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Interspecies spread of CTX-M-32 extended-spectrum β-lactamase and the role of the insertion sequence IS*1* in down-regulating $bla_{\text{CTX-M}}$ gene expression

Ana Fernández[1], Emilia Gil[2], Mónica Cartelle[1], Astrid Pérez[1], Alejandro Beceiro[1], Susana Mallo[1], María Mar Tomás[1], Francisco J. Pérez-Llarena[1], Rosa Villanueva[1] and Germán Bou[1]*

[1]Servicio de Microbiología-Unidad de Investigación, Complejo Hospitalario, Universitario Juan Canalejo, La Coruña, Spain; [2]Laboratorio de Microbiología, Hospital S. Rafael, La Coruña, Spain

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***Objectives*: To characterize the extended-spectrum β-lactamases (ESBLs) as well as their genetic environment in different isolates of Enterobacteriaceae from a patient with repeated urinary tract infections.**

***Methods*: Two isolates of *Escherichia coli* and one *Proteus mirabilis*, all with ESBL phenotypes, were studied. Conjugation experiments and restriction fragment length polymorphisms (RFLPs) were performed. Cloning of the *bla* genes was by plasmid restriction and fragments ligation. Antibiotic susceptibility testing was by Etest. The genetic environment was analysed by direct sequencing of the DNA surrounding the *bla* gene. RT–PCR was performed to study the differences in the $bla_{\text{CTX-M}}$ gene expression.**

***Results*: The *bla* gene was transferred by conjugation from the three clinical isolates, which by RFLP showed the same plasmid. The *bla* gene and surrounding sequences were cloned, an ~9 kbp *Acc*I fragment was sequenced and the $bla_{\text{CTX-M-32}}$ gene was identified. The MICs of ceftazidime for transconjugants and transformants bearing the $bla_{\text{CTX-M-32}}$ gene were lower than those previously reported. Analysis of the DNA surrounding the ESBL gene revealed a new genetic structure with two insertion sequences, IS*5* and IS*1*, located immediately upstream of the $bla_{\text{CTX-M-32}}$ gene; IS*1* was located between the *bla* gene and IS*5*, and within the −10 and −35 promoter boxes of the $bla_{\text{CTX-M-32}}$ gene. Microbiological and biochemical studies revealed lower $bla_{\text{CTX-M-32}}$ gene expression in bacterial isolates with IS*1* between the promoter boxes.**

***Conclusions*: Data suggest putative *in vivo* horizontal $bla_{\text{CTX-M-32}}$ gene transfer between two different genera of Enterobacteriaceae. A new complex structure, IS*5*–IS*1*, was detected upstream of the *bla* gene and IS*1* negatively modulated expression of the $bla_{\text{CTX-M-32}}$ gene because its location modified the *bla* promoter region.**

Keywords: *in vivo* spreading, interspecies dissemination, ESBL expression

Introduction

The production of β-lactamases is the predominant cause of resistance to β-lactam antibiotics in Gram-negative bacteria.[1] The β-lactamases are classified, according to the scheme of Ambler,[2] into four classes, designated A to D, on the basis of their amino acid sequences. Another classification scheme is that of Bush *et al.*,[3] in which representative β-lactamases belonging to all molecular classes are described with separation into groups primarily on the basis of published functional characteristics. According to this classification scheme, class A extended-spectrum β-lactamases (ESBLs) are included in group 2be of the scheme of Bush *et al.*[3] They are mainly encoded by transferable plasmids, exhibit extended-spectrum activities, are capable of hydrolysing some broad-spectrum cephalosporins and are susceptible to clavulanic acid.

The CTX-M-type β-lactamases represent a rapidly emerging group with a typical phenotype of ESBL resistance, but are not

*Corresponding author. Tel: +34-981-178359; Fax: +34-981-178216; E-mail: germanbou@canalejo.org

TEM or SHV derivatives.[4] The CTX-M β-lactamases are probably the most widespread ESBLs at present.[4] In the past few years, ESBLs of the CTX-M type have been increasingly reported in members of the family Enterobacteriaceae worldwide.[4–12]

Microbial drug resistance is an inescapable consequence of the use and overuse of antimicrobial agents in the treatment of patients.[13] This is one of the most obvious risk factors for the dissemination of genes encoding ESBLs. Nevertheless, the investigation of other factors may be critical for predicting the potential spread and evolution of ESBL-producing strains. For example, analysis of the genetic environment of ESBLs may help to explain the dissemination of ESBLs.[14–17] Different elements may be involved in the mobilization and expression of bla_{CTX-M} genes.[4,14–17] Insertion sequences (ISs) are also an important source of genetic plasticity.

