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# TEM-28 from an *Escherichia coli* Clinical Isolate Is a Member of the His-164 Family of TEM-1 Extended-Spectrum β-Lactamases

PATRICIA A. BRADFORD,* NILDA V. JACOBUS, NIRAJA BHACHECH, AND KAREN BUSH

*Wyeth-Ayerst Research, Lederle Laboratories, Pearl River, New York 10965*

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**TEM-28 (pI 6.1), expressed by an *Escherichia coli* clinical isolate, is a novel β-lactamase which hydrolyzed ceftazidime, cefotaxime, and aztreonam with rates of 25, 1.1, and 5.6, respectively, relative to that for benzylpenicillin (100). The nucleotide sequence of $bla_{TEM-28}$ differed from that of $bla_{TEM-1}$ by two base changes, resulting in amino acid substitutions of Arg-164 to His and Glu-240 to Lys.**

Although extended-spectrum β-lactamases were first detected in European centers (7), there has been an increase in the incidence of outbreaks of ceftazidime-resistant organisms in the United States (2, 10, 13, 17, 21). In these outbreaks, several new extended-spectrum β-lactamases from clinical strains isolated in the United States have been described (3, 13, 17). In this study we report the finding of yet another novel extended-spectrum β-lactamase from an American clinical isolate.

A clinical isolate of *Escherichia coli*, strain 2300, was isolated in December 1992 from the urine of a patient in a long-term-care facility in southern California. The strain was selected as part of a study involving the evaluation of test methods for piperacillin-tazobactam. Antibiotic susceptibility was determined in broth microdilution tests with commercially prepared panels (Microscan, Sacramento, Calif.). Tests were performed by standard methods and interpreted according to National Committee for Clinical Laboratory Standards guidelines (12). As shown in Table 1, strain 2300 was resistant to amoxicillin-clavulanate, ampicillin-sulbactam, ceftazidime, aztreonam, and ciprofloxacin but was susceptible to piperacillin-tazobactam, cefotaxime, cefotetan, imipenem, and chloramphenicol.

Isoelectric focusing (IEF) of the β-lactamases was performed by the method of Matthew et al. (9) with an LKB Multiphor apparatus with prepared PAGplates (pH 3.5 to 9.5; Pharmacia LKB, Piscataway, N.J.). Crude preparations of β-lactamases from the clinical isolate and a reference strain of *E. coli* expressing the TEM-6 β-lactamase (kindly provided by A. Bauernfeind) were obtained from a freeze-thaw extract prepared in 0.2 M sodium acetate, pH 5.5 (5). The isoelectric point of each enzyme was confirmed by activity staining with nitrocefin (Becton Dickinson Microbiology Systems, Cockeysville, Md.) following IEF. The pI of the enzyme was calculated by two methods: by measuring the pH gradient of the IEF gel with a surface pH electrode and by comparing the pI with those of known β-lactamase standards. IEF revealed that strain 2300 expressed a single β-lactamase with a pI of 6.1.

Plasmid isolation, restriction enzyme digestions, recombinant DNA techniques, and transformations of plasmid DNA were performed as described by Sambrook et al. (18). Plasmid analysis of strain 2300 revealed multiple plasmids, ranging in size from approximately 10 to >50 kb. The ceftazidime resistance was not transferred by filter mating to a susceptible *E. coli* strain. However, when plasmid DNA from the clinical strain was transformed into *E. coli* DH5α, transformants that were ceftazidime resistant could be selected. These transformants each possessed a single plasmid of approximately 10 kb which was designated pCLL3412.

Plasmid DNA from a transformant containing pCLL3412 was isolated and sequenced directly. One strand of the entire TEM gene was sequenced with a nested set of primers complementary to the TEM-1 coding sequence (16). These were selected so that they did not anneal to regions of the gene corresponding to base pair changes associated with previously determined extended-spectrum TEM gene DNA sequences. DNA sequencing was performed on double-stranded plasmid DNA with a Sequenase kit (United States Biochemical, Cleveland, Ohio) with $^{35}$S-dATP label (Amersham, Arlington Heights, Ill.) according to the manufacturer's instructions.

Nucleotide sequencing revealed a $bla_{TEM}$-type gene, derived from a TEM-1 Tn*2*. As shown in Table 2, the amino acid sequence of the pI 6.1 β-lactamase, derived from nucleotide

TABLE 1. MICs of various β-lactam agents for clinical isolate *E. coli* 2300 and its related transformant

| *E. coli* strain | MIC (μg/ml)[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AMC | SAM | PTZ | TIC | TIM | CAZ | CTX | ATM | CTT | IPM | CAM | CIP |
| 2300(pCLL3412) | 32 | >32 | 8 | >128 | 128 | >32 | 8 | >32 | ≤4 | ≤0.5 | 8 | >4 |
| DH5α | 2 | 2 | ND[b] | ≤16 | ≤16 | ≤1 | ≤2 | ≤1 | ≤4 | ≤0.5 | 2 | ≤0.25 |
| DH5α(pCLL3412) | 8 | 16 | 2 | >128 | 32 | >32 | ≤2 | 32 | ≤4 | ≤0.5 | 4 | ≤0.25 |

[a] AMC, amoxicillin-clavulanate; SAM, ampicillin-sulbactam; PTZ, piperacillin-tazobactam; TIC, ticarcillin; TIM, ticarcillin-clavulanate; CAZ, ceftazidime; CTX, cefotaxime; ATM, aztreonam; CTT, cefotetan; IPM, imipenem; CAM, chloramphenicol; CIP, ciprofloxacin.
[b] ND, not determined.

