An IS1-like element is responsible for high-level synthesis of extended-spectrum β -lactamase TEM-6 in *Enterobacteriaceae*

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Resistance of *Escherichia coli* strain HB251 to the newer β -lactam antibiotics, in particular ceftazidime and aztreonam, results from production of the extended-spectrum β -lactamase TEM-6. The corresponding structural gene, *blaT-6*, and its promoter region were amplified by the polymerase chain reaction. Analysis of the sequence of the amplification product showed that *blaT-6* differed by two nucleotide substitutions from *blaT-1*, the gene encoding TEM-1 penicillinase in plasmid pBR322. The mutations led to the substitution of a lysine for a glutamic acid at position 102 and of a histidine for an arginine at position 162 of the unprocessed TEM-1 protein. The presence of a 116 bp DNA insert upstream from *blaT-6* resulted in the creation of hybrid promoter P₆ in which the –10 region was that of TEM-1 promoter P₃ whereas the –35 canonical sequence TTGACA was provided by the right end of the insert. P₆ was found to be 10 times more active than P₃ and to confer higher levels of antibiotic resistance upon the host. Analysis of the sequence of the insert indicated that the 116 bp fragment is related to insertion sequence IS1 but differs from it by three internal deletions that removed regions encoding the transposase. The distribution of the IS1-like element in clinical isolates of *Enterobacteriaceae* was studied by the polymerase chain reaction and by DNA–DNA hybridization. The element appeared to be widespread and was detected in strains producing TEM-6 or other TEM variants.

Introduction

β -Lactamases catalyse hydrolysis of the β -lactam ring in penicillins and cephalosporins. Among Gram-negative bacteria, plasmid-encoded penicillinases TEM-1 and TEM-2 are the most prevalent β -lactamases (Medeiros, 1989). These enzymes hydrolyse penicillins and the so-called first-generation cephalosporins but not the novel broad-spectrum second- and third-generation cephalosporins, such as cefotaxime and ceftazidime. Plasmid-

mediated resistance to cefotaxime and other recently developed cephalosporins was first described in 1983 in *Serratia marcescens*, *Klebsiella pneumoniae* and *K. ozaenae* (Knothe *et al.*, 1983). Kliebe *et al.* (1985) showed that resistance was due to production of a new β -lactamase, SHV-2, that differed by a single mutation from the SHV-1 penicillinase commonly found in *Klebsiella* spp. (Barthélémy *et al.*, 1988). More recently, other extended-spectrum plasmid-mediated β -lactamases that are variants of TEM-type penicillinases have been detected in strains highly resistant to third-generation cephalosporins (for a review, see Philippon *et al.*, 1989). Enzymes TEM-3 and TEM-4 are characterized by similar levels of activity against cefotaxime and ceftazidime (Paul *et al.*, 1989; Sirot *et al.*, 1987) whereas TEM-5, -6, -7 and -9 were found in strains that are preferentially resistant to ceftazidime and aztreonam (Bauernfeind & Hörl, 1987; Collatz *et al.*, 1989; Petit *et al.*, 1988; Spencer *et al.*, 1987). With the exception of TEM-6, all these variants have been characterized at the molecular level. They differ from TEM-1 or TEM-2 by

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Abbreviations: CAT, chloramphenicol acetyltransferase; IC₉₉, 99% inhibitory concentration.

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several point mutations that are mostly involved in the extension of the substrate range of the enzymes (Collatz *et al.*, 1989; Mabilat & Courvalin, 1990; Mabilat *et al.*, 1990; Sougakoff *et al.*, 1988b, 1989).

β -Lactamase TEM-6 was detected in *Escherichia coli* HB251, which is resistant to ceftazidime and aztreonam but susceptible to other third-generation cephalosporins (Bauernfeind & Hörl, 1987). The enzyme is characterized by an isoelectric point of 5.9 and the corresponding gene is transferable by conjugation, together with chloramphenicol, kanamycin and sulphonamide resistance determinants, to other *E. coli* strains. DNA-DNA hybridization with a probe specific for TEM-1 indicated that the gene encoding TEM-6, designated *blaT-6*, is closely related to the structural *blaT* genes for TEM-type penicillinases (Sougakoff *et al.*, 1988a).

