Available online at www.sciencedirect.com
ScienceDirect
C. R. Biologies 330 (2007) 565–570
COMPTES RENDUS BIOLOGIES
http://france.elsevier.com/direct/CRASS3/

Microbiology / Microbiologie

Cefotaxime and ceftazidime-resistant *Escherichia coli* isolate producing TEM-15 β-lactamase from a Tunisian hospital

Chedly Chouchani *, Nahed Ben-Achour, Arij M'charek, Omrane Belhadj

Laboratoire de biochimie et de biotechnologie, faculté des sciences de Tunis, 2092 El-Manar II, Tunisia

Received 13 November 2006; accepted after revision 27 March 2007

Available online 17 May 2007

Presented by Jean Rosa

Abstract

A clinical isolate of *Escherichia coli* LBT04 was found to have a high-level resistance to broad-spectrum β-lactams. Analysis of this strain by the disk diffusion test revealed synergies between clavulanic acid and ceftazidime, cefotaxime. Clavulanic acid decreased the MICs of ceftazidime, cefotaxime, and ceftriaxone, which suggested that LBT04 produced an extended-spectrum β-lactamase. These resistances were carried by a 1080-bp chromosomal gene that encoded a β-lactamase with a pI of 6.3. Cloning and sequencing experiments showed that this β-lactamase revealed identity with the $bla_{\text{TEM-1}}$ gene encoding the TEM-1 β-lactamase, except for a replacement of the Glu residue at position 104 by Lys, and of the Gly residue at position 238 by Ser. These two mutations were encountered in TEM-15 β-lactamase, but this is the first description of this enzyme in the *E. coli* species in Tunisian hospitals. ***To cite this article: C. Chouchani et al., C. R. Biologies 330 (2007).***

Keywords: *E. coli*; Cefotaxime; Ceftazidime; TEM-15; ESBL

1. Introduction

Extended-spectrum β-lactamases (ESBLs) are enzymes that confer resistance to oxyimino-β-lactams such as cefotaxime, ceftazidime, ceftriaxone, and the monobactam aztreonam, but not to cephamycins or carbapenems [1]. The first ESBLs, which are predominantly derivatives of plasmid-mediated TEM or SHV β-lactamases, arise through mutations that introduce one or more amino acid substitutions that alter the configuration or binding properties of the active site [2], resulting in an expansion of the substrate range of the enzymes (G.A. Jacoby and K. Bush, website http://www.lahey.org/studies/webt.htm).

ESBL-producing clinical isolates are frequently associated with nosocomial outbreaks [3,4], with production detected most commonly in *Escherichia coli* [5,6] in addition to other members of the *Enterobacteriaceae* family [7] and *Pseudomonas aeruginosa* [8]. Novel ESBLs continue to be reported at an alarming rate (Jacoby and Bush website) [9,10]. The incidence of resistance to extended-spectrum β-lactam antibiotics is increasing in Tunisia, and recent studies revealed the involvement of TEM-138 encoding plasmid in *Salmonella enterica* serovar Infantis [11]. In this study, we reported that TEM-15 has been the most commonly identified ESBL in *Escherichia coli*

* Corresponding author.
E-mail address: chdoula77@yahoo.fr (C. Chouchani).

1631-0691/$ – see front matter
doi:10.1016/j.crvi.2007.03.017

isolate. However, *E. coli* isolates usually show different resistance patterns for oxyimino-cephalosporins and monobactam; no data have been published yet about the prevalence and characteristics of ESBLs among *Escherichia coli* isolates in Tunisian hospitals.