Another plasmid-borne bla_{CTX-M}-type gene, $bla_{CTX-M-32}$, was recently detected in an *Escherichia coli* isolate from La Coruña, Spain.[18] This gene, like $bla_{CTX-M-15}$, $bla_{CTX-M-16}$, $bla_{CTX-M-19}$ and $bla_{CTX-M-27}$,[19–22] codes for an ESBL that has the ability to hydrolyse ceftazidime, and, therefore, strains of *E. coli* harbouring the gene are resistant to this antibiotic. The CTX-M-15, CTX-M-16 and CTX-M-27 as well as CTX-M-32 genes harbour an Asp-240→Gly substitution. *E. coli* expressing CTX-M-32 shows ceftazidime MICs higher than 256 mg/L.[18]

The present study describes intra- and interspecies transmission of the $bla_{CTX-M-32}$ gene as well as description of its genetic environment. In addition, the role of a specific IS in down-regulating $bla_{CTX-M-32}$ gene expression, and therefore in altering ceftazidime MICs, is also reported.

Materials and methods

Bacterial strains and susceptibility testing

An ESBL-producing strain of *E. coli* (EC1) was isolated from a patient admitted to the Juan Canalejo Hospital (La Coruña, Spain) with urinary tract infection (UTI) and who was previously treated with amoxicillin/clavulanic acid. Two months later, an ESBL-producing strain of *Proteus mirabilis* (PM1) was isolated from the same patient in a new episode of UTI. An ESBL-producing strain of *E. coli* (EC2) was isolated at the same time from the skin of the same patient. The bacteria were identified with API-20 E systems (bioMérieux SA, Marcy l'Étoile, France).

The MICs of the β-lactams amoxicillin, amoxicillin plus clavulanic acid, piperacillin, cefotaxime, cefotaxime plus clavulanic acid, ceftazidime, ceftazidime plus clavulanic acid, cefepime, aztreonam and imipenem were determined by Etest (AB Biodisk, Solna, Sweden), following the manufacturer's instructions. The ESBL phenotype was determined with the corresponding Etest strips; cefotaxime, ceftazidime and cefepime with clavulanic acid.

DNA extraction

Bacterial chromosomal DNA was obtained with the MasterPure™ Genomic DNA Purification Kit Epicentre® (Biotechnologies, Madison, WI, USA) preparation kit. The purity and integrity of chromosomal DNA were checked by electrophoresis in 0.7% agarose gels in Tris/borate/EDTA (TBE) buffer prior to manipulation. Plasmid DNA was extracted from clinical strains, transconjugants and transformants with the High Pure plasmid isolation kit (Roche Diagnostics GmbH, Mannheim, Germany).

Conjugation experiments, cloning experiments and DNA sequencing

Transfer of resistance by conjugation was attempted with *E. coli* XL1-Blue MRF' Kan strain (Stratagene Europe, Amsterdam, The Netherlands) as recipient. Overnight mating experiments were performed at 37°C, and the transconjugants TEC1, TEC2 and TPM1, obtained from EC1, EC2 and PM1, respectively, were selected on MacConkey agar plates supplemented with ampicillin (50 mg/L) and kanamycin (50 mg/L).

Cloning procedures were performed as described by Sambrook *et al.*[23] Restriction enzymes and T4 DNA ligase were purchased from Roche Diagnostics GmbH and were used as specified by the manufacturer.

For cloning the *bla* gene, plasmid DNA from transconjugant TPM1 was digested with *Acc*I. The resulting fragments were ligated into pBGS18,[24] and the ligation mixture was transformed into *E. coli* TG1 [D(*lac-pro*) *supE thi hsdDS*/F9 *traD36 proA*1*B*1 *lacL lacZ*M15] made competent by the calcium chloride method. After transformation, a few clones grew on Luria–Bertani (LB) agar plates supplemented with kanamycin (50 mg/L) and ampicillin (50 mg/L). These transformants harboured an identical plasmid (pAF-1) with an insert of ~9 kbp. Double-stranded templates were subjected to nucleotide sequencing by the method of Sanger *et al.*[25]

The BLAST program on the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov) was used for database searches.

