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TEM-103/IRT-28 β-Lactamase, a New TEM Variant Produced by *Escherichia coli* BM4511

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Clinical isolate *Escherichia coli* BM4511 was resistant to broad-spectrum penicillins in the presence or in the absence of β-lactamase inhibitors but remained susceptible to cephalosporins. Resistance was due to production of a new TEM-type β-lactamase, designated TEM-103/IRT-28, characterized by the Arg_{275}Leu substitution and encoded by the ca. 62-kb pIP845 conjugative plasmid of the IncI1 incompatibility group.

Among the mechanisms responsible for resistance of *Escherichia coli* to β-lactam–β-lactamase inhibitor combinations, hyperproduction of TEM-1 penicillinase and alteration in the outer membrane proteins limiting the entry of the drugs were reported first (16, 19). Inhibitor-resistant TEM (IRT) β-lactamases have been isolated since the beginning of the 1990s and are mostly produced by strains of *E. coli* but also by clinical isolates of *Klebsiella* spp. and *Proteus mirabilis* (2). These enzymes confer resistance to penicillins and their combinations with β-lactamase inhibitors (3, 6, 13). IRT variants are derivatives of TEM β-lactamases, with mutations at various amino acid positions (69, 130, 244, 275, and 276, according to Ambler's numbering [1]) that have been shown, or postulated, to play a role in determining resistance to inhibitors. These substitutions decrease the affinity for β-lactams and alter the way in which the enzymes interact with suicide inactivators (9). We report the genetic characterization of a new TEM variant, TEM-103/IRT-28, in a clinical isolate of *E. coli* resistant to amoxicillin-clavulanate and susceptible to cephalosporins.

E. coli BM4511 was isolated from the surgery wound of an 82-year-old woman suffering from colon cancer and hospitalized in Hospital de Basurto, Bilbao, Spain. The MICs of various β-lactams, alone or in combination with β-lactamase inhibitors used at fixed concentrations (clavulanic acid, 2 μg/ml; tazobactam, 4 μg/ml; sulbactam, 8 μg/ml), were determined by the microdilution method in Mueller-Hinton broth (Difco) with an inoculum of approximately 5×10^4 cells/well. BM4511 was resistant to penicillins (MICs ≥ 1,024 μg/ml) but susceptible to cephalosporins (MICs ≤ 4 μg/ml). Clavulanic acid was unable to restore susceptibility of the strain to penicillins, but tazobactam reduced significantly the piperacillin MIC (Table 1). These data were compatible with the production of an IRT β-lactamase. The presence of a bla_{TEM} gene in BM4511 was revealed by PCR amplification (data not shown) using primers TEM-C and TEM-H (12). To determine the pI of the enzyme, crude sonic bacterial extracts were applied to Phast-gels with different pH gradients in a Phast System (Pharmacia) and compared with TEM-30/IRT-2 (pI 5.2), TEM-1 (pI 5.4), and TEM-2 (pI 5.6). The strain was shown to produce a β-lactamase with an isoelectric point of 5.2 (data not shown). All IRT-type β-lactamases reported so far, except for TEM-59/IRT17 (pI 5.6), have a pI of either 5.2 or 5.4.

Resistance to amoxicillin-clavulanate was transferred by conjugation from BM4511 to *E. coli* JM83 at an approximate frequency of 10^{-7} per donor. Analysis of the donor and of a transconjugant after digestion with *Eco*RI revealed the presence of a single plasmid, pIP845, with a size of approximately 62 kb. By reciprocal conjugation with *E. coli* BM21 (5) containing plasmids belonging to various incompatibility groups, pIP845 was found to be of incompatibility group I1; the size of these plasmids ranges between 55 and 80 kb (A. Labigne, personal communication).

