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TEM-168, a Heretofore Laboratory-Derived TEM β-Lactamase Variant Found in an *Escherichia coli* Clinical Isolate[▽]

There have been over 160 variants of the TEM-1 and TEM-2 penicillinases described, most of which also exhibit activity against extended-spectrum cephalosporins (www.lahey.org/Studies/). It was noted in 1994 that a T265M mutation (Ambler numbering) was often associated with active-site mutations E104K, R164S, G238S, and E240K in some TEM variants (4). Comparison of MICs and kinetic studies of TEM-1 variants with the amino acid substitutions T265M, G238S, G238S:T265M, G238S:E240K, and G238S:G240K:T265M constructed by in vitro mutagenesis showed that the T265M mutation had no measurable effect on the TEM-1 wild type or on the G238S or G238S:240K variant. To date, no naturally occurring T265M variant of TEM-1 has been reported, though currently, 22 TEM variants contain an M265 residue along with other residue changes (www.lahey.org/Studies/).

Escherichia coli N08-1503 obtained by the National Microbiology Laboratory for advanced testing was resistant to ampicillin, cefazolin, and ampicillin-sulbactam, as determined by the submitting laboratory. Antimicrobial testing was carried out by disk diffusion and broth microdilution according to CLSI guidelines and Etests (AB Biodisk) according to manufacturer's instructions (Table 1) (1). The CLSI methods indicated that *E. coli* N08-1503 did not harbor an extended-spectrum β-lactamase (ESBL), though a reduced susceptibility to ceftazidime was noted. Analysis using the ESBL Etest strips yielded similar results. Interestingly, the cefepime ESBL Etest strip yielded a positive result. This strip is recommended outside of the United States when testing a strain with an inducible *ampC* gene (e.g., *Enterobacter*) or when a nondeterminable result is obtained. Broth microdilution confirmed the reduced susceptibility to cefepime and synergy with clavulanic acid, though the cefepime MICs were one or two doubling dilutions higher than the Etest results were. Resistance to amoxicillin-clavulanic acid and piperacillin-tazobactam and intermediate susceptibility to cefoxitin were also noted for *E. coli* N08-1503 and DH10B transformed with a plasmid (pT168) harboring $bla_{\text{TEM-168}}$ (see below).

PCR analysis results for β-lactamase genes were positive for TEM and negative for SHV, CTX-M, OXA-1, and CMY-2 types. Sequence analysis of the TEM amplicon revealed a translation product with a single amino acid change from TEM-1, a T261M change (T265M by Ambler numbering) caused by a C782T transition. This variant has been assigned the name TEM-168 (www.lahey.org/Studies). In the $bla_{\text{TEM-168}}$ promoter region, we detected the G162T change that defines the strong *P4* promoter (3, 5). Plasmid analysis showed that an ~13-kb plasmid, pT168, harboring $bla_{\text{TEM-168}}$ could be transformed into *E. coli* DH10B. Isoelectric focusing of crude extracts from *E. coli* N08-1503 and the DH10B transformant identified a single band of β-lactamase activity with a pI of 5.4. The resistance to β-lactam inhibitor combinations was likely due to the hyperproduction of plasmidic TEM-168 expressed from the *P4* promoter, similar to what has been shown for TEM-1 (5, 6). Since the TEM-168 did not test positive with standard ESBL tests, it is easy to understand why this variant has not been described previously in clinical isolates. It was the unusual result with the cefepime ESBL Etest strip that made us continue our investigation to further characterize the TEM harbored by *E. coli* N08-1503.

Nucleotide sequence accession number. The sequence of $bla_{\text{TEM-168}}$ has been assigned accession number FJ919776 in the GenBank database.

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TABLE 1. Antimicrobial data for *E. coli* N08-1503 and the transformants harboring pT168[a]

E. coli strain	Disk diameter (mm)						MIC (μg/ml)														
							Broth microdilution								ESBL Etest						Etest		
	Cefotaxime[b]	Cefotaxime-CLA[c]	Ceftazidime[b]	Ceftazidime-CLA[c]	Cefpodoxime[b]	Cefpodoxime-CLA	Cefotaxime[b]	Cefotaxime-CLA	Ceftazidime[b]	Ceftazidime-CLA[c]	Cefpodoxime[b]	Cefpodoxime-CLA	Cefepime	Cefepime-CLA	Cefotaxime	Cefotaxime-CLA[d]	Ceftazidime	Ceftazidime-CLA[d]	Cefepime	Cefepime-CLA[e]	Amoxicillin-CLA	Pipercillin-TZB[g]	Cefoxitin
N08-1503	23 (pos)	24 (neg)	8 (pos)	11 (neg)	20 (neg)	15	≤0.25 (neg)	≤0.12	4 (pos)	2 (neg)	8 (pos)	0.5	8	≤0.125	0.25	0.094 (neg)	3	0.75 (neg)	2	0.094 (pos)	32	>256	12
(pT168) DH10B	26 (pos)	30 (neg)	8 (pos)	10 (neg)	20 (neg)	16	≤0.25 (neg)	≤0.12	4 (pos)	1 (neg)	4 (neg)	0.25	4	≤0.125	0.25	0.094 (neg)	3	1 (neg)	2	0.094 (pos)	64	>256	6
(pT168) DH10B	29 (neg)	33	16 (pos)	18 (neg)	27 (neg)	21	≤0.25 (neg)	≤0.12	≤0.25 (neg)	0.25	1 (neg)	≤0.125	≤0.25	≤0.125	<0.25	0.094 (neg)	1	0.38 (neg)	<0.25	<0.25 (neg)	3	1.5	8

[a] CLA, clavulanic acid; TZB, tazobactam; pos, positive; neg, negative.
[b] Data in parentheses are the interpretations for the screen for ESBL according to CLSI document M100-S19 (2).
[c] Data in parentheses are the interpretation for confirmation of ESBL according to CLSI document M100-S19 (2).
[d] Data in parentheses are the interpretation for confirmation of ESBL according to the manufacturer's instructions in document 75001448-MH0383 (Etest for detection of ESBL; AB bioMérieux, Solna, Sweden). The overall interpretation of the phenotype is non-ESBL producer as both strips were negative.
[e] Data in parentheses are the interpretation for confirmation of ESBL according to the manufacturer's instructions in document 75002213-MH0344 (Etest for detection of ESBL; AB bioMérieux, Solna, Sweden). The overall interpretation of the phenotype is ESBL producer as one strip was positive.