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TEM-158 (CMT-9), a New Member of the CMT-Type Extended-Spectrum β-Lactamases[▽]

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TEM-158 was found to include the substitutions previously observed for TEM-12 and TEM-35. This enzyme presented hydrolytic activity against ceftazidime and a high level of resistance against clavulanate, which can alter its detection. Its discovery highlights the need for accurate detection methods.

Since the mid-1990s, a new subgroup of TEM β-lactamases that comprises enzymes harboring both extended-spectrum β-lactamase (ESBL)-type and inhibitor-resistant TEM (IRT)-type substitutions has emerged. These new β-lactamases, called complex mutants, were identified in different *Enterobacteriaceae* species (4, 6–11). They confer different levels of resistance to clavulanic acid and to oxyimino-cephalosporins, depending on the mutations harbored.

Escherichia coli BER1 was isolated from a stool specimen from a patient hospitalized in an intensive care unit of the University Hospital of Clermont-Ferrand, France. This patient had been treated with an amoxicillin-clavulanate combination for an aspiration pneumonia for 10 days. *E. coli* BER1 harbored a high level of resistance to penicillins and penicillin-clavulanate combinations and was in the intermediate range for ceftazidime. The French double-disk synergy test was negative for *E. coli* BER1. CLSI MIC testing was not reproducibly positive. A modified double-disk test with a 20-mm interdisk distance was positive between ceftazidime- and amoxicillin-clavulanate-containing disks (Fig. 1).

E. coli BER1 produced two β-lactamases, of pI 5.2 and pI 5.4. The genes encoding resistance to β-lactam antibiotics were transferred by conjugation to rifampin-resistant *E. coli* C600. A plasmid-content analysis revealed the transfer of an 85-kb plasmid, designated pBER1. The transconjugant *E. coli* C600 (pBER1) produced only one β-lactamase, of pI 5.2. TEM-specific PCR experiments were performed with the transconjugant as previously described (8). The nucleic acid sequence

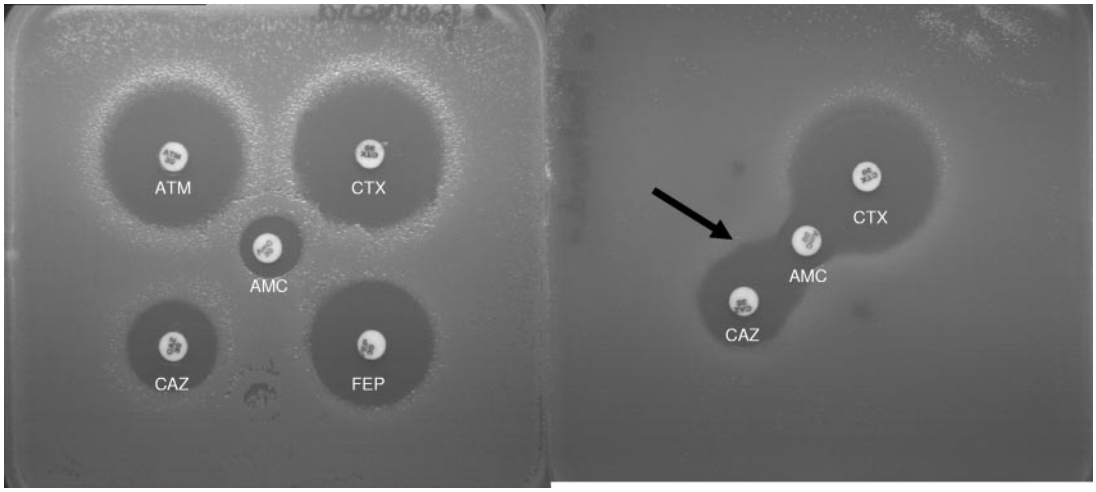

FIG. 1. Comparison of the synergy tests performed with a 30-mm interdisk distance (left), following Comité de l'Antibiogramme de la Société Française de Microbiologie recommendations (3), and with a 20-mm interdisk distance (right) for the clinical TEM-158-producing *E. coli* strain BER1. ATM, aztreonam; CTX, cefotaxime; AMC, amoxicillin-clavulanate; CAZ, ceftazidime; FEP, cefepime. The black arrow indicates a synergy.