In this study, the molecular characterization of *blaT-6* and of its promoter region is presented. We found upstream from *blaT-6* a 116 bp DNA element related to insertion sequence *IS1*. The major features of the active hybrid-promoter P_6 created by insertion of this DNA fragment have been determined. The distribution of the *IS1*-like element was studied in clinical isolates that produced TEM-6 or other TEM variants.

Methods

Strains, plasmids and media. *E. coli* HB251, harbouring plasmid pIP1844 [*Tra*⁺, *blaT-6* (TEM-6), Ap^r Cm^r Km^r Su^r, approx. 200 kb], and recipient strain *E. coli* A15, plasmid-free and resistant to nalidixic acid, have been described (Bauernfeind & Hörl, 1987). *E. coli* THB251 corresponds to transconjugant A15(pIP1844) and produces a β -lactamase with an isoelectric point of 5.9 (Bauernfeind & Hörl, 1987). Plasmids pBR322 (Ap^r Tc^r, 4.3 kb) (Bolivar *et al.*, 1977) and pKK232-8 (Ap^r, 4.6 kb) (Brosius, 1984) were from our laboratory collection. *E. coli* HB101 (Boyer & Roulland-Dussoix, 1969) was the host strain for recombinant plasmids. *E. coli* BM2570 has been previously described (Brisson-Noël *et al.*, 1988). The strains isolated in the Federal Republic of Germany were obtained in an intensive care unit from patients treated with ceftazidime alone or combined with tobramycin (Bauernfeind *et al.*, 1989). *E. coli* THB82, 87, 91, 102, 103, 107, 114, 136 and 137 are transconjugants derived from these strains by mating with *E. coli* A15 and selecting for ampicillin and nalidixic acid resistance. Clinical isolates resistant to β -lactams were described previously (Mabilat & Courvalin, 1990). Strains were grown in brain heart infusion broth and agar (Difco), containing ampicillin at 100 μ g ml⁻¹ or chloramphenicol at 10 μ g ml⁻¹ when required.

Mating experiments. Donor and recipient strains were mixed in the same ratio and mating was performed on a nitrocellulose filter for 48 h. Transconjugants were selected on agar containing nalidixic acid (50 μ g ml⁻¹) and ampicillin (100 μ g ml⁻¹).

Determination of 99% inhibitory concentrations (IC₉₉) of antibiotics. Concentrations of antibiotics that inhibited 99% of bacterial growth were determined as described by Chabbert & Derlot (1983).

Preparation of DNA and nucleic acid techniques. Plasmid pIP1844 DNA was prepared by caesium chloride/ethidium bromide ultracentrifugation as described by Labigne-Roussel *et al.* (1981). Digestion with

restriction endonucleases (Amersham), cloning experiments and electrophoresis in 0.8% agarose gels were performed as described by Maniatis *et al.* (1982).

Amplification and sequencing of DNA and computer analysis of sequence data. The polymerase chain reaction (PCR) with oligonucleotides A, B, C, E and G was performed as described by Mabilat *et al.* (1990), using 25 ng plasmid DNA as a template. PCR with oligonucleotides F, J and K was carried out in a final volume of 100 μ l containing 10 μ l of a 1:1000 diluted overnight culture lysed by heating at 95 °C for 3 min. The 35 amplification cycles were performed as follows: annealing at 55 °C for 1 min, extension step at 72 °C for 30 s and denaturation at 94 °C for 30 s. The *Taq* DNA polymerase was purchased from Pelkin-Elmer, Cetus Co. PCR-amplified DNA was purified (Maniatis *et al.*, 1982) and sequenced directly with T7 polymerase (Sequenase) using the protocol of Tabor & Richardson (1987) as described previously (Mabilat *et al.*, 1990). Oligonucleotides A to G used as amplification and/or sequencing primers were described by Mabilat *et al.* (1990). Amplification primers J (5'-d{GGTAATGATACCAACGT}-3') and K (5'-d{GGTAATGCTGTCAACCT}-3') were also used. DNA sequences were compared using the algorithm of Wilbur & Lipman (1983).