* Corresponding author. Phone: (914) 732-4396. Fax: (914) 732-5671.
Electronic mail address: bradforp@war.wyeth.com.com.

TABLE 2. Amino acid substitutions of TEM-type β-lactamases at critical positions

| β-Lactamase | pI | Residue at amino acid no.[a] | | | | Reference |
|---|---|---|---|---|---|---|
| | | 104 | 164 | 240 | 265 | |
| TEM-1 | 5.4 | Glu | Arg | Glu | Thr | 19 |
| TEM-6 | 6.1 (5.9)[b] | **Lys** | **His** | Glu | Thr | 6 |
| TEM-10 | 5.6 | Glu | **Ser** | **Lys** | Thr | 16 |
| TEM-26 | 5.6 | **Lys** | **Ser** | Glu | Thr | 13 |
| TEM-27 | 5.9 | Glu | **His** | **Lys** | **Met** | 11 |
| TEM-28 | 6.1 | Glu | **His** | **Lys** | Thr | This study |

[a] Amino acids shown in boldface type represent sequence changes from that of TEM-1 (19).

[b] The pI of TEM-6 has been reported to be 5.9 (1); however, this enzyme comigrates with TEM-28 in our IEF gels. The pI of TEM-28 was calculated to be 6.1 by two different methods: pH measurement of gel and comparison with reference β-lactamase standards.

sequencing, differed from that of TEM-1 by two substitutions: histidine for arginine at position 164 and lysine for glutamate at position 240. This indicates a novel TEM-type enzyme which has been designated TEM-28. The nucleotide sequence reported here will appear in the GenBank nucleotide sequence database under accession no. U37195. TEM-28 is very similar to the recently described TEM-27 enzyme, which has identical substitutions at positions 164 and 240 but also has a substitution of a methionine for threonine at position 265 (11). The TEM-28 β-lactamase shares the histidine-164 substitution with TEM-6. However, TEM-6 has a lysine-for-glutamate substitution at position 104 instead of position 240, similar to the Ser-164-containing TEM-26. TEM-11 and TEM-16 also each possess a histidine residue at position 164; however, these two β-lactamases are derived from TEM-2 (8). TEM-28 is also similar to TEM-10, which has the lysine-for-glutamate substitution at position 240 but has a serine at position 164.

Purified β-lactamases were prepared for kinetic assays as follows. Crude preparations of β-lactamases from the reference *E. coli* clinical isolate expressing TEM-6 and *E. coli* 2300 expressing TEM-28 were obtained from sonic extracts prepared in 0.05 M phosphate buffer, pH 7.0 (5). Both TEM-6 and TEM-28 β-lactamases were initially purified by Sephadex G-75 chromatography in 0.05 M phosphate buffer, pH 7.0 (20). The TEM-28 β-lactamase was further purified by ion exchange chromatography on CM Sephadex C-50 (Pharmacia LKB) in 0.05 M phosphate buffer, pH 5.9. The enzyme was eluted from the column by stepwise elution at pH 6.1 followed by pH 6.3. The homogeneous enzyme (>95% purity by Coomassie blue staining of both IEF and sodium dodecyl sulfate-polyacrylamide gels) had a $k_{cat}$ of 140 $s^{-1}$ for benzylpenicillin. As shown in Fig. 1, the purified enzyme from strain 2300 comigrated with the partially purified TEM-6 β-lactamase. TEM-6 had previously been reported to have a pI of 5.9 (1).

Antibiotics used in hydrolysis assays were as follows: tazobactam (Taiho Laboratories, Tokushima, Japan), potassium clavulanate (Beecham Laboratories, Bristol, Tenn.), sulbactam (Pfizer Inc., New York, N.Y.), ceftazidime (Glaxo Group Research Ltd., Greenford, England), cefotaxime (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.), aztreonam and benzylpenicillin (Bristol-Myers Squibb, Princeton, N.J.), and cephaloridine (Eli Lilly, Indianapolis, Ind.). Initial hydrolysis rates were monitored spectrophotometrically at 25°C in 0.05 M phosphate buffer, pH 7.0 (15). The computer program ENZPACK (Biosoft, Cambridge, England) was used to calculate kinetic parameters by five methods of calculation (direct linear plot, Lineweaver-Burk, Hanes-Woolf, Eadie-Hofstee, and the