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TABLE 1. MICs of β-lactam antibiotics for *E. coli* strains[a]

β-Lactam antibiotic(s)	MIC (μg/ml) for *E. coli* strain (plasmid)							
	BER1 (pBER1)	C600 (pBER1)	$DH_5\alpha$ (pBK-TEM-158)	$DH_5\alpha$ (pBK-TEM-12)	$DH_5\alpha$ (pBK-TEM-35)	$DH_5\alpha$ (pBK-TEM-1)	$DH_5\alpha$ (pBK-CMV)	C600
Amoxicillin	>2,048	>2,048	>2,048	>2,048	>2,048	>2,048	4	4
Amoxicillin + CLA	1,024	1,024	1,024	64	>2,048	16	4	4
Ticarcillin	>2,048	>2,048	>2,048	>2,048	>2,048	>2,048	2	2
Ticarcillin + CLA	512	512	512	32	>2,048	32	2	2
Piperacillin	2,048	2,048	2,048	>2,048	>2,048	512	2	2
Piperacillin + TZB	128	128	512	2	>2,048	2	2	2
Cephalothin	32	16	32	8	16	4	4	4
Cefuroxime	8	4	8	8	8	4	4	4
Cefoxitin	4	4	4	4	4	4	4	4
Cefotaxime	0.25	0.25	0.25	0.12	0.06	0.06	0.06	0.06
Cefotaxime + CLA	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Ceftazidime	16	16	32	32	0.25	0.12	0.12	0.12
Ceftazidime + CLA	4	2	8	0.5	0.12	0.12	0.12	0.12
Aztreonam	1	1	1	4	0.12	0.12	0.12	0.12
Aztreonam + CLA	0.12	0.25	0.25	0.12	0.12	0.12	0.12	0.12
Cefepime	4	4	4	1	<0.06	<0.06	<0.06	<0.06
Cefepime + CLA	0.25	0.12	0.25	0.12	<0.06	<0.06	<0.06	<0.06
Imipenem	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

[a] CLA, clavulanic acid at 2 μg/ml; TZB, tazobactam at 4 μg/ml.

of the PCR product revealed a new bla_{TEM}-type gene called $bla_{TEM\text{-}158}$. $bla_{TEM\text{-}158}$ harbored a promoter, P_3. The sequence of $bla_{TEM\text{-}158}$ showed a pattern of silent mutations identical to that of $bla_{TEM\text{-}1b}$ (5). The novel resulting enzyme, designated TEM-158, combined the mutations of IRT TEM-35 (IRT-4) (Met69Leu and Asn276Asp) and that of ESBL TEM-12 (Arg164Ser) (1, 2). This enzyme is the ninth member of the complex mutant TEM-derived subgroup (4, 6–11). *E. coli* $DH_5\alpha$ clones producing TEM-158, TEM-12, TEM-35, and TEM-1 were obtained as previously described (8). *E. coli* BER1, its clone *E. coli* $DH_5\alpha$ ClBER1, and its transconjugant C600 (pBER1) demonstrated high levels of resistance to penicillins, similar to those of the *E. coli* clones producing TEM-12 and TEM-35 (2,048 to >2,048 μg/ml) (Table 1). They were also in the intermediate range or resistant to ceftazidime (16 to 32 μg/ml) and to cephalothin (16 to 32 μg/ml). The MICs of cefotaxime, aztreonam, and cefepime were in the susceptible range (0.25 to 4 μg/ml) but higher than those for *E. coli* $DH_5\alpha$ (<0.06 to 0.12 μg/ml). MICs of cefuroxime, cefoxitin, and imipenem were closely similar to those of *E. coli* $DH_5\alpha$ (0.25 to 8 μg/ml). Clavulanate and tazobactam did not restore susceptibility to penicillins (128 to 1,024 μg/ml). ClBER1 MICs of penicillin-inhibitor combinations were lower than those of the TEM-35-producing clone (512 to 1,024 versus >2,048 μg/ml) but higher than those of the TEM-12-producing clone (512 to 1,024 versus 2 to 64 μg/ml). The ClBER1 MICs of cephalosporins were closely similar to those of the TEM-12-producing clone (0.25 to 32 μg/ml), but the addition of clavulanate only slightly decreased the MICs of oxyimino-β-lactams, in contrast to what was observed with *E. coli* $DH_5\alpha$ (pBK-TEM-12) (0.06 to 8 versus <0.06 to 0.5 μg/ml).

The different enzymes were purified to homogeneity, and their kinetic constants were determined by computerized microacidimetry as previously described (8). TEM-158 harbored 4- to 81-fold lower activity against penicillins than TEM-1, TEM-35, and TEM-12 (Table 2). TEM-158 K_m values for penicillins were closer to those of TEM-1 (K_m values, 24.8 to 142.6 versus 15 to 55 μM) than to those of TEM-35 (K_m values, 140 to 320 μM) and TEM-12 (K_m values, 7 to 15 μM). Overall, the catalytic efficiency of TEM-158 against penicillins was 8- to 129-fold lower than that of TEM-1, TEM-35, or TEM-12. The hydrolytic activity of TEM-158 against cephalothin was 176- to 635-fold lower than that of TEM-1, TEM-35, or TEM-12. However, TEM-158 K_m for this substrate was closer to those of

