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Biochemical Characterization of the TEM-107 Extended-Spectrum β-Lactamase in a *Klebsiella pneumoniae* Isolate from South Korea▽

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The TEM-107 extended-spectrum β-lactamase detected in a *Klebsiella pneumoniae* clinical isolate had a Gly238Ser substitution compared to the TEM-43 β-lactamase. The MIC of ceftazidime was higher (64 μg/ml) than that of cefotaxime (2 μg/ml) for the isolate. Clavulanic acid reduced the MIC of ceftazidime 64-fold.

Numerous TEM-type extended-spectrum β-lactamases (ESBLs) have been reported since the first detection of TEM-12 in 1982 (7). It was reported that TEM-52 is a predominant TEM-type ESBL in South Korea (6). TEM-52-producing isolates had the unusual feature of decreased susceptibility to moxalactam, and the enzyme hydrolyzed cefotaxime more efficiently than it hydrolyzed ceftazidime (6, 8). TEM-107 (GenBank accession no. AY101764) is a novel ESBL found in a *Klebsiella pneumoniae* isolate (YMC 99/12/94) that colonized the throat of a 17-year-old hospitalized Korean girl with diarrhea. TEM-107 has an additional Gly238Ser substitution (Table 1) compared to TEM-43 (13). Although TEM-107 has only a single additional amino acid substitution (Arg164His) compared to TEM-52, the isolate showed a higher resistance to ceftazidime than to cefotaxime (6, 8). Therefore, we were interested in determining the biochemical characteristics of TEM-107.

The antimicrobial agents used in this study were ampicillin, cephalothin, and colistin (Sigma Chemical, St. Louis, MO), piperacillin, tazobactam, and tigecycline (Wyeth, Pearl River, NY), cefotetan (Kukje, Sungnam, South Korea), cefotaxime (Handok, Seoul, South Korea), cefuroxime, ceftazidime, and clavulanic acid (GlaxoSmithKline, Greenford, United Kingdom), cefepime and aztreonam (Bristol-Myers Squibb, Plainsboro, NJ), moxalactam (Eli Lilly, Indianapolis, IN), cefoxitin, ertapenem, and imipenem (Merck Sharp & Dohme, Rahway, NJ), and meropenem (Sumitomo, Tokyo, Japan). The MICs of antimicrobial agents were determined by the CLSI agar dilution method (2). ESBL production was tested by the double disk synergy test (12). *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as control strains.

Resistance to β-lactams was transferred to *E. coli* J53 recipient by agar mating and using Mueller-Hinton agar containing ceftazidime (2 μg/ml) and sodium azide (100 μg/ml). The *K. pneumoniae* isolate with TEM-107 and the transconjugant exhibited the typical ESBL phenotype (Table 2) and gave positive results by the double-disk synergy test. The MICs of ceftazidime and cefotaxime for the clinical isolate were 64 μg/ml and 2 μg/ml, respectively, and the MICs decreased by at least 8-fold when clavulanic acid or tazobactam was also included. It was reported that the MIC of cefotaxime was higher than that of ceftazidime for *K. pneumoniae* isolates with TEM-52 (6). Two amino acid substitutions in TEM variants, Glu104Lys and Gly238Ser, were associated with high-level resistance to cefotaxime (5). Both TEM-107 and TEM-43 have Arg164His substitutions, a change frequently observed in TEM-type ESBLs (1, 3, 13), which confer a higher level resistance to ceftazidime than to cefotaxime (5). The MIC of moxalactam was <1 μg/ml for the TEM-107-producing isolate.

Preparation of recombinant plasmid and transformation of competent *E. coli* were performed as described previously (10). Briefly, the $bla_{\text{TEM-107}}$ open reading frame was amplified by PCR using primers ET9-F (F stands for forward) (5′-GGG GCA TAT GAG TAT TCA ACA TTT TCG-3′) and ET9-R (R stands for reverse) (5′-CCG GAT CCT TAC CAA TGC TTA ATC AGT-3′); each of these two primers was designed to include restriction sites NdeI and BamHI, respectively, allowing it to be cloned in the pET9a vector, and it was subsequently transformed in *E. coli* XL-1 Blue or BL21(DE3) laboratory strains.