Repetitive extragenic palindromic (REP)-PCR

Amplification reactions were performed in a final volume of 50 μL as previously reported.[26] The previously described primer pairs[26] were used to amplify putative REP-like elements in the genomic bacterial DNA.

Plasmid restriction fragment length polymorphisms (RFLPs)

Plasmid DNA from *E. coli* XL1-Blue MRF' Kan transconjugants, TEC1, TEC2 and TPM1 was isolated. The DNA was then independently digested with *Hin*dIII, *Hin*cII, *Bam*HI and *Acc*I restriction enzymes, and the resulting fragments separated by electrophoresis on a 0.8% agarose gel. The DNA fragments were then visualized by staining with ethidium bromide (50 mg/L).

Isoelectric focusing (IEF) assay and β-lactamase detection

β-Lactamases were characterized by IEF[27] of ultrasonicated bacterial extracts. Cultures of strains grown on LB medium were harvested and cell extracts were prepared by sonication. The β-lactamases were analysed by IEF of cell extracts on polyacrylamide gels containing ampholytes (range of pH 3–9) (PhastGel; Amersham Pharmacia Biotech, Piscataway, NJ, USA) in a PhastSystem apparatus (Amersham Pharmacia Biotech). The focused β-lactamases were detected by overlaying the gel with nitrocefin (0.5 mg/mL) in 100 mM phosphate buffer (pH 7.0). β-Lactamases with a known pI (CTX-M-8, 8.9; VIM-2, 5.1; and SHV-1, 7.6) were electrophoresed in parallel as controls.

For biochemical experiments to assess activity towards nitrocefin, protein extracts were obtained as described earlier for IEF

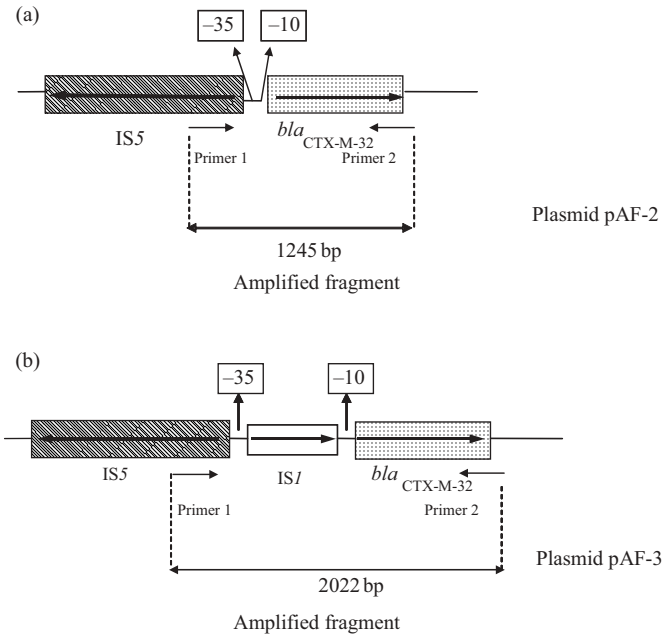

Figure 1. Schematic map of the region surrounding the $bla_{CTX\text{-}M\text{-}32}$ gene in (a) plasmid pMC-2[18] and (b) the plasmid reported in the present study. Recombinant plasmids pAF-2 and pAF-3 harboured 1245 and 2022 bp DNA fragments, respectively. Open reading frames and genes are shown as boxes with an arrow indicating the orientation of each coding sequence and the gene name is shown under the corresponding box. The relative location of promoter −35 and −10 boxes is indicated with respect to the $bla_{CTX\text{-}M\text{-}32}$ gene. Primers 1 and 2 (indicated by arrows) are those used to amplify the target region, which includes IS*1* in plasmid pAF-3 (b).

assays, but with the presence of protease inhibitors aprotinin, pepstatin and leupeptin (20 mg/L final concentration each). Results were the mean value of three independent experiments.

Detection of ESBL genes by PCR

To confirm the presence of the $bla_{CTX\text{-}M\text{-}32}$ gene in the clinical isolates (EC1, EC2 and PM1) as well as their transconjugants (TEC1 and TEC2), a PCR assay was performed with CTX-M group 1 specific primers TestF (5′-ATGGTTAAAAAATCACTGCG-3′) and TestR (5′-TTACAAACCGTTGGTGAC-3′), using standard conditions. An amplicon band of 876 bp was considered as a positive result.