Nucleic acid hybridization. For Southern hybridization (Southern, 1975), DNA restriction fragments fractionated by agarose gel electrophoresis were transferred to Nytran membranes (Schleicher & Schuell). Prehybridization and hybridization were carried out at 65 °C in 6 $\times$ SSC (1 $\times$ SSC is 0.15 M-NaCl, 0.015 M-sodium citrate), 0.5% sodium dodecyl sulphate and 0.05% nonfat dry milk (Johnson *et al.*, 1984). Colony hybridization was as described by Grunstein & Hogness (1975). Probes were labelled with [α -³²P]dATP (Amersham) by nick-translation (Maniatis *et al.*, 1982). The probe specific for *blaT* genes was a 347 bp fragment internal to *blaT-1* obtained by PCR using primers C and E (Fig. 1). The *IS1*-specific probe consisted of the 0.5 kb *Pst*I-*Bst*NI fragment of plasmid pAT69 (Brisson-Noël *et al.*, 1988).

Enzyme assays and analytical isoelectric focusing. Chloramphenicol acetyltransferase and β -lactamase activities were assayed using the protocol of Lupski *et al.* (1984). The isoelectric point of β -lactamases was determined on polyacrylamide gels by the method of Matthew & Hedges (1976). The enzymes were revealed with nitrocefin (Glaxo) (Barthélémy *et al.*, 1978).

Results

Comparative analysis by agarose gel electrophoresis of plasmid DNA from wild strain *E. coli* HB251 and from transconjugant *E. coli* THB251 (data not shown) indicated that *blaT-6* was part of a plasmid, named pIP1844, of about 200 kb. The presence of a *blaT* gene on pIP1844 was confirmed by DNA-DNA hybridization. The *blaT* probe specific for TEM-type β -lactamases hybridized to a 7 kb *Bam*HI-generated fragment and to *Eco*RI and *Hind*III fragments of approximately 12 kb (data not shown).

Amplification and sequencing of blaT-6 and of its promoter and comparison with blaT genes

We amplified by PCR (Saiki *et al.*, 1988), using oligonucleotide primers A and B (Mabilat *et al.*, 1990) and plasmid pIP1844 DNA as a template (Fig. 1), a

Fig. 1. Strategy for amplification and nucleotide sequencing of *blaT-6* and comparison with *blaT-1*. Heavy line, homologous DNA in pIP1844 (top) and pBR322 (bottom). Hatched line, 116 bp DNA insert upstream from *blaT-6*. Open boxes, genes *blaT-6* in pIP1844 and *blaT-1* in pBR322. Letters in the boxes indicate nucleotides at positions 512 and 693. The corresponding amino acids are represented under open triangles. Arg, arginine; Glu, glutamic acid; His, histidine; Lys, lysine. The numbering system is that of Sutcliffe (1978). P_6 and P_3 , promoters of *blaT-6* and *blaT-1*, respectively. Arrows, primers for amplification and/or sequencing.

Fig. 2. DNA sequence upstream from *blaT-6*. The nucleotide sequence of pIP1844 is indicated by lower-case letters. The first nucleotide in the sequence, as presented, corresponds to that at position 130 in *blaT-1* (Sutcliffe, 1978). The -35 and -10 regions of the promoter of *blaT-1* (Chen & Clowes, 1984) and the -35 consensus sequence of *blaT-6* are underlined. The 116 bp DNA fragment inserted in the promoter region of *blaT-6* is represented by boxed upper-case letters. Bold letters represent the 9 bp directly repeated sequences. Arrows, primers J and K used for amplification.