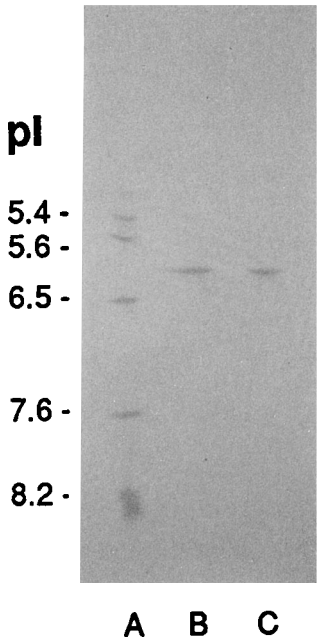


FIG. 1. IEF of purified TEM-28 β-lactamase and reference standards. Lane A, β-lactamase standards TEM-1 (pI 5.4), TEM-2 (pI 5.6), K1 (pI 6.5), SHV-1 (pI 7.6), and P99 (pI 8.2); lane B, purified TEM-28; lane C, partially purified TEM-6.

method of Wilkinson). Each substrate was analyzed on at least 2 days, with either cephaloridine or benzylpenicillin included as a reference each day. Mean coefficients of variation were 22% for relative values for maximum rate of hydrolysis ($V_{max}$) and 36% for $K_m$ values. Inhibition studies were performed by incubating the inhibitor with purified enzyme for 10 min prior to the addition of 50 μg of nitrocefin per ml (4). The concentration required for inhibition of 50% of the enzyme activity was determined graphically.

The biochemical characteristics of the β-lactamase with a pI of 6.1 were similar to those of TEM-6 and TEM-10 (Table 3) in that ceftazidime hydrolysis was faster than hydrolysis of either aztreonam or cefotaxime. The $K_m$ values of the three enzymes were quite similar for each of the substrates tested. In addition, the 50% inhibitory concentration values for all of these inhibitors with TEM-28 were nearly identical to those of the TEM-6 β-lactamase (Table 4). TEM-6 and TEM-28 were more susceptible to sulbactam inhibition than were TEM-1 and TEM-10. Inhibition by clavulanic acid and tazobactam was

TABLE 3. Substrate profiles of TEM β-lactamases

| Antibiotic | Result for β-lactamase (strain) | | | | | |
|---|---|---|---|---|---|---|
| | TEM-6 (HB 251) | | TEM-10 (KC 2)[a] | | TEM-28 (2300) | |
| | Relative $V_{max}$ | $K_m$ (μM) | Relative $V_{max}$ | $K_m$ (μM) | Relative $V_{max}$ | $K_m$ (μM) |
| Benzylpenicillin | 100 | 6.9 | 100 | 6.0 | 100 | 5.8 |
| Cephaloridine | 28 | 42 | 59 | 62 | 33 | 34 |
| Ceftazidime | 57 | 240 | 120 | 150 | 25 | 170 |
| Cefotaxime | 1.4 | 18 | 5.5 | 46 | 1.1 | 31 |
| Aztreonam | 8.6 | 35 | 19 | 28 | 5.6 | 24 |

[a] Data for TEM-10 are from reference 15; in that study assays were performed in this laboratory under conditions identical to those in this study.

TABLE 4. Inhibition of purified β-lactamases by commercially available β-lactamase inhibitors

| *E. coli* strain | β-Lactamase | $IC_{50}$[a] (nM) | | |
|---|---|---|---|---|
| | | Clavulanic acid | Sulbactam | Tazobactam |
| SC10404 | TEM-1[b] | 15 | 560 | 6.6 |
| HB 251 | TEM-6 | 1.5 | 120 | 7.1 |
| KC 2 | TEM-10[c] | 4.4 | 940 | 87 |
| 2300 | TEM-28 | 2.4 | 130 | 9.6 |

[a] $IC_{50}$, 50% inhibitory concentration.
[b] Data for TEM-1 are from reference 3; in that study all assays were performed in this laboratory under conditions identical to those in this study.
[c] Data for TEM-10 are from references 13 and 15.

much more effective for all the enzymes than was that by sulbactam.

The predicted amino acid sequence for the novel TEM-28 β-lactamase shows that this enzyme is most similar to the TEM-6, TEM-10, and TEM-27 extended-spectrum β-lactamases, differing from TEM-10 and TEM-27 by only one amino acid residue and from TEM-6 by two amino acids. It is interesting that the substrate profile for TEM-28 is also similar to those of TEM-6, TEM-10, TEM-26, and TEM-27. In the past, the Ser-164-containing TEM-10 and TEM-26 β-lactamases have been among the most frequently isolated extended-spectrum β-lactamases in the United States (2, 10, 13–17, 21, 22), whereas the His-164-containing β-lactamases have rarely been described in this country. The TEM-28 enzyme represents the development of a new enzyme which has both functional and molecular properties in common with these previously described enzymes. The substitution of histidine at position 164 as seen in TEM-6 and TEM-28 appears to correlate with lower levels of ceftazidime hydrolysis than when serine is at the same position in TEM-10 and TEM-26. In addition, the His-164 mutants are more susceptible to inhibition by the sulfone inhibitors than are the Ser-164 enzymes. The higher level of efficiency of the Ser-164 mutants for ceftazidime hydrolysis may explain why TEM-10 and TEM-26 are the predominant extended-spectrum β-lactamases in the United States.

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