2. Materials and methods

2.1. Bacterial strains and plasmid

Escherichia coli strain LBT04, isolated from the stool culture, was randomly selected from the outbreak isolates. *Escherichia coli* strain LBT04 was characterized by conventional tests on API 20 E gallery and on specific reactive media. *E. coli* HB101 (rec A1, F^-, lac Y, rpsL20, thi-1, IncFI, str^r), *E. coli* DH10B (F^-mcrAΔ (mrr$^-$hsd RMS-mcrBC) Φ80 lacz ΔM15 Δlac X74 rec A1 end A1 ara D139 Δ (ara, leu) 7697 gal U gal Kλ^- rspL nupG (Invitrogen, Life Technologies), and *E. coli* DH5α (rec A1, F^-, end A1, gyr A96, thi-1, hsd R17, rK^-, mK+, sup E44, rel A1, Δlac U69, Φ80 laz ΔM15) were used as hosts for plasmids. Plasmid pGEM-T Easy (Promega, USA) was used as cloning vector.

2.2. Antimicrobial susceptibility testing

We used the disk diffusion assay with MH agar in accordance with CA-SFM Guidelines (communiqué 2006; http://www.sfm.asso.fr/sect4/atbfr.htm). MICs of selected antimicrobial agents were determined by broth microdilution agents in cation-adjusted Muller–Hinton broth [12]; they were inoculated and analysed using Guidelines of CA-SFM.

2.3. Isoelectric focusing of β-lactamases

Crude β-lactamase preparation was extracted by a sonication method [12]. Analytical isoelectric focusing was performed as described previously [13]. Supernatants of sonicates were subjected to isoelectric focusing for 3 h by using a 111Mini IEF Cell (Bio-Rad), and a gradient made up of polyampholytes with a pH range of 3 to 10 (Bio-Rad). Extracts from TEM-1-, TEM-2-, TEM-3- and SHV-1-producing strains were used as standards for pIs of 5.4, 5.6, 6.3, and 7.6, respectively. β-Lactamases activities were revealed with the iodine method [14], with cephaloridine (0.1 mg/ml) in phosphate buffer (50 mM; pH 7) [7].

2.4. Analysis of plasmids and transfer of resistance

Analysis of plasmids was performed by the alkaline lysis method, as described by Sambrook et al. [15]. Extracts were run on 0.8% agarose gel at 7 V/cm and stained with ethidium bromide. Conjugation experiments were carried out with *E. coli* HB101, as previously described [16]. The transconjugants were selected on LB agar supplemented with streptomycin (100 μg/ml) and ampicillin (100 μg/ml).

The plasmid of the clinical isolate was electroeluted and transformed into *E. coli* DH10B (Electro Max^{TM} DH10B^{TM} Cells; Invitrogen) by electroporation using a Bio-Rad gene pulser (set at 2.5 kV, 25 μF and 350 Ω). Transformants were selected by plating on LB agar plates containing 100 μg/ml ampicillin.

2.5. Total DNA extraction

Total DNA of *E. coli* LBT04 was extracted as previously described [4] using the Wizard® Genomic DNA purification Kit (Promega, USA) according to the manufacturer's instructions.

2.6. Southern hybridization

Total DNA was digested with *Hind*III, *Pvu*I and *Xba*I. After agarose gel electrophoresis, restriction products were transferred to a nylon membrane by the vacuum transfer method, as described previously [17] and hybridized with a CDP-labelled probe (the probe was a PCR product of the bla_{TEM} gene) using the AlkPhos Direct Kit (Amersham, Life Science, Germany). Hybridization was achieved at 55 °C by overnight incubation. A 2.3-kb fragment corresponding to a hybridization spot was eluted and purified using a GFX PCR DNA and Gel Band Purification Kit (Amersham, Life Science, Germany). This purified DNA was used to amplify the bla_{TEM} gene of the *E. coli* LBT04 strain.

2.7. DNA amplification

PCR amplification of the TEM gene was carried out on a Tricycler-DNA, (Biometra®). The PCR mixture contained, in a total volume of 50 μl: 10 pmol of each primer; 0.2 pmol of deoxyribonucleoside triphosphates; 1 U of Taq DNA Polymerase (Promega) and 10 pmol of 2.3 purified fragment. The following oligonucleotides primers specific for the TEM genes were obtained from Eurogentec (Belgium).