1.2 kb DNA fragment including *blaT-6* that was sequenced directly. Substitutions in *blaT-6* relative to *blaT-1* encoding TEM-1 are indicated in Fig. 1. The genes differ at positions 512 and 693, where a transition of A in *blaT-6* to G in *blaT-1* had occurred. In *blaT-6*, mutations A_{512} and A_{693} determine a lysine at amino acid number 102 and a histidine at residue 162 instead of glutamic acid and arginine, respectively, in TEM-1. Comparison of sequences upstream from *blaT-6* and *blaT-1* revealed that the promoter region of *blaT-6* differed from that of *blaT-1* by insertion of a 116 bp DNA fragment (Fig. 1).

Sequence analysis of the 116 bp DNA fragment inserted into the promoter region of *blaT-6*

As shown in Fig. 2, the 116 bp insertion occurred between the -35 and -10 regions of promoter P_3 of *blaT-1*, which was consequently disrupted. However, analysis of the sequence indicated that another pro-

Fig. 3. Sequence comparison between IS/ and the 116 bp insert in pIP1844. Identities are indicated by dots. Dashes represent gaps introduced to ensure optimal homology. Numbering begins at the first nucleotide of each sequence. The -35 region of the 116 bp insert (positions 103-108) is underlined. The terminal inverted repeats of IS/ are boxed.

moter, designated P_6 , was formed. It is composed of the -10 region of P_3 and of a -35 TTGACA sequence provided by the right end of the insert. The -10 and -35 sequences of the hybrid promoter of *blaT-6* are separated by 18 nucleotides, a spacing 1 bp longer than that in P_3 (Chen & Clowes, 1984).

Another feature of the DNA sequence upstream from *blaT-6* is the presence of a 9 bp sequence (CCGCTCATG) that is directly repeated at the boundary of the insert (Fig. 2). Insertion of IS elements is accompanied by a short (2-12 bp) duplication of target DNA so that the transposed element is flanked by direct repeats (Grindley & Reed, 1985). It therefore appears that the presence of the 116 bp DNA fragment in the β -lactamase promoter results from a transposition event. The sequence of the 116 bp DNA fragment was compared with those of known IS elements and homology with IS/ (Ohtsubo & Ohtsubo, 1978) was found (Fig. 3). Segments from bp 1 to 31 and from bp 94

Table 1. Chloramphenicol resistance and CAT versus β -lactamase activity (Lupski *et al.*, 1984) in *E. coli* HB101 harbouring pKK232-8 containing different *blaT* promoters

Promoter	Nucleotide sequence		IC ₉₉ of Cm ($\mu\text{g ml}^{-1}$)	10 ⁻³ × CAT/ β -lactamase activity
	-35 region	-10 region		
-	-	-	5	<1
P ₃	TTCAAA	GACAAT	80	33
P ₆	TTGACA	GACAAT	800	306

to 116 of the insert shared 71% and 74% similarity with the left and right ends of *IS1*, respectively. Another region, nucleotides 32-44, was highly similar (85%) to the 182-194 bp region in *IS1*. The degree of similarity between nucleotides 45-93 of the insert and 670-718 of *IS1* was lower (37%).

Analysis of the strength of hybrid promoter P₆ of *blaT*-6

As already mentioned, the *IS1*-like element contributed a -35 sequence, TTGACA, to P₆ (Fig. 2). This is equivalent to the consensus sequence for *E. coli* promoters (Hawley *et al.*, 1983). The hexanucleotide TTCAAA in the -35 region of P₃ is less related to the consensus. Since the -35 region is known to play an important role in promoter strength (Moran *et al.*, 1982), we compared the level of expression of P₆ of *blaT*-6 and of P₃ of *blaT*-1. The 0.34 kb DNA fragment including P₆ of pIP1844 that was obtained after amplification with oligonucleotides A and G and digestion with *SspI* and the 0.19 kb *EcoRI*-*SspI* fragment of pBR322 that contains P₃ (Fig. 1) were inserted into the *SmaI* site of the promoter-probe plasmid pKK232-8 (Brosius, 1984).