The products from TEC1 and TEC2 were sequenced to confirm the precise $bla_{CTX\text{-}M\text{-}32}$ gene.

Promoter analysis of the $bla_{CTX\text{-}M\text{-}32}$ gene

To confirm the functionality of the $bla_{CTX\text{-}M\text{-}32}$ promoter and the role of the IS*1* insertion in separating −35 and −10 regions and, therefore, in reducing *bla* gene expression, a set of experiments was performed with the $bla_{CTX\text{-}M\text{-}32}$ gene as gene reporter. For this, a PCR assay was carried out with the oligonucleotides Primer1 (5′-AAAGGATCCGCTGAATTCACTATCGGCG) and Primer2(5′-AAAGAATTCCCGTTTCCGCTATTACAAAC), which amplify a DNA region including promoter and the whole $bla_{CTX\text{-}M\text{-}32}$ gene. Plasmid pMC-2[18] (Figure 1), which harbours the originally described $bla_{CTX\text{-}M\text{-}32}$ gene, and plasmid pAF-1 reported here were used as templates. Amplicons from pMC-2 and pAF-1 (1245 and 2022 bp, respectively) were then digested with *Bam*HI and *Eco*RI, ligated into pBGS18, transformed into *E. coli* TG1 and the transformants were selected on LB plates with 50 mg/L kanamycin and 50 mg/L ampicillin. The resulting recombinant plasmids, containing amplicons from pMC-2 and pAF-1, were designated as pAF-2 and pAF-3, respectively (Figure 1). The MICs of β-lactams for *E. coli* TG1 harbouring pAF-2 and pAF-3 were determined, as was the specific activity towards 100 μM nitrocefin with protein extracts from these transformants.

Real-time RT–PCR was also performed to determine the expression of the $bla_{CTX\text{-}M\text{-}32}$ gene. Total RNA was isolated using TRIZOL® Reagent (Invitrogen) according to the manufacturer's instructions and treated with RNase-free DNase I. The concentration of RNA was determined spectrophotometrically and 1 μg of RNA was reverse transcribed into single-stranded cDNA using a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH), according to the manufacturer's instructions. The cDNAs were quantified by real-time PCR amplification with specific primers (CTX-M-32-F 5′-TATAATCCGATTGCGGAAAAG and CTX-M-32-R 5′-CGGTACGGTCGAGACGGAA, GapA-F 5′-GTCCGTCAAAGACAACACTCCG and GapA-R 5′-CGATGATGCCGAAGTTATCG) using the Light Cycler® 480 (Roche Diagnostics GmbH), with initial incubation at 95°C for 10 min, followed by 45 cycles of 10 s at 95°C, 10 s at 60°C and 10 s at 72°C. The expression levels were normalized against the *gapA* housekeeping gene (coding for glyceraldehyde-3-phosphate dehydrogenase A).

Results

Antimicrobial susceptibilities

The antibiotic susceptibility profiles of *E. coli* isolates EC1 and EC2 and *P. mirabilis* isolate PM1 and of their ESBL-producing transconjugants showed resistance to most of the β-lactam antibiotics tested, with the exception of β-lactam/β-lactamase inhibitor combinations and imipenem (Table 1). A moderate degree of resistance to ceftazidime, aztreonam and cefepime was also observed. For these bacterial strains, the MIC of cefotaxime was higher than that of ceftazidime, suggesting the presence of a CTX-M-type enzyme.

Detection of the CTX-32 ESBL gene in TEC1, TEC2 and TPM1

A *bla* gene was detected by PCR with specific oligonucleotides of group 1 CTX-M enzymes, in clinical strains EC1, EC2 and PM1, as well as with their transconjugants TEC1, TEC2 and TPM1. Sequencing of the amplicons obtained from TEC1, TEC2 and TPM1 revealed the presence of the $bla_{CTX\text{-}M\text{-}32}$ gene in all cases. IEF showed that all three clinical strains and their transconjugants had a unique major β-lactamase band of pI 9.0, corresponding to CTX-M-32 enzyme.

REP-PCR

A REP-PCR assay was carried out to discount any epidemiological relationship between clinical *E. coli* strains EC1 and EC2. More than two band differences were detected between EC1 and EC2, therefore suggesting that there was no genetic relationship between them (data not shown).

