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TEM-187, a New Extended-Spectrum β-Lactamase with Weak Activity in a *Proteus mirabilis* Clinical Strain

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A *Proteus mirabilis* clinical strain (7001324) was isolated from urine sample of a patient hospitalized in a long-term-care facility. PCR and cloning experiments performed with this strain identified a novel TEM-type β-lactamase (TEM-187) differing by four amino acid substitutions (Leu21Phe, Arg164His, Ala184Val, and Thr265Met) from TEM-1. This characterization provides further evidence for the diversity of extended-spectrum β-lactamases (ESBL) produced by *P. mirabilis* and for their potential spread to other *Enterobacteriaceae* due to a lack of sensitive detection methods used in daily practice.

Before the CTX-M era, TEM-type extended-spectrum β-lactamases (TEM-ESBL) were the most prevalent mechanism of resistance to β-lactam antibiotics in *Enterobacteriaceae*. They emerged from the parental penicillinases TEM-1 and TEM-2 (1). Since the 1990s, different enzymes that combine novel substitutions leading to an ESBL pattern in *Proteus mirabilis* isolates such as TEM-10, TEM-24, TEM-52, TEM-72, TEM-87, and TEM-92 have been reported (2–6).

In 2002, during a study on ESBL detection and the agreement between four different techniques, Leverstein-van Hall et al. identified a novel TEM variant that associated the Arg164His substitution observed in numerous TEM-ESBL with Leu21Phe and Thr265Met substitutions (7). This β-lactamase, designated TEM-75, was produced by *Escherichia coli* or *Klebsiella pneumoniae* strains and is easily detected by different methods; the ESBL-Etest method was considered the best. Recently, we reported on an ESBL-producing *Proteus mirabilis* isolate incorrectly detected as a TEM-24-producing clone recovered from urine of spinal cord injury patients (8). During this outbreak period, one patient was previously infected by a new TEM-derived ESBL called TEM-187 with a new combination of four substitutions in bla_{TEM} gene.

In this study, we characterized the genetic support and the enzymatic activity of TEM-187. A *Proteus mirabilis* clinical strain (7001324) was isolated from a urine sample of a patient hospitalized in the Physical Medicine Department at Nantes University Hospital, France. This patient had been treated with different antibiotics for urinary tract colonization/infections in the previous months. *P. mirabilis* 7001324 harbored a high level of resistance to amoxicillin and ticarcillin but was fully susceptible to penicillin-clavulanate combinations and expanded-spectrum cephalosporins according to the results determined with a Vitek2 automated system with an AST-N103 card (bioMérieux, Marcy l'Etoile, France) or with a Phoenix automated system with an NMIC-93 gallery (BD Diagnostics, Sparks, MD), using a standard protocol. The double-disk synergy test (Mast Cica-β ESBL test) was negative for *P. mirabilis* 7001324 (9). Alone, a modified double-disk test with a 35-mm interdisk distance between ceftazidime- and amoxicillin-clavulanate-containing disks was positive. β-Lactam MICs were determined by a microdilution method on Mueller-Hinton agar (BD) with an inoculum of 10^4 CFU per spot (Table 1). *P. mirabilis* 7001324 produces only one β-lactamase, which has a pI of 5.7, as previously described (10). Plasmid DNA was extracted from the clinical strain by the method of Kieser (11). The plasmid size was determined by comparison with those of plasmids of reference strain *E. coli* NCTC 50192 as previously described (8). Plasmid content analysis revealed one plasmid of about 75 kb. The TEM-187-harboring plasmid did not transfer in mating experiments despite three attempts (12). However, an *E. coli* TOP10 transformant harboring the parental phenotype of resistance to β-lactams was obtained throughout electroporation of plasmid DNA. TEM-specific PCR and sequencing experiments were performed on the clinical strain *P. mirabilis* 7001324 and on the transformant and confirmed the presence of $bla_{TEM-187}$. This bla_{TEM} gene possesses a pattern with four mutations (Leu21Phe, Arg164His, Ala184Val, and Thr265Met) compared with bla_{TEM-1} and a promoter sequence, P_a/P_b (13, 14). This new TEM-encoding gene was cloned into a modified plasmid vector, pET-9a, as previously described (15). The recombinant plasmid was transformed into *E. coli* strain BL21(DE3) (Novagen, Darmstadt, Germany). *E. coli* clones were selected on Mueller-Hinton agar supplemented with 30 μg/ml kanamycin and 0.5 μg/ml ceftazidime. Direct sequencing was performed on three independent PCR products, which were obtained from the recombinant *E. coli* BL21. These PCR products were sequenced by dideoxy chain termination on both strands with an Applied Biosystems sequencer (ABI 377) (16). A TEM-producing *E. coli* BL21(DE3) clone was used to overproduce TEM-187, as previously described (15). Bacteria were disrupted by sonication. TEM purification was carried out as previously described by ion-exchange chromatography