TEM-D 5′ ATAAAATTCTTGAAGACGAAAG 3′

TEM-R 5′ TTACCAATGCCTTAATCAGTGA 3′

The PCR programme consisted of an initial denaturing at 94 °C for 12 min, followed by 30 cycles of 60 s at 94 °C, 60 s at 55 °C, and 90 s at 72 °C. A final extension was performed at 72 °C for 10 min [18].

2.8. Cloning of the $bla_{\text{TEM-15}}$ gene

The $bla_{\text{TEM-15}}$ and 214 bp upstream region was amplified by PCR. The PCR product was purified using a GFX column (Amersham Biosciences, Germany), ligated into pGEM-T Easy cloning vector, and transformed into *E. coli* DH5α competent cells. Antimicrobial susceptibilities of the *E. coli* DH5α harbouring the recombinant plasmid (pGEM-T Easy + $bla_{\text{TEM-15}}$) were shown in Table 2.

2.9. Sequencing

The resulting plasmids were isolated and the sequence of the PCR-generated insert was determined using T7 and SP6 primers. The sequences were performed by the dideoxy-chain-termination procedure of Sanger et al. [19] on an ABI 1377 automatic sequencer with the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit, with Ampli Taq DNA polymerase.

3. Results

3.1. Properties of E. coli LBT04

LBT04 was isolated in a paediatric ward of a Tunisian hospital, from the stool culture. Analysis of this strain by the conventional disk diffusion antibiotic susceptibility test suggested that β-lactams resistance was due to the presence of the ESBL. Synergies were observed between clavulanic acid, cefotaxime, and ceftazidime. LBT04 was also resistant to chloramphenicol, gentamicin, and tetracycline. The MICs of β-lactams for LBT04 indicated that this strain was resistant to all antibiotics tested, except imipenem and streptomycin (Table 1). These results suggested that *E. coli* LBT04 produced an ESBL.

3.2. β-Lactamase characterization

Analytical isoelectric focusing of crude β-lactamases extracts of *E. coli* LBT04 strain revealed that this strain produces two β-lactamases, with pIs of 5.4 and 6.3. A single β-lactamase with a pI of 5.4 was transferred to transconjugants and transformants (Fig. 1). Extended spectrum β-lactam resistance was not conjugatively transferred to *E. coli* HB101 from the LBT04 isolate. The transconjugants and transformants confirmed to be extended-spectrum β-lactams susceptible (Table 1).

Table 1
MICs of various antimicrobial agents obtained for the clinical isolate *E. coli* LBT04, their transconjugants and transformants, and the *E. coli* recipients

Antibiotics	*E. coli*				
	LBT04	LBT04 × HB101	HB101	DH10B/pLBT04	DH10B
Ampicillin	>256	>256	4	>256	2
Oxacillin	>256	>256	<1	>256	2
Ticarcillin	>256	>256	2	>256	2
Ticarcillin + CLA	32	128	<1	64	<1
Benzylpenicillin	>256	>256	<1	>256	<1
Imipenem	<1	2	1	<1	1
Cephalothin	>256	>256	2	>256	<1
Cephaloridine	>156	128	1	>256	1
Cefotaxime	>256	2	1	4	4
Ceftazidime	>256	2	<1	2	1
Ceftriaxone	>256	< 1	<1	2	1
Cefuroxime	>256	4	2	<1	2
Cefpirome	256	2	2	2	<1
Cefoxitin	<1	<1	2	2	4
Streptomycin	8	>256	>256	2	1
Chloramphenicol	>256	<1	<1	<1	2
Nalidixic acid	16	2	1	2	2
Tetracycline	16	<1	2	<1	<1

Table 2
Antimicrobial susceptibilities of the clinical strain LBT04, *E. coli* DH5α harbouring the recombinant plasmid (pGEM-T Easy + bla_{TEM-15}), and the reference strain