TEM-107 β-lactamase was produced and purified using the following procedure. *E. coli* BL21(DE3) (pET9a/$bla_{\text{TEM-107}}$) was grown in 6 liters of ZYP-5052 autoinducing rich medium (11) supplemented with 100 μg/ml kanamycin, 50 μg/ml ampicillin, and 2 mg/ml oxgall (Difco Laboratories, Detroit, MI) at 35°C for 24 h. With the addition of oxgall to the medium, most β-lactamase activity was found in the culture supernatant (4). The proteins were precipitated by the addition of ammonium sulfate (70% saturation) to the supernatant, and the solution was mixed for 45 min at 4°C. Precipitated proteins were separated by centrifugation and resuspended in 50 mM phosphate buffer (pH 7.0). The sample was desalted using a HiPrep 26/10 column and loaded on a Hitrap ANX FF column using 50 mM Tris buffer (pH 7.5). β-Lactamase-containing fractions, eluted using a linear NaCl gradient, were pooled and loaded onto a Resource Q column (bed volume, 1 ml) using 20 mM Tris buffer (pH 7.5) for further purification. The purity of the preparation was estimated to be higher than 95% by so-

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TABLE 1. Amino acid substitutions in TEM-107 compared to the amino acids in TEM-1, TEM-43, and TEM-52

β-Lactamase	Residue at amino acid position:			
	104	164	182	238
TEM-1	Glu	Arg	Met	Gly
TEM-43	Lys	His	Thr	Gly
TEM-52	Lys	Arg	Thr	Ser
TEM-107	Lys	His	Thr	Ser

dium dodecyl sulfate-polyacrylamide gel electrophoresis. Isoelectric focusing showed that the protein had an approximate pI of 6.0 (data not shown).

For enzyme assays, purified TEM-107 β-lactamase was diluted in 50 mM phosphate buffer (pH 7.0) containing 20 μg/ml of bovine serum albumin (BSA). Kinetic parameters were determined as described previously with some modifications (10). Briefly, the initial rates of hydrolysis for β-lactam substrates were measured at 30°C using a spectrophotometer (UV-2401; Shimadzu Corp., Tokyo, Japan). Ten microliters of enzyme was added to 600 μl of antibiotic solution in 50 mM phosphate buffer (pH 7.0). The steady-state kinetic parameters were computed using the Hanes-Wolff linearization of the Michaelis-Menten equation. For poor substrates with K_m values lower than 10, K_m was measured as the competitive inhibition constant (K_i) determined in competition experiments using 20 μM nitrocefin as the reporter substrate.

TEM-107 efficiently hydrolyzed several β-lactams (Table 3). In terms of turnover rates, TEM-107 was clearly more active on ceftazidime (k_{cat}, 16 s^{-1}) than on cefotaxime, cefepime, or aztreonam (k_{cat}, ≤3 s^{-1}). Hydrolysis of moxalactam and cefoxitin could not be observed with enzyme concentrations up to 585 nM in the reaction mixture, although these two compounds were able to interact with TEM-107, as revealed by competition assays (K_i, 26 and 48 μM, respectively). The k_{cat} value of TEM-107 for aztreonam was 2 s^{-1}, whereas the catalytic activity of TEM-52 was very low (6). Improved catalytic efficiency was reported against ceftazidime and aztreonam by an Arg164His change in TEM-109 (9), which is also present in TEM-107.

The k_{cat}/K_m values of TEM-107 were slightly higher for cefotaxime, ceftazidime, cefepime, and aztreonam (1.2×10^5 to 1.8×10^5 $M^{-1} \cdot s^{-1}$) than for cephalothin and cefuroxime (6.7×10^4 to 7.9×10^4 $M^{-1} \cdot s^{-1}$) (Table 3). The k_{cat}/K_m value of TEM-107 for ceftazidime (1.8×10^5 $M^{-1} \cdot s^{-1}$) was slightly higher than that for cefotaxime (1.2×10^5 $M^{-1} \cdot s^{-1}$), which was similar to that of TEM-43 (13).