In this replicon, the *SmaI* site is located upstream from a promoterless chloramphenicol acetyltransferase (CAT) gene and expression of the enzyme is dependent upon transcription originating in the inserts. The promoter strength was expressed by the ratio of CAT activity to β -lactamase activity (Lupski, 1984) and was also assessed by the level of chloramphenicol resistance of the host (Table 1). When P₆ was fused to the CAT gene, there was a 10-fold higher CAT/ β -lactamase ratio and chloramphenicol resistance level as compared to the fusion with P₃.

Distribution of the *IS1*-like element among *Enterobacteriaceae*

β -Lactamase TEM-6 was first detected in two *E. coli* strains isolated in the Federal Republic of Germany (Bauernfeind & Hörl, 1987). Since 1987, outbreaks of infections due to enterobacteria resistant to ceftazidime have been observed in this country (Bauernfeind *et al.*, 1989). Nine transconjugants in *E. coli* A15 expressing resistance to ceftazidime were chosen because they were shown to produce TEM-6, as judged by analytical isoelectric focusing (data not shown). Since the study of *blaT*-6 from pIP1844 showed that an *IS1*-like element was inserted into the promoter region for the gene, we searched, by DNA amplification, for the 116 bp insert in the promoter region of *blaT*-6 in the transconjugants. Oligonucleotides J and K (Fig. 2) will only amplify the *IS1*-like element, whereas primers J and F (Fig. 1) allow amplification of a 525 bp fragment only when the 116 bp insert is present in the promoter region. Resolution of amplified DNA by agarose gel electrophoresis (Fig. 4) showed that the *IS1*-like element was present in the promoter region of the gene for TEM-6 in each transconjugant.

Fig. 4. Agarose gel electrophoresis of DNA fragments obtained by PCR with oligonucleotides J and K (left) or J and F (right). Lanes: 1, THB251; 2, THB82; 3, THB87; 4, THB91; 5, THB102; 6, THB103; 7, THB107; 8, THB114; 9, THB136; 10, THB137. *PstI*-generated fragments of bacteriophage λ were used as molecular size standards. The size of the amplified fragments is indicated on the right. No amplification product was observed when DNA of *E. coli* BM2570 harbouring *IS1* was used as a template (data not shown).

Table 2. Distribution of the *ISI*-like element in clinical isolates resistant to β -lactam antibiotics

Species	No. of strains			
	Producing TEM β -lactamases*		Producing non-TEM β -lactamases*	
	+	–	+	–
<i>Citrobacter freundii</i>		3	1	
<i>Enterobacter cloacae</i>	5	8	3	2
<i>Escherichia coli</i>	1	45	2	6
<i>Klebsiella pneumoniae</i>	93	43	16	12
<i>K. oxytoca</i>	4	2	3	
<i>K. ozaenae</i>			2	
<i>Proteus</i> spp.		2		
<i>Serratia marcescens</i>		6		1
<i>Salmonella typhimurium</i> and non-typable <i>Salmonella</i>			6	

* +, Positive hybridization with the *ISI*-like probe; –, negative hybridization with the *ISI*-like probe.