Table 1. MICs of β-lactam antibiotics (mg/L) for the bacterial isolates under study

Antibiotics	*E. coli* EC1	*E. coli* EC2	*P. mirabilis* PM1	*E. coli* TEC1[c]	*E. coli* TEC2[c]	*E. coli* TPM1[c]	*E. coli* TG1[a] (pAF-1)	*E. coli* TG1[a,b] (pAF-2)	*E. coli* TG1[a,b] (pAF-3)	*E. coli* TG1	*E. coli* XL1-Blue[c]
AMX	>256	>256	>256	>256	>256	>256	>256	>256	>256	3	4
AMX/CLA	8	8	4	16	4	8	8	4	8	4	4
PIP	>256	>256	>256	>256	>256	>256	>256	>256	>256	0.38	0.38
CTX	>256	>256	>256	>256	>256	>256	>256	>256	>256	0.02	0.02
CTX/CLA	0.12	0.06	0.03	0.06	0.06	0.12	0.12	0.12	0.12	0.02	0.02
CAZ	8	8	8	16	8	8	32	>256	4	0.06	0.06
CAZ/CLA	0.25	0.25	0.06	0.5	0.25	0.5	0.12	0.12	0.12	0.06	0.06
ATM	32	16	8	32	32	32	32	>256	8	0.03	0.03
FEP	8	8	8	4	8	8	16	>256	4	0.03	0.02
IPM	0.25	0.25	2	0.25	0.25	0.25	0.25	0.25	0.25	0.12	0.12

AMX, amoxicillin; CLA, clavulanic acid; PIP, piperacillin; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; FEP, cefepime; IPM, imipenem.
[a]*E. coli* TG1 transformed with the indicated plasmids.
[b]Identical MIC values were obtained with three independent transformants.
[c]TEC1, transconjugant from EC1; TEC2, transconjugant from EC2; TPM1, transconjugant from PM1; XL1-Blue, transconjugant strain.

RFLP analysis of ESBL-encoding plasmids

As the $bla_{\text{CTX-M-32}}$ gene was demonstrated to be encoded by conjugative plasmids in the clinical strains of *E. coli* (EC1 and EC2) and *P. mirabilis* (PM1), an attempt was then made to assess whether or not these plasmids were genetically related, thus raising the possibility of intra- and interspecies transmission in the patient.

The restriction patterns with *Bam*HI, *Acc*I, *Hin*dIII or *Hin*cII of the three plasmids obtained from transconjugants TEC1, TEC2 and TPM1 were very similar (data not shown), which provides strong evidence that the same plasmid (~20 kbp) was present in different strains of Enterobacteriaceae isolated from the same patient.

Identification of the genetic structures surrounding the CTX-M-32 ESBL gene

As several ESBL genes may be transposon- or integron-borne,[14–17] the surrounding sequences of the $bla_{\text{CTX-M-32}}$ gene were explored to detect any potential genetic structures able to mobilize the ESBL gene. For this, the *Acc*I fragment of ~9 kbp cloned from TPM1 (pAF-1 plasmid) was used as template for nucleotide sequencing of the surrounding region of the $bla_{\text{CTX-M-32}}$ gene. The full sequence of ~5 kbp is shown in Figure 2.

There are some interesting features worthy of mention: as previously reported,[18] the inverted repeat right (IRR) sequence of IS*Ecp1B* was detected 80 bp upstream of the ATG start codon of $bla_{\text{CTX-M-32}}$ (Figure 2); in this fragment, the −35 and −10 promoter sequences provided by this IRR were physically separated and therefore modified by the presence of the IS IS*1*[24] (Figure 2a and b); IS*1* was bracketed by a 5 bp duplicated target site of the putative insertion site for the DNA fragment resulting from an IS*1*-mediated transposition process. IS*1* also contained two imperfect 18 bp inverted-repeat sequences (four mismatches), inverted repeat left (IRL) (left) and IRR (right) surrounding an InsA–InsB protein that encoded a putative transposase, the integrity of which is essential for transposition;[28,29] no putative promoter sequences were found in the 80 bp sequence that separated the IRR of IS*Ecp1B* from the ATG site of the $bla_{\text{CTX-M-32}}$ gene. Our research team[18] and others[17] have previously demonstrated that this IRR provided −35 and −10 promoter sequences for the expression of $bla_{\text{CTX-M}}$ genes. However, in the bacterial strains under study, the IS IS*1* is located between the −10 and −35 promoter sequences, and it is therefore plausible that this genetic event is responsible for the reduced $bla_{\text{CTX-M-32}}$ gene expression. This hypothesis is supported by the fact that MICs of ceftazidime were lower than for previously reported bacterial strains bearing $bla_{\text{CTX-M-32}}$ (>256 mg/L when compared with 8–32 mg/L for the strains reported here); the IS*1* was downstream of a *tnpA* gene that encoded the transposase of IS*5*, which we have previously reported to be associated with $bla_{\text{CTX-M-32}}$;[18] a truncated ORF-477 was found downstream of the $bla_{\text{CTX-M-32}}$ gene.