TABLE 3. Kinetic properties of the purified TEM-107 β-lactamase with various β-lactam compounds[a]

Substrate	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1} \cdot s^{-1}$)
Nitrocefin	72	5	1.4×10^7
Ampicillin	5	1[b]	4.2×10^6
Piperacillin	11	3[b]	3.3×10^6
Cephalothin	1	10	7.9×10^4
Cefuroxime	2	34	6.7×10^4
Cefotaxime	3	22	1.2×10^5
Ceftazidime	15	86	1.8×10^5
Cefepime	3	26	1.3×10^5
Aztreonam	2	17	1.2×10^5
Moxalactam	ND[c]	26[b]	ND
Cefoxitin	ND	48[b]	ND

[a] The standard deviations were below 20%.
[b] These values are K_i (μM).
[c] ND, not determinable.

TABLE 2. MICs of β-lactams and other antimicrobial agents for an isolate, a transconjugant, and a cloned strain with $bla_{TEM-107}$

Antimicrobial agent[a]	MIC (μg/ml)					
	K. pneumoniae (TEM-107)	*E. coli* transconjugant (TEM-107)	*E. coli* J53 recipient	*E. coli* XL1-Blue (TEM-107)	*E. coli* XL1-Blue	*K. pneumoniae* (TEM-52)[b]
Ampicillin	1,024	256	4	32	4	>256[c]
Ampicillin-CLA	4	4	2	4	2	6[c]
Piperacillin	128	64	1	32	1	NT
Piperacillin-TZB	4	2	1	0.5	0.5	NT
Cephalothin	32	32	16	16	8	>256
Cefuroxime	8	8	8	8	4	NT
Cefotaxime	2	1	0.12	0.12	≤0.06	>256
Cefotaxime-CLA	0.12	0.12	0.12	≤0.06	≤0.06	NT
Ceftazidime	64	32	0.25	4	0.25	192
Ceftazidime-CLA	1	1	0.25	0.25	0.12	NT
Cefepime	2	1	0.12	0.12	≤0.06	NT
Cefepime-CLA	≤0.25	≤0.25	0.12	0.12	≤0.06	NT
Azteonam	8	8	0.25	0.5	0.12	16
Moxalactam	1	1	1	0.5	0.5	4
Cefoxitin	8	8	8	8	8	NT
Cefotetan	0.25	0.5	0.5	0.5	0.25	8
Ertapenem	0.03	0.03	0.008	NT	NT	NT
Imipenem	0.12	0.25	0.25	0.25	0.25	NT
Meropenem	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06	NT
Tigecycline	0.5	0.12	0.12	NT	NT	NT
Colistin	0.5	0.5	0.5	NT	NT	NT

[a] Abbreviations: CLA, clavulanic acid (1/2 concentration of antibiotics); TZB, tazobactam (4 μg/ml constant concentration); NT, not tested.
[b] Based on data from reference 7.
[c] These two values are the MICs of amoxicillin and amoxicillin-CLA instead of ampicillin and ampicillin-CLA, respectively.

The 50% inhibitory concentrations (IC_{50}s) were determined by measuring residual enzyme activities after preincubating the enzyme with various concentrations of β-lactamase inhibitors for 10 min at 30°C. The IC_{50} of clavulanic acid was 0.55 ± 0.05 μM, as measured using ceftazidime or cefotaxime as the reporter substrate (data not shown), confirming that TEM-107 is efficiently inhibited by clavulanic acid, which is in good agreement with the MIC values obtained for the TEM-107-producing clinical isolate or transconjugant. We considered that prevalence of TEM-107-producing clinical isolates is difficult to suspect, because the phenotype does not differ from those of most ESBLs. Among 22 ESBL genes sequenced in a South Korean surveillance study in 2000, there was another isolate with TEM-107 (*Enterobacter cloacae*), and the most prevalent one was TEM-52 (5 isolates) (2a).

In conclusion, the MICs of ceftazidime were much higher than those of cefotaxime for a TEM-107-producing clinical isolate and a transconjugant, which is in good agreement with the higher turnover rate of TEM-107 with ceftazidime than with cefotaxime.

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