Colony hybridization using the 116 bp fragment amplified with primers J and K as a probe was used to study the distribution of the *ISI*-like element in 266 β -lactam-resistant clinical isolates that originated from various hospitals in five countries (Table 2). The probe did not hybridize to DNA from *E. coli* BM2570, which harbours *ISI* (data not shown), indicating that it is specific for the *ISI*-like element. Among the strains tested, 218 produced TEM-type enzymes whereas the remainder (48) encoded non-TEM β -lactamases (Mabilat & Courvalin, 1990). The *ISI*-like element was found in 103 (47%) isolates harbouring TEM variants and in 27 (56%) strains producing non-TEM enzymes (Table 2). In TEM-producing strains, homology with the *ISI*-like probe was detected in 93 (68%) *K. pneumoniae* isolates. One of 46 *E. coli* isolates hybridized with the probe and the *ISI*-like element was not found in *Citrobacter freundii*, *Proteus* spp., *Salmonella typhimurium* and *Serratia marcescens*. Among the isolates producing non-TEM β -lactamases, 16 (57%) *K. pneumoniae* strains hybridized with the probe. The *ISI*-like element was also found in *E. coli*, *Enterobacter cloacae*, *K. oxytoca* and *K. ozaenae*. The only strain of *C. freundii* tested hybridized with the probe whereas that of *S. marcescens* did not.

Discussion

E. coli HB251, a strain that is highly resistant to ceftazidime and aztreonam, was isolated from a patient treated with ceftazidime. Cephalosporin resistance in this strain is due to production of the extended-spectrum β -lactamase TEM-6 encoded by the approximately 200 kb plasmid pIP1844.

TEM-type β -lactamases that hydrolyse third-generation cephalosporins differ from penicillinases TEM-1

and TEM-2 by a few amino acid substitutions (Collatz *et al.*, 1989; Mabilat & Courvalin, 1990; Mabilat *et al.*, 1990; Sougakoff *et al.*, 1988b, 1989). Determination of the sequence of *blaT-6* encoding TEM-6 in *E. coli* HB251 showed that the resistance gene differed from *blaT-1* specifying TEM-1 by two point mutations (Fig. 1). The nucleotide substitutions correspond, in the primary structure of TEM enzymes, to a lysine and a histidine in TEM-6 in place of a glutamic acid and an arginine in TEM-1 at positions 102 and 162, respectively (Fig. 1). Lysine 102 has been previously detected in extended-spectrum β -lactamases TEM-3, -4 and -9, whereas the Arg to His substitution at amino acid position 162 has not previously been reported in a TEM variant of clinical origin. This histidine, however, was the only change in an extended-spectrum variant hydrolysing ceftazidime and aztreonam which was obtained by ultraviolet mutagenesis of the structural gene for a TEM-type penicillinase (W. Sougakoff, S. Goussard & P. Courvalin, unpublished result). Since lysine 102 is known to be implicated in the hydrolysis of ceftazidime and aztreonam by TEM variants (Sougakoff *et al.*, 1988b) the high-level resistance of strains producing TEM-6 (Philippon *et al.*, 1989) may therefore reflect a combination of the effects of each substitution. A similar phenomenon has already been proposed to account for the high catalytic activity towards ceftazidime of the extended-spectrum β -lactamase TEM-9 (Mabilat *et al.*, 1990). This enzyme also differs from TEM-1 by two amino acid substitutions, a lysine at position 102 (as in TEM-6) and a serine at position 162 which confers resistance to ceftazidime in TEM-7 (Collatz *et al.*, 1989). The location of residues 102 and 162 in the three-dimensional structure of the class A β -lactamase of *Staphylococcus aureus* PC1 (Herzberg & Moul, 1987) indicates that these amino acids could be part of the substrate-binding

site (Sougakoff *et al.*, 1989); however, the mechanism by which substitutions in these positions expand the substrate range of the enzyme is still unknown.

