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New Variant of TEM-10 β-Lactamase Gene Produced by a Clinical Isolate of *Proteus mirabilis*

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A clinical isolate of *Proteus mirabilis* was found to produce a new variant of the TEM-10 β-lactamase gene. This is the first report of TEM-10 production by *P. mirabilis* and the first report of extended-spectrum β-lactamase production by an isolate of this species recovered in the United States.

The plasmid-mediated extended-spectrum β-lactamases (ESBLs) found among isolates of the family *Enterobacteriaceae* are recent derivatives of TEM-1, TEM-2, and SHV-1 β-lactamases. These enzymes have been reported to cause failures during treatment with extended-spectrum cephalosporins and aztreonam (3). Because ESBLs are most frequently produced by *Klebsiella pneumoniae* and *Escherichia coli* (3, 6, 7), it may seem to be cost-effective for laboratories to confine ESBL screening tests to these two species. This report concerns the production of a novel gene encoding the TEM-10 β-lactamase in an isolate of *Proteus mirabilis* from a patient at a hospital in which an ESBL-producing *K. pneumoniae* strain had been detected previously. Our findings suggest that other species of the family *Enterobacteriaceae* should also be considered as possible producers of ESBLs, even when ESBL-producing *K. pneumoniae* and *E. coli* have not been commonly encountered in an institution.

P. mirabilis 177 was cultured from the urine (10^4 CFU/ml) and sputum of a 48-year-old female in the medical intensive care unit of the St. Louis University Health Sciences Center, St. Louis, Mo., on 27 February 1994. Antibiotic susceptibility tests with the Vitek GNS card (Biomérieux Vitek, Inc., St. Louis, Mo.) and Microscan MKD MIC microdilution test (Baxter Microscan, West Sacramento, Calif.) performed at the center indicated resistance to multiple antibiotics, including gentamicin, tobramycin, fluoroquinolones, trimethoprim-sulfamethoxazole, and, possibly, ceftazidime and aztreonam (Table 1). The isolate was susceptible to ceftazidime and aztreonam in the Vitek GNS card test but resistant by microdilution testing. However, the microdilution MICs were very difficult to ascertain because of the trailing endpoints. Thus, there was uncertainty about the accuracy of these results.

ESBL screening tests were equivocal (three-dimensional test) or positive (double-disk test and Vitek experimental ESBL card) (4, 13, 16). A crude cell sonicate was analyzed for β-lactamase activity by isoelectric focusing and a spectrophotometric assay (17). Isoelectric focusing indicated production of a β-lactamase of pI 5.58 which cofocused with TEM-10 and TEM-26 and a second β-lactamase of pI 8.1 which was only detected when the sonicate was concentrated threefold. TEM-10, TEM-26, and the pI 5.58 enzyme hydrolyzed cefotaxime in a cefotaxime-isoelectric focusing overlay technique (17). In the spectrophotometric hydrolysis assay, the crude sonicate from *P. mirabilis* 177 hydrolyzed ceftazidime more rapidly than cefotaxime.

On the assumption that the strain contained bla_{TEM}, PCR was performed with primers that are complementary to nucleotides 165 to 182 and 1077 to 1092 (Sutcliffe numbering) (15). DNA was isolated from the *Proteus* strain by alkaline lysis (12). PCR was performed with the extracted DNA, and the expected 927-nucleotide fragment was generated. This DNA fragment was inserted into the plasmid pBC KS^+ (Stratagene), which had been digested with *Sma*I (New England Biolabs). Transformants were initially selected on agar plates containing 100 μg of ampicillin per ml. The selected transformants were then tested for ceftazidime and cefotaxime resistance. The presence of the 927-bp bla_{TEM} insert was confirmed by restriction enzyme mapping of the recombinant plasmids. Nucleotide sequencing of double-stranded plasmid DNA was performed by dideoxy-chain termination (14) with [^{35}S]dATP and the Sequenase kit (United States Biochemical). Custom-synthesized oligonucleotide primers specific for the bla_{TEM} gene were used for sequencing. Two independent bla_{TEM} inserts were sequenced to ensure that any nucleotide differences observed were not due to PCR errors.