To confirm that IS*1* was interrupting the −10 and −35 regions of the $bla_{\text{CTX-M-32}}$ promoter and therefore modulating $bla_{\text{CTX-M-32}}$ gene expression, the MICs were determined for *E. coli* TG1 harbouring pAF-2 and pAF-3 (Figure 1). The MICs of ceftazidime for pAF-2 and pAF-3 transformants were >256 and 4 mg/L, respectively (Table 1). The MICs of aztreonam and

(a)

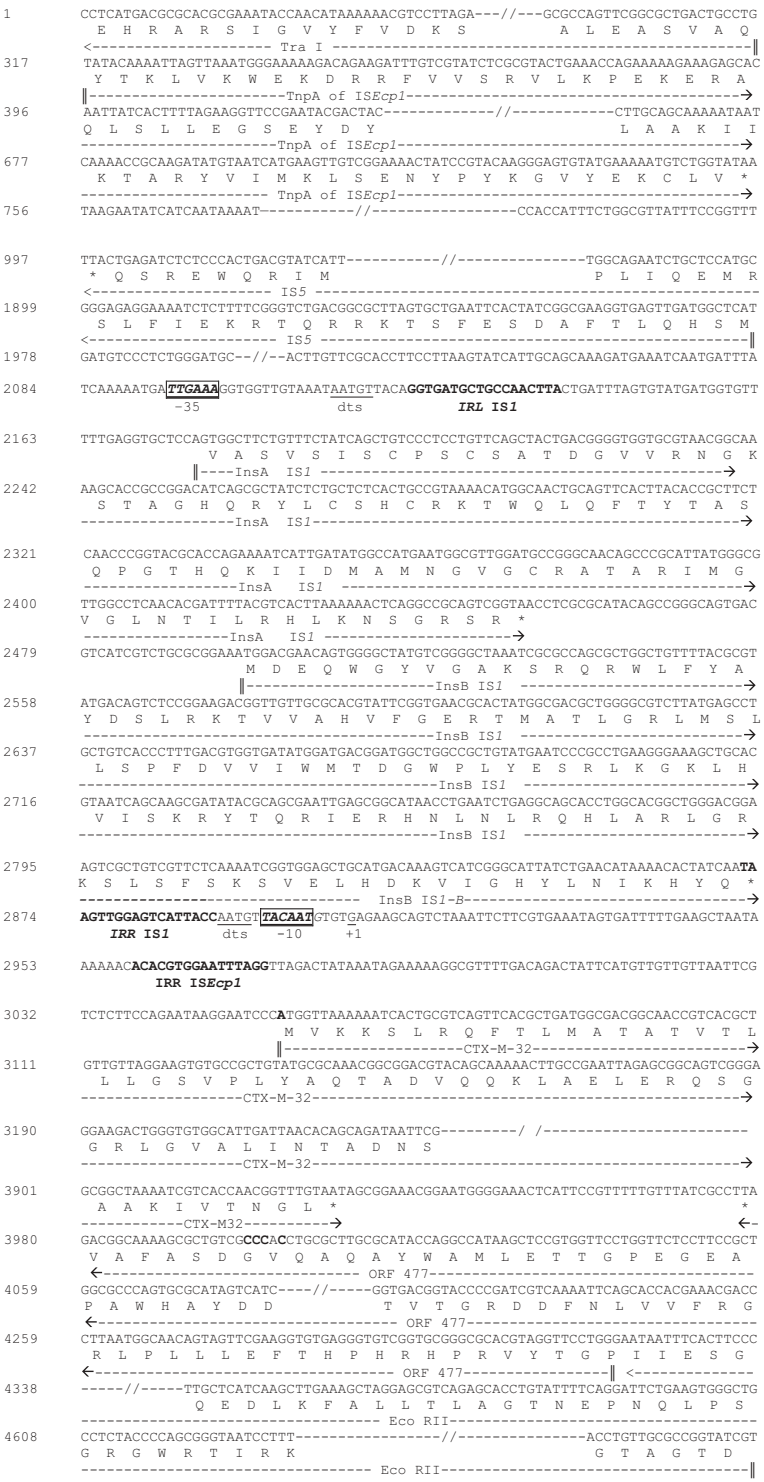

Figure 2. (a) Nucleotide sequence of ∼5 kbp DNA fragment of pAF-1 plasmid. The deduced amino acid sequence is indicated by single-letter code below the nucleotide sequence. Stop codons are indicated by asterisks. The −35 and −10 promoter sequences of the $bla_{\text{CTX-M-32}}$ gene are boxed and indicated by bold letters, and the +1 position of the transcriptional start of the $bla_{\text{CTX-M-32}}$ gene (determined on the basis of that described for $bla_{\text{CTX-M-19}}$) is also indicated. The IRR sequence of IS*Ecp1* and the IRL and IRR of IS*1* are also indicated in bold type. The 5 bp duplicated target sites of the putative insertion site for the DNA fragment resulting from an IS*1*-mediated transposition process are underlined and indicated as dts. Different gene products are indicated below the amino acid sequence where appropriate. (b) Schematic map of the genetic environment surrounding the $bla_{\text{CTX-M-32}}$ gene. Open reading frames and genes are shown as boxes with an arrow indicating the orientation of each coding sequence and the gene name shown over the corresponding box. IRL and IRR motifs of IS*1* are