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TABLE 1 MICs of β-lactam antibiotics for *Proteus mirabilis* clinical isolate, *E. coli* TOP10 plus p-TEM-187, and *E. coli* TOP10

Antibiotic(s)[a]	MIC (μg/ml) for strain: *P. mirabilis* 7001324	*E. coli* TOP10 (p-TEM-187)	*E. coli* TOP10
Amoxicillin	>512	>512	2
Amoxicillin + CLA	8	8	2
Ticarcillin	>512	>512	1
Ticarcillin + CLA	1	8	1
Piperacillin	>512	>512	1
Piperacillin + TZB	0.25	1	1
Cephalothin	4	4	4
Cefoxitin	1	2	2
Cefotaxime	0.06	0.12	<0.06
Cefotaxime + CLA	<0.06	<0.06	<0.06
Ceftazidime	0.5	2	<0.06
Ceftazidime + CLA	<0.06	0.12	<0.06
Aztreonam	<0.06	0.12	0.06
Cefepime	0.5	0.25	0.06
Imipenem	0.25	0.25	0.06
Ertapenem	<0.06	0.06	0.06

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

with a Q Sepharose column (Amersham Pharmacia Biotech, Orsay, France) and gel filtration chromatography with a Superose 12 column (Amersham Pharmacia Biotech), using a fast protein liquid chromatography system (15). The total protein concentration was estimated using the Bio-Rad protein assay (Bio-Rad, Richmond, CA), with bovine serum albumin (Sigma Chemical Co.) used as a standard. The level of purity was estimated to be >90% by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (15). Michaelis constant (K_m) and catalytic activity (k_{cat}) values were determined with purified extracts using a computerized microacidimetric method (17). The 50% inhibitory concentrations (IC_{50}s) were determined for clavulanic acid and tazobactam, as previously described (18), with 100 μM benzylpenicillin as the reporter substrate and 10 min of incubation. The kinetic parameters of the TEM-1 and TEM-24 ESBLs are presented in Table 2 for comparison (19).

According to automated susceptibility testing, *P. mirabilis* 7001324 harbored a penicillinase pattern without any coresistance except a reduced susceptibility to netilmicin and tobramycin. On the other hand, determinations of kinetic parameters revealed that TEM-187 harbored activity against penicillins that was 1.7- to 5-fold lower than that of TEM-1 (Table 2). TEM-187 K_m values for penicillins were close to those of TEM-1 (K_m, 19.3 to 36.2 versus 15 to 55 μM), except for those for ticarcillin. Overall, the catalytic efficiency of TEM-187 against penicillins was 1.9- to 7.6-fold lower than that of TEM-1. The hydrolytic activity of TEM-187 against cephalothin was close to that of TEM-1 (k_{cat}, 199.2 versus 165 s^{-1}), whereas its Michaelis constant for this substrate was 2.5-fold lower than that of TEM-1.

In contrast to TEM-1, TEM-187 displayed hydrolytic activity against oxyimino β-lactams, especially cefotaxime, ranging from 2.6 to 12.5 s^{-1} and its K_m values for these substrates were low, ranging from 27.8 to 81.6 μM. Considering its low activity against oxyimino β-lactams, TEM-187 could be a first step in the evolution of TEM enzymes from penicillinase to ESBL.

