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Molecular Characterization of a TEM-21 β-Lactamase in a Clinical Isolate of *Morganella morganii*

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A clinical isolate of *Morganella morganii*, with reduced susceptibility to expanded-spectrum cephalosporins and aztreonam, was found to produce an extended-spectrum β-lactamase with a pI of 6.4. The nucleotide sequence of the encoding gene was that of the gene encoding TEM-21. This is the first molecular characterization of an extended-spectrum β-lactamase in *M. morganii*.

Morganella morganii is an opportunistic pathogen, commonly found in human feces, which is responsible for infections mainly of the urinary tract (17). This species is intrinsically susceptible to antibiotics active against gram-negative bacilli except for fosfomycin, colistin, and some β-lactams due to a chromosomal cephalosporinase (20–22). Additional resistances to β-lactams are usually related to the mutational overproduction of the species-specific AmpC enzyme (13). The occurrence of an extended-spectrum β-lactamase (ESBL) in *M. morganii* was not reported until very recently (7, 8, 18), and the enzymes produced have not been characterized at the molecular level (10).

An ESBL-producing strain of *M. morganii* (Mm126) has been isolated together with a methicillin-resistant strain of *Staphylococcus aureus* from the knee puncture of a 24-year-old male patient hospitalized in a plastic surgery unit. By the disk diffusion method, this strain was demonstrated to have reduced susceptibility to expanded-spectrum cephalosporins and aztreonam; an ESBL was determined to be present by a very slightly positive result for a double disk test (12) for synergy between these antibiotics and clavulanic acid. This isolate was also resistant to most aminoglycosides (streptomycin, spectinomycin, kanamycin, gentamicin, sisomicin, tobramycin, dibekacin, and netilmicin), sulfamethoxazole, trimethoprim, and quinolones. Transconjugants of an azide-resistant mutant of *Escherichia coli* C600 were obtained on agar containing azide (300 μg/ml) and ticarcillin (100 μg/ml), using the plate-mating method (11), at a frequency of 2.7×10^{-4}. Plasmid DNA prepared from Mm126 by an alkaline lysis method (6) was used to transform *E. coli* HB101, and transformants were selected on Mueller-Hinton agar supplemented with 50 μg of ampicillin/ml. Resistance to aminoglycosides, chloramphenicol, and sulfonamides was cotransferred with the ESBL in the transconjugants and the transformants. The MICs of various β-lactams, alone or in combination with clavulanic acid at 2 μg/ml, have been determined by the agar dilution technique in Mueller-Hinton medium with a final inoculum of 10^4 CFU per spot (11). They showed that the *E. coli* transformant and transconjugant were much more resistant to expanded-spectrum cephalosporins and aztreonam, and more susceptible to the drugs used in combination with clavulanic acid, than the clinical isolate Mm126 (Table 1). Isoelectric focusing of crude β-lactamase extracts was performed on polyacrylamide gels containing ampholines (Serva, Westbury, N.Y.) with a pH range of 4 to 9, and the expression was revealed by the iodine procedure in gels by successive use of benzylpenicillin (75 μg/ml), which is hydrolyzed by all β-lactamases, and ceftriaxone (125 μg/ml), which is a substrate only for the ESBLs (3, 5). Strain Mm126 and its transconjugant expressed a single β-lactamase revealed by both gels, with an isoelectric point (pI) estimated at 6.4 by comparison with those of known β-lactamases (TEM-3 and TEM-16, pI 6.3; TEM-24, pI 6.5). In addition, strain Mm126 produced a nontransferable β-lactamase with a pI of 7.1, which was revealed only by benzylpenicillin and probably corresponds to the chromosomal enzyme. Plasmid DNA analysis of the clinical isolate Mm126 showed three

TABLE 1. MICs of β-lactam agents for clinical isolate Mm126 and its related transconjugant and transformant

Strain	MIC (μg/ml)										
	Ticarcillin		Cefotaxime		Ceftazidime		Cefepime		Aztreonam		Imipenem alone
	Alone	With CA[a]	Alone	With CA	Alone	With CA	Alone	With CA	Alone	With CA	
M. morganii Mm126	>1,024	32	2	2	1	1	0.2	≤0.01	0.5	0.1	2
E. coli Tc[b]	>1,024	32	8	0.05	16	0.2	0.5	≤0.01	8	0.02	0.2
E. coli Tf[c]	>1,024	32	8	0.05	16	0.2	0.5	≤0.01	8	0.05	0.2

[a] CA, clavulanic acid (2 μg/ml).
[b] Tc, transconjugant.
[c] Tf, transformant.

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TABLE 2. Nucleotide sequences used for amplification and/or sequencing reactions

Primer[a]	Sequence	Position[b]	Reference
A2	5′-d(GTATCCGCTCATGAGACAAT)-3′	148	This study
E	5′-d(CCAATGCTTAATCAGTGA)-3′	1066	This study
C	5′-d(GGGCAAGAGCAACTCGG)-3′	461	9
D	5′-d(CAGCAATGGCAACAACGTTG)-3′	753	9
F	5′-d(CAACGTTGTTGCCATTGCTGCAG)-3′	772	This study
G	5′-d(ACCGAGTTGCTCTTGCCC)-3′	478	This study

[a] Primers A2 and E were used for amplification and sequencing. Primers C, D, F, and G were used for sequencing.
[b] The numbers correspond to the positions of the first 5′-end base of the oligonucleotide according to the numbering system of Sutcliffe (19).