indicated by vertical arrows. IRR of IS*Ecp1* is also indicated by a vertical arrow (irr). The promoter boxes of $bla_{\text{CTX-M-32}}$ are indicated as P(−10) and P(−35). t, truncated proteins. Horizontal arrows below indicate oligonucleotides used for sequencing of nucleotides.

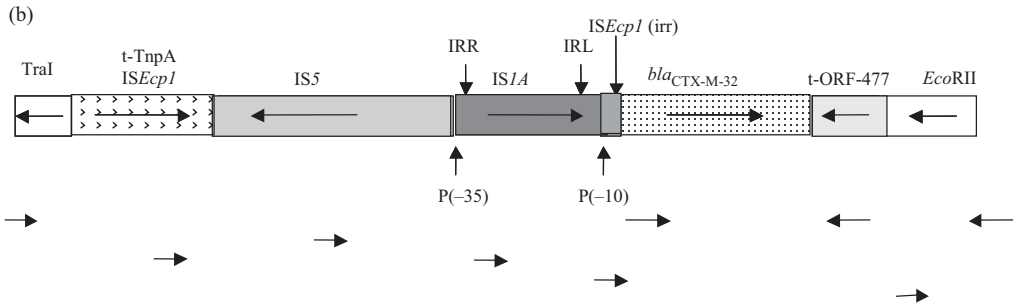

Figure 2. Continued.

cefepime for pAF-3 transformants were also lower. Moreover, specific activity towards nitrocefin was $1.9 \pm 0.05 \times 10^{-4}$ and $8.9 \pm 2.2 \times 10^{-6}$ μmol/s · μg of protein with cell extract from transformants pAF-2 and pAF-3, respectively. There were no differences between pAF-2 and pAF-3 plasmids in terms of the sequence of nucleotides in $bla_{\text{CTX-M-32}}$ or additional regulatory sequences (data not shown). RT–PCR analysis showed ≥10 times higher expression of $bla_{\text{CTX-M-32}}$ in *E. coli* TG1 expressing the *bla* gene with its intact promoter (pAF-2 plasmid) with respect to that with IS*1* modifying the promoter region (pAF-3 plasmid).

These results were further supported by cloning the $bla_{\text{CTX-M-32}}$ gene under the control of the previously described CTX-M-14 gene promoter (positions 1502–1740 of the sequence with EMBL database accession no. AF252622, which have been demonstrated to drive high levels of gene transcription) into pBGS18 to obtain full ESBL expression. After transformation of different clones harbouring $bla_{\text{CTX-M-32}}$ under the CTX-M-14 gene promoter into *E. coli* TG1, the resulting MICs of ceftazidime were >256 mg/L (data not shown).