Finally, TEM-187 harbored a susceptibility to clavulanic acid close to that of TEM-1 (IC_{50}s, 0.1 versus 0.08 μM) but was more susceptible to tazobactam than TEM-1 (IC_{50}s, 0.002 versus 0.13 μM).

TEM-187 differs from the penicillinase TEM-1 by four substitutions: L21F, located in the signal peptide and previously observed in different TEM enzymes such as TEM-25 (20), R164H (an ESBL substitution previously observed in different TEM enzymes such as TEM-29), and A184V plus T265M. Among them, the R164H substitution confers improved activity against oxyimino β-lactams through enhanced flexibility of the Ω loop as observed in the crystal structure of TEM-64 (21). The role of A184V is unclear: this substitution was first observed in TEM-116, which is considered an ESBL (22) or a penicillinase (23). There are no enzymatic data for TEM-116 or other TEM enzymes which harbor this substitution to confirm its role. However, as residue 184 is not located in the active site, this substitution is probably not essential for the enzymatic activity of TEM-187.

Because of its enzymatic characteristics, TEM-187 was quite difficult to detect as an ESBL due to very low expanded-spectrum cephalosporin MICs compared with those seen with CTX-M or TEM/SHV ESBL (Table 1), especially with automated instruments. Different expert systems revealed only a penicillinase pattern, without any alert on expanded-spectrum cephalosporin MICs. This difficulty was previously observed with other TEM-type ESBL such as the TEM-24 or CMT type, especially TEM-125 (23–25). As with the clinical TEM-125-producing strain TO799, TEM-187 was not easy to detect following either the American

TABLE 2 Kinetic parameters of TEM-187 β-lactamase compared with TEM-1 and TEM-24[a]

β-Lactam	TEM-187 k_{cat} (s^{-1})	TEM-187 K_m (μM)	TEM-187 k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)	TEM-1 k_{cat} (s^{-1})	TEM-1 K_m (μM)	TEM-1 k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)	TEM-24 k_{cat} (s^{-1})	TEM-24 K_m (μM)	TEM-24 k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)
Benzylpenicillin	446.9 ± 7	19.3 ± 2	23.2	1,500	34	44	20	9	2.2
Amoxicillin	223.4 ± 4	22.9 ± 2	9.8	1,125	15	75	6	55	0.11
Ticarcillin	81.6 ± 2	73.2 ± 4	1.1	135	36	4	10	60	0.16
Piperacillin	360.6 ± 4	36.2 ± 3	10	1,250	55	23	6	6	1
Cephalothin	199.2 ± 6	97.5 ± 10	2	165	242	0.7	30	30	1
Cefuroxime	12.5 ± 1	81.6 ± 10	0.15	<0.1	ND		45	330	0.14
Ceftazidime	2.6 ± 0.2	42.9 ± 6	0.06	<0.1	ND		120	180	0.67
Cefotaxime	12.5 ± 1	81.6 ± 5	0.15	<0.1	ND		8,5	25	0.34
Aztreonam	2.6 ± 0.3	27.8 ± 4	0.09	<0.1	ND		16	55	0.29

[a] ND, not determined. TEM-1 data are from reference 15; TEM-24 data are from reference 19.

CLSI or the French Comité de l'Antibiogramme de la Société Française de Microbiologie recommendations (26, 27).

The emergence of ESBL such as TEM-187 with weak activity without any other resistance markers could lead either to an underestimation of the strains detected or to a diffusion of the plasmid to other *Enterobacteriaceae*. The observation of this new ESBL member in *P. mirabilis* highlights the need for an assessment of accurate and relevant ESBL detection methods, including in *P. mirabilis* without coresistance. Microbiologists and physicians should be aware of this ESBL with weak activity in *Proteus mirabilis* clinical isolates, even in the case of a multisusceptibility pattern.

Nucleotide sequence accession number. The nucleotide sequence data reported in this work are available in GenBank under accession number HM246246.

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