Fragments of pIP845 DNA generated by double digestion with *Eco*RI and *Bam*HI were inserted into pBGS18+ (17), and the resulting recombinant plasmids were introduced into *E. coli* TOP10 (Invitrogen) with selection on ampicillin at 100 μg/ml. The smallest plasmid able to confer resistance to amoxicillin-clavulanate to the new host, pAT779, had a 1.3-kb insert. Direct sequencing of the resistance gene, designated $bla_{TEM-103}$, using an automated sequencer (CEQ 2000 DNA analysis system; Beckman Coulter) revealed that it was identical to bla_{TEM1-B} except for a single G-to-T change at position 1020 (according to Sutcliffe's numbering [18]) (Table 2), which leads to the amino acid substitution of Arg to Leu at position 275 (according to Ambler's numbering [1]). The change of arginine, a basic positively charged amino acid, to leucine, an uncharged amino acid, accounts for the decrease in pI from 5.4 to 5.2 (9). Substitutions that lead to an inhibitor-resistant phenotype occur at a few specific sites within the TEM enzymes: Met-69, Arg-244, Arg-275, and Asn-276 (14). To the best of our knowledge, the Arg_{275}Leu substitution has not yet been observed alone in TEM-type β-lactamases, and the enzyme was designated TEM-103/IRT-28 according to the TEM and IRT nomenclature (G. Jacoby and K. Bush, Amino acid sequences for TEM, SHV, and OXA extended-spectrum

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TABLE 1. MICs of β-lactams against strains

E. coli strain	MIC (μg/ml)[a]										
	AMX	AMX + CLA	TIC	TIC + CLA	AMP	AMP + SUL	PIP	PIP + TZB	CEF	CFZ	FOX
BM4511, *bla*$_{TEM-103}$	>1,024	64	>1,024	64	>1,024	32	64	16	8	4	4
JM83	8	8	4	4	8	4	2	2	4	2	2
JM83 (pIP845), *bla*$_{TEM-103}$	>1,024	32	>1,024	32	>1,024	32	64	8	8	4	4
TOP10	4	4	4	4	4	4	4	4	4	1	2
TOP10 (pAT779), *bla*$_{TEM-103}$	>1,024	1,024	>1,024	>1,024	>1,024	>1,024	128	16	16	8	4

[a] AMP, ampicillin; AMX, amoxicillin; CEF, cephalothin; CFZ, cefazolin; CLA, clavulanic acid (2 μg/ml); FOX, cefoxitin; PIP, piperacillin; SUL, sulbactam (8 μg/ml); TIC, ticarcillin; TZB, tazobactam (4 μg/ml).

β-lactamases [http://www.lahey.org/studies/webt.html; last accessed 10 July 2002]).

The $Arg_{275}Leu$ substitution has been detected in TEM-38/IRT-9 associated with $Met_{69}Val$ (9) and in TEM-68 associated with $Gly_{238}Ser$, $Glu_{240}Lys$, and $Thr_{265}Met$ (7). Another mutation at this position, $Arg_{275}Gln$, was found in TEM-45/IRT-14 (4), TEM-82/IRT-24 (11), and TEM-83/IRT-25 (11) enzymes associated with $Met_{69}Leu$, $Met_{69}Val$, and $Met_{69}Ile$ substitutions, respectively; TEM-83 has, in addition, a $Trp_{165}Cys$ substitution. These β-lactamases, except for TEM-68, are enzymes with strong inhibitor-resistant activity because the Arg275 substitution is associated with substitutions at position 69 known to confer resistance to inhibitors (10). Therefore, the contribution of the Arg275 mutation to the IRT phenotype was not known.

Argnine 275 is located at the beginning of the C terminus of the α-11 helix, and its side chain is close to the guanidinium group of Arg244. A kinetic study of the $Arg_{275}Leu$ variant has shown the involvement of this change in resistance of the TEM β-lactamases to inactivation by clavulanic acid (15). This could be related to electrostatic interactions with Arg244 and/or to a possible displacement of the water molecule involved in the inactivation process of clavulanic acid (6). Variant TEM-68 (7) combines mutations responsible for the enlargement of the substrate profile ($Gly_{238}Ser$, $Glu_{240}Lys$, and $Thr_{265}Met$) with a change ($Arg_{275}Leu$) conferring resistance to inhibitors. Fiett et al. (7) attributed the inhibitor-resistant activity of TEM-68 to the $Arg_{275}Leu$ substitution.

