele codifies a functional IntI3 integrase.

Among class 1 integrons, four versions of P_c have been identified, which were classified according to their activity (11, 45). In3-p22K9 showed a second version of P_c for class 3 integrons, differing by a C/A transversion in the −10 region from the previously reported version (10). The −35 region of the class 3 promoter (TAGACA) differs from the strong promoter of class 1 integrons (TTGACA) by a T/A transversion at the second nucleotide. Previously, the promoter of a plasmid (pRMH262) containing this mutation showed an intermediate activity, as opposed to the strong activity of the promoter from pRMH280, whose sequence was $^{-35}$(TTGACA) (11). To ascertain if the C/A transversion influences the expression of the inserted gene cassettes, as previously determined for other mutations in the P_c promoters of class 1 integrons, further studies are needed.

Cassette fusion may occur by a deletion event with endpoints in two adjacent gene cassettes, resulting in the truncation of one or both genes, or by loss of the 59-base element from one cassette, resulting in retention of both gene coding regions (45). In the present study we described the first fused cassette containing truncated versions of bla_{OXA-10}-type and *aac(6′)-Ib* genes. Although several start codons have been proposed for the *aac(6′)-Ib* genes (6), the fusion of the bla_{OXA-10}-type and *aac(6′)-Ib* cassettes placed a translational start codon (ATG)

in frame for the *aac(6′)-Ib* gene, resulting in a fused AAC(6′)-Ib enzyme that conferred resistance to kanamycin. Similarly, in pSTI1 the *aac(6′)-Ib* gene was fused with the 5′ end of bla_{OXA-1}, resulting in a fused 188-amino-acid protein (6).

This is the first time that a class 3 integron has been found and characterized in Europe, suggesting wide dissemination of integrons belonging to this class. However, it was not found in strains collected during the same period at Hospital Santa Maria, namely *Escherichia coli*, *Enterobacter cloacae,* and nonclonal *K. pneumoniae* strains.

In summary, the molecular characterization of the extended-spectrum β-lactamase gene carried by *K. pneumoniae* FFUL 22K revealed the presence of a bla_{GES-1} gene cassette inserted into a class 3 integron, carried by a 9.4-kb plasmid (p22K9). Analysis of the second gene cassette revealed that it was a new fusion cassette that encoded an AAC(6′)-Ib enzyme resulting from translational fusion between a bla_{OXA-10}-type and an *aac(6′)-Ib* gene cassette.

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