bands. In contrast, the related transconjugant and transformant exhibited only the band with the highest molecular weight. The size of the plasmid pMm126 has been estimated after enzymatic restriction to be about 40 kb. Based on the acidic pI of the enzyme, this strain was expected to produce an ESBL of the TEM family. Thus, PCR was performed on DNA of the transformant with primers A2 and E, which are specific for the bla_{TEM} gene (9) (Table 2). A PCR product of 918 bp was sequenced on both strands by automated fluorescent sequencing by the dye-terminator method (Perkin Elmer) with six oligonucleotides (Table 2). The nucleotide sequence analysis showed that it differed from the TEM-1 sequence by four substitutions leading to the amino acid replacements Gln→Lys-39, Glu→Lys-104, His→Arg-153, and Gly→Ser-238 (positions are according to the numbering system of Ambler et al. [1]) and by four silent mutations at positions 346 (A→G), 436 (C→T), 682 (T→C), and 925 (G→A) (positions are according to Sutcliffe's numbering system [19]) (Table 3). These substitutions are identical to those previously described for TEM-21 (2, 4) except for the silent mutation at position 682 (T→C). Indeed, the complete nucleotide sequence of the gene encoding TEM-21 has not been published to date, and the sequence for the segment containing position 682 has not been reported.

ESBLs are found most frequently in *Klebsiella pneumoniae* and occasionally in *E. coli*; other species of the family *Enterobacteriaceae* are rarely producers of ESBLs, except in an epidemic situation, and the corresponding enzymes are uncommon (7, 8, 18). Among members of *Proteae*, ESBLs have been detected in *Proteus mirabilis*: a clinical isolate synthesizing the TEM-10 enzyme (15) and an outbreak due to TEM-3 and CTX-1-producing strains (14) have been reported. In a recent study, designed to improve the detection of ESBLs, several strains of *M. morganii* were shown to produce this type of enzyme: the double disk synergy test was not found to be as reliable as with other enterobacteria, possibly due to coproduction of the chromosomal enzyme, and the MICs of expanded-spectrum cephalosporins were low and not significantly reduced by the inclusion of 2 μg of clavulanic acid/ml (10); these isolates harbored SHV-derived ESBLs, based on the basic pI of the enzymes. Strain Mm126 produces a TEM-21 β-lactamase. This ESBL is very uncommon. Indeed, its detection has been reported for only a single clinical strain of *K. pneumoniae* isolated from the urine of a patient hospitalized in a pediatric unit in Tunisia (4). This strain exhibited a cefotaxime-hydrolyzing phenotype, and the MICs of cefotaxime and ceftazidime (both 64 μg/ml) and aztreonam (32 μg/ml) were higher and the synergy with clavulanic acid was greater than for Mm126, but the susceptibilities to β-lactam agents of the corresponding *E. coli* transconjugants were very similar to each other (MICs of cefotaxime, ceftazidime, and aztreonam of 8, 8, and 4 μg/ml, respectively). The TEM-21 enzyme has been reported to have a pI of 6.4, and its substrate profile is marked by substantial hydrolysis rates for cefotaxime and ceftazidime (maximum rates of metabolism relative to that for benzylpenicillin [set at 100] of 493 and 57, respectively); the enzyme was inhibited by cloxacillin, clavulanic acid, and sulbactam, but not by chloride ions (4). Like Mm126, the TEM-21-producing *K. pneumoniae* strain harbored three plasmids. The enzyme was encoded by a highly transferable plasmid (frequency of 10^{-3}), which was larger (150 kb) than pMm126. The two plasmids carried identical resistance determinants, notably for aminoglycosides (amikacin susceptibility), except for an additional resistance to tetracyclines encoded by the plasmid of the *K. pneumoniae* strain.

TABLE 3. Nucleotide differences among bla_{TEM} genes

Nucleotide position[a]	Nucleotide (amino acid)[b]		
	bla_{TEM-1}	bla_{TEM-21}[c]	bla_{TEM-21}[d] of Mm126
317	C (Gln-39)	A (Lys-39)	A (Lys-39)
346	A	G	G
436	C	T	T
512	G (Glu-104)	A (Lys-104)	A (Lys-104)
660	A (His-153)	G (Arg-153)	G (Arg-153)
682	T	—[e]	C
914	G (Gly-238)	A (Ser-238)	A (Ser-238)
925	G	A	A

[a] Nucleotide numbering is according to the system of Sutcliffe (19).
[b] Amino acid numbering is according to the system of Ambler et al. (1).
[c] Mutations determined by PCR-restriction fragment length polymorphism (2).
[d] Mutations determined by DNA sequencing.
[e] —, not determined.

Finally, mutations in the gene encoding TEM-21 have been identified by PCR-restriction fragment length polymorphism (2). This method demonstrated that the gene for TEM-21 differed from that for TEM-3 by a single mutation (substitution of G for A) at position 660, bringing about an amino acid substitution, arginine for histidine, at position 153. Mm126 was probably a contaminant in a nosocomial wound infection. Indeed, *M. morganii* has been found rarely, as the causative agent of septic arthritis (16). In addition, Mm126 was isolated only once, together with a methicillin-resistant strain of *S. aureus* found several times at the same site, from this quadriplegic patient, hospitalized to undergo skin grafting on a sore. The source of the ESBL in Mm126 remains unknown: there was no outbreak due to ESBL-producing strains in the unit, and SHV-4 is the ESBL most commonly found in the hospital (5). In fact, ESBLs may emerge as a response to the selective pressure of the 7-oxyimino cephalosporins. Indeed, the Tunisian strain was isolated from a patient who had been treated with cefotaxime and gentamicin (4). In the present case, the patient had received ceftazidime and amikacin, but only for 3 days, 3 weeks prior to the isolation of Mm126. This study is the first molecular characterization of an ESBL in a clinical isolate of *M. morganii* and the first description of the complete sequence of TEM-21.

Nucleotide sequence accession number. The sequence of the 918-bp PCR product has been deposited in GenBank under accession no. AF052748.

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