Antibiotics	*E. coli*		
	LBT04	DH5α (pGEM + bla_{TEM-15})	DH5α
Ampicillin	>256	128	2
Oxacillin	>256	>256	4
Ticarcillin	>256	>256	1
Ticarcillin + CLA	32	128	<1
Benzylpenicillin	>256	>256	2
Imipenem	<1	<1	<1
Cephalothin	>256	>256	2
Cephaloridine	>156	>256	<1
Cefotaxime	>256	256	2
Ceftazidime	256	256	2
Ceftriaxone	>256	128	<1
Cefuroxime	>256	128	4
Cefpirome	256	64	2
Cefoxitin	<1	<1	<1
Streptomycin	8	2	2
Chloramphenicol	>256	<1	<1
Nalidixic acid	16	2	2
Tetracycline	16	<1	<1

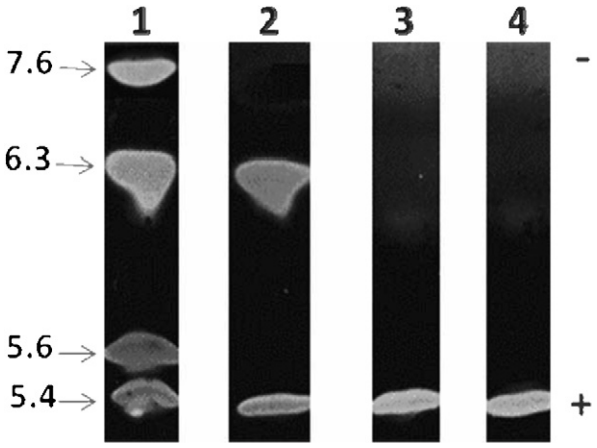

Fig. 1. IEF of crud extract of *E. coli* LBT04 (lane 2), their transformants (lane 3), and their transformants (lane 4). β-Lactamase standards TEM-1 (pI 5.4), TEM-2 (pI 5.6), TEM-3 (pI 6.3) and SHV-1 (pI 7.6) (lane 1).

3.3. DNA characterization

The single plasmid extracted from LBT04 strain was transferred to *E. coli* DH10B by transformation. Gene encoding for an ESBL type is not located in this plasmid. Hybridization methods show that the ESBL-encoding gene was located on a 2.3-kb fragment (Fig. 2) and confirm that the extended-spectrum β-lactamase was encoded by a chromosomal gene.

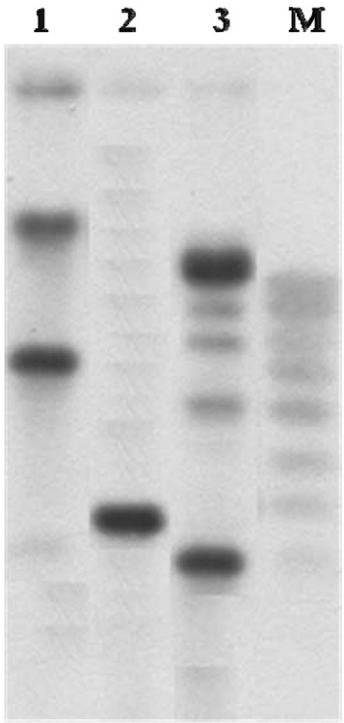

Fig. 2. Hybridization pattern with the TEM probe after *Hind*III, *Pvu*I, and *Xba*I digestion of total DNA. Lane: **1**, *Hind*III fragments; **2**, *Xba*I fragments; **3**, *Pvu*I fragments; **M**, 10-kb DNA marker.

3.4. Gene characterization

PCR amplification specific for the bla_{TEM} genes on both the LBT04 strain gave a fragment of the expected size of 1080 bp. Cloning and sequencing on both strands of the complete gene revealed that it was identical to bla_{TEM-1}, except for two-point mutations. These mutations are reflected in a change of two amino acids. The mutations consisted of a replacement of the Glu residue at position 104 (GAG) by Lys (AAG) and of the Gly residue at position 238 (GGT) by Ser (AGT). Multiple-sequence alignments of the DNA sequence and of the amino-acid sequence of this β-lactamase against that of TEM-1 was performed with the website: http://prodes.toulouse.inra.fr/multalin/multalin.html, and the blasts of the amino acid sequences of this β-lactamase were performed with the website: http://www.ncbi.nlm.nih.gov/.