TABLE 2. Kinetic parameters of β-lactamases TEM-158, TEM-12, TEM-35, and TEM-1[a]

β-Lactam antibiotic	TEM-158			TEM-12			TEM-35[b]			TEM-1		
	k_{cat}	K_m	k_{cat}/K_m	k_{cat}	K_m	k_{cat}/K_m	k_{cat}	K_m	k_{cat}/K_m	k_{cat}	K_m	k_{cat}/K_m
Benzylpenicillin	18.5	38.1	0.49	80	7	11	1,050	140	7.5	1,500	34	44
Amoxicillin	14.4	24.8	0.58	60	7.5	8	900	245	8.5	1,125	15	75.0
Ticarcillin	4.2	142.6	0.029	19	12	1.6	125	320	0.4	135	36	3.8
Piperacillin	17.1	46.0	0.37	89	15	6	945	320	2.9	1,250	55	23
Cephalothin	0.26	170.4	0.0015	46	327	0.02	52	1,200	0.04	165	242	0.7
Ceftazidime	1.7	184.1	0.009	11.1	254	0.04	<0.1	ND	ND	<0.1	ND	ND
Cefotaxime	0.08	207.5	0.0004	10.6	320	0.03	<0.1	ND	ND	<0.1	ND	ND
Aztreonam	0.06	75.6	0.0008	2	247	0.008	<0.1	ND	ND	<0.1	ND	ND

[a] k_{cat} values are expressed in s^{-1}; K_m values are expressed in μM; k_{cat}/K_m values are expressed in $s^{-1} \cdot \mu M^{-1}$; ND, not determined.
[b] TEM-35 kinetic values were previously determined by Sirot et al. (11).

TABLE 3. IC_{50}s of clavulanic acid and tazobactam for TEM-158, TEM-12, TEM-35, and TEM-1

β-Lactamase	IC_{50} (μM)	
	Clavulanic acid	Tazobactam
TEM-158	8.6	0.24
TEM-12	0.02	0.13
TEM-35[a]	27	1.8
TEM-1	0.08	0.13

[a] TEM-35 IC_{50}s were previously determined by Sirot et al. (11).

TEM-1 and TEM-12 than to that of TEM-35 (K_m, 170.4 versus 242, 327, and 1,200 μM, respectively). Overall, TEM-158 exhibited low catalytic efficiency against cephalothin, closer to that of TEM-12 and TEM-35 than to that of TEM-1 (k_{cat}/K_m values, 0.0015, 0.02, 0.04, and 0.7 $s^{-1} \cdot \mu M^{-1}$). In contrast to TEM-1 and TEM-35, TEM-158 displayed hydrolytic activity against oxyimino-β-lactams, especially ceftazidime, but its activity was 6- to 132-fold lower than that of the ESBL TEM-12. K_m values for ceftazidime and cefotaxime were similar for TEM-158 and TEM-12. The catalytic efficiency of TEM-158 against oxyimino-β-lactams was 4- to 75-fold lower than that of TEM-12. Finally, TEM-158 was 100- to 400-fold less susceptible to clavulanic acid and 2- to 4-fold less susceptible to tazobactam than TEM-1 and TEM-12 (Table 3). However, its level of resistance to inhibitor was three- to sevenfold lower than that of the IRT TEM-35.

TEM-158 appears to be close to CMT-type enzymes TEM-121, TEM-125, and TEM-152 (K_{cat} values, 40, 3.7, and 16 s^{-1}, respectively), which all are active against ceftazidime, and also had a resistance level to clavulanic acid close to that of an IRT-type enzyme (50% inhibitory concentrations [IC_{50}s], 1, 13.6, and 1 μM, respectively) (7, 8, 10).

Because of its enzymatic characteristics, TEM-158 was difficult to detect as an ESBL. This difficulty was previously observed with other CMT-type ESBLs, especially TEM-125 (7, 8, 10). As with the clinical TEM-125-producing strain TO799, it was not easy to reproducibly detect *E. coli* BER1 as an ESBL producer when following the American CLSI or the French Comité de l'Antibiogramme de la Société Française de Microbiologie recommendations (8). The presence of Met69Leu, Asn276Asp, and Arg164Ser substitutions in TEM-125 and TEM-158 could explain the closely similar behavior of these enzymes. The discovery of TEM-158 confirms the emergence of this subgroup of atypical ESBLs. The difficulties in detecting these enzymes could be responsible for an underestimation of their number. The observation of a new member of the CMT subgroup, which includes IRT and ESBL properties, highlights the need for an assessment of ESBL detection methods.

Nucleotide sequence accession number. The GenBank accession number for $bla_{TEM-158}$ is EF534736.

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