A summary of the nucleotide sequence of the *Proteus* bla_{TEM} gene is shown in Table 2. The gene encodes the extended-spectrum TEM-10 β-lactamase (9). The TEM-10 enzyme has previously been identified in both *K. pneumoniae* and *E. coli* (8–10). This represents the first report of TEM-10 in *P. mirabilis*, although rare occurrences of other ESBLs in *P. mirabilis* have been reported recently (5, 11, 18). It is interesting that the bla_{TEM-10} gene reported here has a DNA sequence different from those of any of the previously reported bla_{TEM-10} genes (Table 2). Note that the nucleotide changes are silent, so the amino acid sequences are identical. The two bla_{TEM-10} genes reported by Rasmussen et al. (9) appeared to be different, because one originated from bla_{TEM-1A} and the other originated from bla_{TEM-1B}. The origins of the bla_{TEM-10} gene reported here are more complex, because it contains nucleotide characteristics of both the bla_{TEM-1A} and bla_{TEM-1B} genes. It is possible that the gene is the product of recombination between a bla_{TEM-1A} gene and a bla_{TEM-10} gene like that on pCLL3405 (Table 2) (9). This recombination event would have occurred between nucleotides 226 and 436. Interestingly, Chanal et al. (2) noted that the bla_{TEM} gene encoding the TEM-24 enzyme

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TABLE 1. Antibiotic susceptibility of *P. mirabilis* 177

Antibiotic	MIC (μg/ml)	
	Microscan MKD MIC microdilution test	Vitek GNS card
Aztreonam	>32	≤8
Cefotaxime	≤1	NT[a]
Cefoxitin	2	≤2
Ceftazidime	>32	≤8
Ceftriaxone	NT	≤8
Imipenem	1	≤4
Mezlocillin	>128	32
Ticarcillin	>128	≥256
Ticarcillin-clavulanate[b]	≤4	≤16
Amikacin	≤4	≤2
Ciprofloxacin	>4	≤4
Gentamicin	>16	≥16
Norfloxacin	NT	≥16
Tobramycin	>16	≥16
Trimethoprim-sulfamethoxazole	NT	≥320

[a] NT, not tested.
[b] Ticarcillin plus 2 μg of clavulanate per ml.

also has a hybrid structure, and recombination between nucleotides 604 and 682 was proposed.

The production of a newly described variant gene encoding TEM-10 by this strain of *P. mirabilis* indicates that the epidemiology of plasmid-mediated ESBLs may be complex. In this case, the original host of the ESBL-encoding plasmid was not identified. Thus, it is not known if this $bla_{\text{TEM-10}}$ gene evolved directly in *P. mirabilis* or if it was acquired from *K. pneumoniae* or *E. coli*. From the clinical laboratory viewpoint, this *P. mirabilis* isolate represents another case that demonstrates the inability of routine antibiotic susceptibility tests to detect ESBLs. It adds further support to recommendations that special tests be used for ESBL detection (3, 4, 6, 13, 16) and indicates the need to use special tests for all isolates of members of the family *Enterobacteriaceae* with phenotypes suggestive of ESBL production (i.e., diminished susceptibility to the newer cephalosporins or aztreonam or resistance to aminoglycosides or trimethoprim-sulfamethoxazole).

TABLE 2. Nucleotide differences between $bla_{\text{TEM-1}}$ and $bla_{\text{TEM-10}}$ genes

Position[a]	Nucleotide (amino acid)[b]				
	$bla_{\text{TEM-1B}}$	$bla_{\text{TEM-10}}$ (pCLL3405)[c]	$bla_{\text{TEM-1A}}$	$bla_{\text{TEM-10}}$ (pCLL3303)[c]	$bla_{\text{TEM-10}}$ (*Proteus*)
226	T (F-8)	T (F-8)	C (F-8)	C (F-8)	C (F-8)
436	T (G-78)	T (G-78)	C (G-78)	C (G-78)	T (G-78)
604	T (A-134)	T (A-134)	G (A-134)	G (A-134)	T (A-134)
692	C (R-164)	A (S-164)	C (R-164)	A (S-164)	A (S-164)
917	G (E-240)	A (K-240)	G (E-240)	A (K-240)	A (K-240)

[a] Nucleotide numbering is according to Sutcliffe (15).
[b] Amino acid numbering is according to Ambler et al. (1).
[c] Data from Rasmussen et al. (9). The same sequence was reported for the MGH-1 gene (10).

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