4. Discussion

In the present study, we have tried to analyse a type of resistance gene; cloning and sequencing experiments showed that the corresponding bla_{TEM} gene was identical to the bla_{TEM-15} gene, but this gene was chromo-

Table 3
Amino-acid substitutions of TEM-type variants at critical positions

β-lactamase	pI	Amino acid at the following position							References or sources
		21	**39**	**104**	**153**	**175**	**182**	**238**	
TEM-1	5.4	Leu	Gln	Glu	His	Asn	Met	Gly	[20]
TEM-3	5.6		***Lys***	***Lys***				***Ser***	[23]
TEM-4	5.9	***Phe***		***Lys***				***Ser***	[29]
TEM-15	6.3		***Lys***					***Ser***	[24]
TEM-20	5.4						***Thr***	***Ser***	[28]
TEM-21	6.4		***Lys***	***Lys***	***Arg***			***Ser***	[28]
TEM-52	6.0			***Lys***	***Thr***			***Ser***	[26]
TEM-138	5.8			***Lys***		***Ile***		***Ser***	[11]

somally located, since the TEM type of resistance has been postulated to be present in *E. coli* strains [20]. Studies with this type of resistance gene may allow a more precise delineation of the correlation, or, possibly, the evolution of different TEM-related mutations among all.

The β-lactamase described in this report, TEM-15, most arose from a TEM-1 ancestor. Sequence analysis revealed that the $bla_{\text{TEM-15}}$ gene of LBT04 differed from $bla_{\text{TEM-1}}$ by two mutations, leading to two amino-acids substitutions: Glu for Lys at position 104 and Gly for Ser at position 238. Since the combination of these two substitutions has been described previously in TEM-15 ESBL, this is the first description of the TEM-15 chromosomally encoding gene in an *E. coli* isolate in Tunisian hospitals. These genes encoding the TEM type ESBLs are supposed to be located on transposons of the Tn*A* family, as are those for the TEM-1 and TEM-2 parental enzymes. However, while the plasmid or chromosomal origin of these genes is usually determined, their precise genetic location is rarely specified [21,22].

TEM-15 is closely related to TEM-3, one of the first ESBLs to have been described [23,24]. The same critical substitutions involved in the extension of the β-lactamase spectrum were present in this enzyme at positions 104 and 238, but TEM-15 differed from TEM-3 by a Gln-to-Lys change at position 39 [25], and from TEM-52 by a Met-to-Thr change at position 182 [26]. Finally, TEM-15 differed from TEM-123 by a Lys-to-Gln change at positions 6 [27]. These results suggest that the combination of Lys 104 and Ser 238 is responsible for the extended spectrum of TEM-15 and is required for extended-spectrum β-lactams (Table 3).

Other TEM-type ESBLs detected in Tunisia during these last 20 years were postulated. We also compare TEM-15, recently detected in Tunisia and described in this report, to these TEM-type ESBLs. TEM-20 and TEM-21 were the first TEM-type ESBLs detected in Tunisia: TEM-15 differed from TEM-20 by a Lys-to-Glu change at position 104 and by a Met-to-Thr change at position 182, and differed from TEM-21 by a Gln-to-Lys change at position 39 and by a His-to-Arg change at position 153 [28]. TEM-15 differed from TEM-4, recently detected in Tunisia, by a Leu-to-Phe change at position 21 [11]. Finally, TEM-15 differed also from TEM-138, more recently detected in Tunisia by an Asn-to-Ile change at position 175 [11].

The molecular characterization of TEM-15 emphasizes the key role of Ser-238, mutation present in all ESBL described in this report, in conferring resistance to extended-spectrum β-lactams antibiotics and provides further insight into the understanding of the catalytic process of class-A β-lactamase. This study is the first description of the $bla_{\text{TEM-15}}$ gene chromosomally located and of the presence of this gene in a clinical isolate of *E. coli*, highlighting once more the broad exchange of resistance genes between *Enterobacteriaceae* families in a Tunisian outbreak ward.