Sequence determination of *blaT-6* and of its promoter region showed that a 116 bp insertion occurred between the -35 recognition site and the -10 Pribnow box of promoter P_3 of the *blaT-1* gene (Fig. 2). Analysis of the sequence of the insert (Fig. 3) suggested that it is derived from the 768 bp insertion sequence *IS1* by two types of events: (i) three internal deletions and (ii) nucleotide substitutions in the remaining portions where the overall similarity was 59%. Nucleotide similarity was highest in the terminal inverted repeats of the two elements (Fig. 3). Transposition of *IS1* results in the duplication of 9 bp of the target (Prentki *et al.*, 1986). Since a 9 bp sequence is repeated directly at the ends of the *IS1*-like element, it is likely that the presence of the 116 bp DNA fragment is the result of a transposition event. The *IS*-related insert does not contain the central region of *IS1* coding for the transposase. Therefore, deletions in the 116 bp insert occurred after insertion of the element or, alternatively, insertion had to be promoted by transposition functions provided *in trans* by another *IS* element such as *IS1* that is common in *E. coli*. In fact, nucleotide sequences homologous to *IS1* were detected in pIP1844 DNA by hybridization using an *IS1*-specific probe (data not shown).

Insertion of the *IS1*-like element between the -35 and -10 sequences of the P_3 promoter of *blaT-1* did not abolish resistance to β -lactam antibiotics. Sequence analysis at the junction of the 116 bp fragment and of the region upstream from *blaT-6* showed that insertion generated a hybrid promoter P_6 in which the TTCAA -35 recognition site of P_3 was replaced by the -35 sequence TTGACA located at the right end of the element (Fig. 2), which is identical to the consensus sequence for *E. coli* promoters. The distance between the -10 and -35 regions in P_6 is 18 bp and corresponds to the preferred spacer (17 or 18 bp) for an *E. coli* promoter (Moran *et al.*, 1982). Comparison of the strength of the promoters and of the antibiotic resistance levels of the hosts (Table 1) indicated that the hybrid promoter of TEM-6 is 10 times stronger than P_3 of *blaT-1*. It therefore appears that acquisition of both a strong promoter and point mutations in the structural gene contribute to the ability of TEM-6-producing bacteria to resist the lethal effect of third-generation cephalosporins. The fact that the strong P_4P_6 promoter, which is 10 times more efficient than P_3 (Chen & Clowes, 1984), has been found upstream from the structural genes for TEM-3, -4, -5, -7 and -9 (Collatz *et al.*, 1989; Mabilat *et al.*, 1990; Sougakoff *et al.*, 1988b, 1989) is consistent with this notion.

Insertion of *IS1* into the promoter P_3 of *blaT-1* in

plasmid pBR322 has been reported (Prentki *et al.*, 1986). The insertion was at the same position as that of the *IS1*-like element upstream in *blaT-6* and resulted in the formation of an active hybrid promoter consisting of the -10 region from P_3 and a -35 TTGGCA region provided by the end of *IS1*. However, gene expression from the resulting hybrid promoter was less efficient than that from P_3 . An explanation for this difference could be that the -35 region of the '*IS1*-hybrid promoter' has a poorer match to the canonical -35 consensus than that in P_6 . Alternatively, features of the regions upstream from the -35 sequences in *IS1* and in the *IS1*-like element might be important for promoter recognition by the RNA polymerase (Moran *et al.*, 1982; Prentki *et al.*, 1986).

The *IS1*-like element was detected by PCR, using two oligonucleotides specific for the ends of the insert (Fig. 2), in transconjugants derived from clinical isolates expressing resistance to ceftazidime and aztreonam by production of TEM-6. Using another pair of primers, J and F (Fig. 1), we found that insertion of the element occurred in the promoter region of *blaT-6* in the nine strains tested (Fig. 4). It is therefore tempting to speculate that presence of the *IS1*-like element into the β -lactamase promoter, which contributes to the survival of the host in the presence of third-generation cephalosporins, was selected by extensive use of these drugs in clinical settings.

Study of the distribution of the *IS1*-like element in enterobacteria of various origins by DNA-DNA hybridization indicated the presence of DNA sequences related to the insert in 47% of the clinical isolates producing a TEM-type enzyme and in 56% of the strains producing non-TEM β -lactamases (Table 2). The *IS1*-like element therefore appears to be widespread in members of the family *Enterobacteriaceae* and not exclusively linked to *blaT-6*.

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