Discussion

We report the identification of the $bla_{\text{CTX-M-32}}$ gene in two different Enterobacteriaceae species (*E. coli* and *P. mirabilis*) as well as in a genetically unrelated strain of *E. coli* isolated from the same patient. The patient had a history of recurrent UTIs and kidney stones, which may have favoured *in vivo* transmission of the plasmid harbouring the $bla_{\text{CTX-M-32}}$ gene between *E. coli* and *P. mirabilis*. The presence of bacteria within the stones suggests that the infecting bacteria play a major role in the structure of the stones. Growth of biofilm may provide organisms with survival advantages and increase their virulence as well as facilitating horizontal gene transfer (i.e. ESBL).[30,31]

There are few examples showing putative *in vivo* transmission of ESBL genes. Mugnaioli *et al.*[32] reported a putative *in vivo* transmission of CTX-M-1 between *E. coli* and *Citrobacter amalonaticus* and *Morganella morganii*, which highlights the ability of CTX-M-type ESBL genes to spread among different species of Enterobacteriaceae.

Analysis of the surrounding sequence of $bla_{\text{CTX-M-32}}$ in the clinical strains under study revealed different ISs. The first was a partially truncated fragment of IS*Ecp1 tnpA* transposase. The next IS found was the complete IS*5*, which has previously been detected upstream of $bla_{\text{CTX-M-32}}$.[18] The IS*1* IS was found between the IS*5* and $bla_{\text{CTX-M-32}}$ and it appeared to be complete, with all elements required for its function. The IS*1* IS has also previously been detected upstream of the $bla_{\text{CTX-M}}$ genes and found to disrupt the IS*Ecp1* element.[14] The ISs, such as IS*1*, are an important source of genetic plasticity in prokaryotes.[28,29] This is the first description of the presence of ISs IS*Ecp1* (truncated), IS*5* and IS*1* together in the same plasmid, upstream of a $bla_{\text{CTX-M}}$ gene. A 281 bp sequence was detected downstream of the *bla* gene and corresponded to a truncated part of ORF-477, which has previously been found in genetic structures surrounding plasmid-borne $bla_{\text{CTX-M}}$ genes in bacterial clinical isolates and described downstream of the $bla_{\text{CTX-M-3}}$ gene of *Kluyvera ascorbata*.[4,14]

The MICs of ceftazidime for transconjugants and transformants harbouring $bla_{\text{CTX-M-32}}$ were not as high as those expected for the CTX-M-32 enzyme,[18] which has been attributed to resistance resulting from ceftazidime hydrolysis.[4,18] A detailed analysis of the upstream sequence surrounding the $bla_{\text{CTX-M-32}}$ genes reported here showed that the IS IS*1* was located between the −35 and −10 promoter boxes, thereby separating and breaking the functionality of the $bla_{\text{CTX-M-32}}$ promoter region,[18] results that were further confirmed in this work. Indeed, the ratio of the relative specific activity against nitrocefin of protein extracts from pAF-2 and pAF-3 transformants (that expressed the originally reported $bla_{\text{CTX-M-32}}$ promoter and that IS*1* truncated, respectively) was ≥20 times higher, which may account for differences in β-lactamase expression, and therefore in the MICs of ceftazidime and other broad-spectrum β-lactams. Therefore, as well as contributing to high-level expression of *bla* genes, e.g. $bla_{\text{CTX-M-19}}$[17] and also $bla_{\text{CTX-M-32}}$,[18] ISs may also reduce or eliminate β-lactamase gene expression by interrupting a specific promoter region.[33]

In summary, putative transmission of a plasmid carrying $bla_{\text{CTX-M-32}}$ between two different genera of Enterobacteriaceae is described in a patient who suffered from repeated UTI. Analysis of the DNA sequence surrounding this $bla_{\text{CTX-M-32}}$ gene revealed a new composite structure formed by IS*Ecp-1*, IS*5* and IS*1*. The IS*1* element was inserted within the promoter region of $bla_{\text{CTX-M-32}}$, thereby reducing ESBL production, modulating antibiotic resistance and resulting in low ceftazidime MICs. These structures may complicate the detection of these ceftazidime-hydrolysing CTX-M-type enzymes.

Nucleotide sequence accession number

The GenBank accession number for the nucleotide sequence reported here is AM420303.

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Transparency declarations

None to declare.

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