Acknowledgements

This work was funded by grants from the Tunisian Ministry of Scientific Research and Technology. Chedly Chouchani was supported by a fellowship from the Tunisian Ministry of Higher Education. We acknowledge the Ministry of Higher Education for their helpful assistance.

References

[1] K. Bush, G.A. Jacoby, A.A. Medeiros, A functional classification scheme for β-lactamases and its correlation with molecular structure, Antimicrob. Agents Chemother. 39 (1995) 1211–1233.
[2] J.M. Ghuysen, Molecular structures of penicillin-binding proteins and β-lactamases, Trends Microbiol. 2 (1994) 372–380.
[3] M. Gniadkowski, I. Schneider, R. Jungwirth, W. Hyryniewicz, A. Bauernfeind, Ceftazidime-resistance *Enterobacteriaceae* isolates from three Polish hospitals: identification of three novel TEM- and SHV-5-type extended spectrum β-lactamases, Antimicrob. Agents Chemother. 42 (1998) 514–520.

[4] C. Pena, M. Pujol, C. Ardanuy, A. Ricart, R. Pallares, J. Linares, J. Ariza, F. Gudiol, Epidemiology and successful control of a large outbreak due to *Klebsiella pneumoniae* producing extended-spectrum β-lactamases, Antimicrob. Agents Chemother. 42 (1998) 53–58.

[5] J. Silva, C. Aguilar, G. Ayala, M.A. Estrada, U. Garza-Ramos, R. Lara-Lemus, L. Ledezma, TLA-1: A new plasmid-mediated extended-spectrum beta-lactamase from *Escherichia coli*, Antimicrob. Agents. Chemother. 44 (2000) 997–1003.

[6] X.Y. Zhou, F. Bordan, D. Sirot, M.D. Kitzis, L. Gutmann, Emergence of clinical isolates of *Escherichia coli* producing TEM-1 derivatives or an OXA-1 β-lactamase conferring resistance to β-lactamase inhibitors, Antimicrob. Agents Chemother. 38 (1994) 1085–1089.

[7] P.E. Coudron, E.S. Moland, C.C. Sanders, Occurrence and detection of extended-spectrum β-lactamases in members of the family *Enterobacteriaceae* at a veterans medical center: seek and you may find, J. Clin. Microbiol. 35 (1997) 2593–2597.

[8] P. Mugnier, P. Dubrous, I. Casin, G. Arlet, E. Collatz, A TEM-derived extended-spectrum β-lactamase in *Pseudomonas aeruginosa*, Antimicrob. Agents Chemother. 40 (1996) 2488–2493.

[9] A. Hossain, M.D. Reisbig, N.D. Hanson, Plasmid-encoded functions compensate for the biological cost of AmpC overexpression in a clinical isolate of *Salmonella typhimurium*, J. Antimicrob. Chemother. 53 (2004) 964–970.

[10] M. Wang, D.F. Sahm, G.A. Jacoby, D.C. Hooper, Emerging plasmid-mediated quinolone resistance associated with the *qnr* gene in *Klebsiella pneumoniae* clinical isolates in the United States, Antimicrob. Agents Chemother. 48 (2004) 1295–1299.

[11] C. Chouchani, R. Berlemont, A. Masmoudi, M. Galleni, J.-M. Frere, O. Belhadj, K. Ben-Mahrez, A novel extended-spectrum TEM-type β-lactamase (TEM-138) from *Salmonella enterica* serovar Infantis, Antimicrob. Agents Chemother. 50 (2006) 3183–3185.

[12] S. Réjiba, F. Limam, C. Belhadj, O. Belhadj, K. Ben-Mahrez, Biochemical characterization of a novel extended-spectrum β-lactamase from *Pseudomonas aeruginosa* 801, Microb. Drug Resistance 8 (2002) 9–13.