Functional characterization of this new inhibitor-resistant β-lactamase should provide further insight into the understanding of the heterogeneity of IRT-type enzymes and into structure-activity relationships of the expanding family of TEM proteins.

TABLE 2. Substitutions in *bla*$_{TEM}$ genes and derived β-lactamases

Region and nucleotide no.[a] (amino acid no.)[b]	Nucleotide (amino acid) in the following gene (protein):							
	bla$_{TEM-1A}$	*bla*$_{TEM-1B}$	*bla*$_{TEM-2}$	*bla*$_{TEM-45}$	*bla*$_{TEM-68}$	*bla*$_{TEM-82}$	*bla*$_{TEM-83}$	*bla*$_{TEM-103}$
Promoter region	P3	P3	Pa and Pb	P4		Pa and Pb	P4	P3
32	C	C	T	C	ND[d]	T	C	C
162	G	G	G	T	ND	G	T	G
175	A	G	A	A	ND	A	A	G
Coding region								
226[c]	C (Phe)	T	C	C	T	C	C	T
317 (39)	C (Gln)	C	A (Lys)	C	C	C	C	C
346[c]	A (Glu)	A	G	G	A	A	G	A
407 (69)	A (Met)	A	A	A	A	G (Val)	T (Ile)	A
409 (69)	G (Met)	G	G	T (Leu)	G	G	G	G
436[c]	C (Gly)	T	T	T	T	C	T	T
604[c]	G (Ala)	T	G	G	G	G	G	T
682[c]	T (Thr)	T	C	C	C	T	C	T
697 (165)	G (Trp)	G	G	ND	G	G	T (Cys)	G
914 (238)	G (Gly)	G	G	ND	A (Ser)	G	G	G
917 (240)	G (Glu)	G	G	ND	A (Lys)	G	G	G
925[c]	G (Gly)	G	A	A	A	G	A	G
929 (244)	C (Arg)	C	C	ND	C	C	C	C
990 (265)	C (Thr)	C	C	ND	T (Met)	C	C	C
1020 (275)	G (Arg)	G	G	A (Gln)	T (Leu)	A (Gln)	A (Gln)	T (Leu)
1022 (276)	A (Asn)	A	A	ND	A	A	A	A
Source or reference	18	8	8	4	7	11	11	This study

[a] Nucleotide numbering is according to J. G. Sutcliffe (18).
[b] Amino acid numbering is according to Ambler et al. (1).
[c] Positions at which only silent mutations occur.
[d] ND, no data.

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REFERENCES

1. **Ambler, R. P., A. F. Coulson, J. M. Frère, J. M. Ghuysen, B. Joris, M. Forsman, R. C. Levesque, G. Tiraby, and S. G. Waley.** 1991. A standard numbering scheme for the class A β-lactamases. Biochem. J. **276:**269–270.
2. **Bermudes, H., F. Jude, E. B. Chaibi, C. Arpin, C. Bebear, R. Labia, and C. Quentin.** 1999. Molecular characterization of TEM-59 (IRT-17), a novel inhibitor-resistant TEM-derived β-lactamase in a clinical isolate of *Klebsiella oxytoca*. Antimicrob. Agents Chemother. **43:**1657–1661.
3. **Bonomo, R. A., S. A. Rudin, and D. M. Shlaes.** 1997. Tazobactam is a potent inactivator of selected inhibitor-resistant class A β-lactamases. FEMS Microbiol. Lett. **148:**59–62.
4. **Caniça, M. M., M. Barthélémy, L. Gilly, R. Labia, R. Krishnamoorthy, and G. Paul.** 1997. Properties of IRT-14 (TEM-45), a newly characterized mutant of TEM-type β-lactamases. Antimicrob. Agents Chemother. **41:**374–378.
5. **Chabbert, Y. A., M. R. Scavizzi, J. L. Witchitz, G. R. Gerbaud, and D. H. Bouanchaud.** 1972. Incompatibility groups and the classification of *fi*$^-$ resistance factors. J. Bacteriol. **112:**666–675.
6. **Chaibi, E. B., D. Sirot, G. Paul, and R. Labia.** 1999. Inhibitor-resistant TEM β-lactamases: phenotypic, genetic and biochemical characteristics. J. Antimicrob. Chemother. **43:**447–458.
7. **Fiett, J., A. Palucha, B. Miaczynska, M. Stankiewicz, H. Przondo-Mordarska, W. Hryniewicz, and M. Gniadkowski.** 2000. A novel complex mutant β-lactamase, TEM-68, identified in a *Klebsiella pneumoniae* isolate from an outbreak of extended-spectrum β-lactamase-producing *Klebsiellae*. Antimicrob. Agents Chemother. **44:**1499–1505.
8. **Goussard, S., and P. Courvalin.** 1999. Update sequence information for TEM β-lactamase genes. Antimicrob. Agents Chemother. **43:**367–370.
9. **Henquell, C., C. Chanal, D. Sirot, R. Labia, and J. Sirot.** 1995. Molecular characterization of nine different types of mutants among 107 inhibitor-resistant TEM beta-lactamases from clinical isolates of *Escherichia coli*. Antimicrob. Agents Chemother. **39:**427–430.
10. **Knox, J. R.** 1995. Extended-spectrum and inhibitor-resistant TEM-type β-lactamases: mutations, specificity, and three-dimensional structure. Antimicrob. Agents Chemother. **39:**2593–2601.
11. **Leflon-Guibout, V., V. Speldooren, B. Heym, and M. H. Nicolas-Chanoine.** 2000. Epidemiological survey of amoxicillin-clavulanate resistance and corresponding molecular mechanisms in *Escherichia coli* isolates in France: new genetic features of bla_{TEM} genes. Antimicrob. Agents Chemother. **44:**2709–2714.
12. **Mabilat, C., and P. Courvalin.** 1990. Development of oligotyping for characterization and molecular epidemiology of TEM β-lactamases in members of the family *Enterobacteriaceae*. Antimicrob. Agents Chemother. **34:**2210–2216.
13. **Medeiros, A. A.** 1997. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. Clin. Infect. Dis. **24**(Suppl. 1)**:** S19–S45.
14. **Nicolas-Chanoine, M. H.** 1997. Inhibitor-resistant β-lactamases. J. Antimicrob. Chemother. **40:**1–3.
15. **Saves, I.** 1995. Evolution de la β-lactamase TEM-1. Ph.D. thesis. University of Toulouse, Toulouse, France.
16. **Speldooren, V., B. Heym, R. Labia, and M. H. Nicolas-Chanoine.** 1998. Discriminatory detection of inhibitor-resistant β-lactamases in *Escherichia coli* by single-strand conformation polymorphism-PCR. Antimicrob. Agents Chemother. **42:**879–884.
17. **Spratt, B. G., P. J. Hedge, S. Heesen, A. Edelman, and J. K. Broome-Smith.** 1986. Kanamycin-resistant vectors that are analogous of plasmids pUC8, pUC9, pEMBL8, and pEMBL9. Gene **41:**337–342.
18. **Sutcliffe, J. G.** 1978. Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322. Proc. Natl. Acad. Sci. USA **75:**3737–3741.
19. **Wu, P. J., K. Shannon, and I. Phillips.** 1994. Effect of hyperproduction of TEM-1 β-lactamase on in vitro susceptibility of *Escherichia coli* to β-lactam antibiotics. Antimicrob. Agents Chemother. **38:**494–498.