[13] T. Ben-Hamouda, T. Foulon, K. Ben-Mahrez, Involvement of SHV-12 and SHV-2a encoding plasmids in outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a Tunisian neonatal ward, Microb. Drug Resist. 10 (2004) 132–138.

[14] C.J. Perret, Iodometric assay of penicillinases, Nature 154 (1954) 1012–1013.

[15] J. Sambrook, E.F. Fritsch, T. Maniatis, Plasmid Vectors, in: Molecular Cloning: A Laboratory Manual, second ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, pp. 1.25–1.74.

[16] T. Ben-Hamouda, T. Foulon, A. Ben-Cheikh-Masmoudi, C. Fendri, O. Belhadj, K. Ben-Mahrez, Molecular epidemiology of an outbreak of multiresistant *Klebsiella pneumoniae* in a Tunisian neonatal ward, J. Med. Microbiol. 52 (2003) 427–433.

[17] T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular cloning: a laboratory manual, Cold Spring Harbor, NY, USA, 1982.

[18] R. AitMhand, A. Soukri, N. Moustaoui, H. Amarouch, N. El-Mdaghri, D. Sirot, M. Benbachir, Plasmid-mediated TEM-3 extended-spectrum β-lactamase production in *Salmonella typhimurium* in Casablanca, J. Antimicrob. Chemother. 49 (2002) 169–172.

[19] F. Sanger, S. Nicklen, A.R. Coulson, DNA sequencing with chain-terminating inhibitors, Proc. Natl Acad. Sci. USA 74 (1977) 5463–5467.

[20] J.G. Sutcliffe, Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322, Proc. Natl Acad. Sci. USA 75 (1978) 3737–3741.

[21] J. Heritage, P.M. Hawkey, N. Todd, I.J. Lewis, Transposition of the gene encoding a TEM-12 extended-spectrum β-lactamase, Antimicrob. Agents Chemother. 36 (1992) 1981–1986.

[22] L. Poirel, T. Naas, D. Nicolas, L. Collet, S. Bellais, J.-D. Cavallo, P. Nordmann, Characterization of VIM-2, a carbapenem-hydolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France, Antimicrob. Agents Chemother. 44 (2000) 891–897.

[23] W. Sougakoff, S. Goussard, G. Gerbaud, P. Courvalin, Plasmid-mediated resistance to third-generation cephalosporins caused by point mutations in TEM-type penicillinase genes, Rev. Infect. Dis. 10 (1988) 879–884.

[24] C. Mabilat, P. Courvalin, Development of 'oligotyping' for characterization and molecular epidemiology of TEM β-lactamases in members of the family *Enterobacteriaceae*, Antimicrob. Agents Chemother. 34 (1990) 2210–2216.

[25] A. Huletsky, J.R. Knox, R.C. Lesvesque, Role of Ser-238 and Lys-240 in the hydrolysis of third-generation cephalosporins by SHV-type β-lactamases probed by site direct mutagenesis and three-dimensional modelling, J. Biol. Chem. 268 (1993) 3690–3697.

[26] C. Poyart, R. Mugnier, G. Quesnes, R. Berche, P. Trieu-Cuot, A novel extended-spectrum TEM-type β-lactamases (TEM-52) associated with decreased susceptibility to moxalactam in *Klebsiella pneumoniae*, Antimicrob. Agents Chemother. 42 (1998) 108–113.

[27] M. Perilli, G. Amicosante, GenBank AY327539.

[28] A. Guillaume, S. Goussard, P. Courvalin, A. Philippon, Sequences of the genes for the TEM-20, TEM-21, TEM-22, and TEM-29 extended-spectrum β-lactamases, Antimicrob. Agents Chemother. 43 (1999) 969–971.

[29] K.V. Venkatachalam, W. Huang, M. LaRocco, T. Palzkill, Characterization of TEM-1 β-lactamase mutants from positions 238 to 241 with increased catalytic efficiency for ceftazidime, J. Biol. Chem. 269 (1994) 23444–23450.