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NOTES

New TEM Variant (TEM-92) Produced by *Proteus mirabilis* and *Providencia stuartii* Isolates

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The sequences of the bla_{TEM} genes encoding TEM-92 in *Proteus mirabilis* and *Providencia stuartii* isolates were determined and were found to be identical. Except for positions 218 (Lys-6) and 512 (Lys-104), the nucleotide sequence of bla_{TEM-92} was identical to that of bla_{TEM-20}, including the sequence of the promoter region harboring a 135-bp deletion combined with a G-162→T substitution. The deduced amino acid sequence of TEM-92 differed from that of TEM-52 by the presence of a substitution (Gln-6→Lys) in the peptide signal.

The extended-spectrum beta-lactamases (ESBLs) observed in *Proteus mirabilis* and rarely in *Providencia stuartii* are often difficult to detect. Their detection requires a modified synergy test (16) because they are usually produced at low levels (5).

We report here on two strains, *P. mirabilis* CF 529 and *P. stuartii* 1606 (Table 1), isolated in 1998 from the urinary tracts of two patients hospitalized at Clermont-Ferrand Hospital and Pitié-Salpêtrière Hospital in Paris, France, respectively. These isolates, in particular, *P. mirabilis* CF 529, were noticed because of their high level of resistance to cefotaxime and the results of the synergy test, which was unequivocally positive with oxyiminocephalosporins and clavulanic acid.

MICs were determined by a dilution method on Mueller-Hinton agar with an inoculum of 10^4 CFU per spot (6). Antibiotics were provided as powders by SmithKline Beecham Pharmaceuticals (amoxicillin, ticarcillin, and clavulanate), Eli Lilly, Paris, France (cephalothin, moxalactam), Roussel-Uclaf (cefotaxime, cefpirome), Glaxo-Wellcome Research and Development (ceftazidime), Bristol-Myers Squibb (aztreonam, cefepime), and Merck Sharp & Dohme (cefoxitin).

Table 2 lists the MICs of aztreonam, cefoxitin, cefotaxime alone and combined with clavulanate at a fixed concentration of 2 μg/ml, ceftazidime, cefepime, cefpirome, and moxalactam for the two strains producing TEM-92, *P. mirabilis* CF 529 and *P. stuartii* 1606. They were compared with those for *P. mirabilis* CF 39 and *P. mirabilis* CF 669, two strains of the same species that produce TEM-3 and TEM-66, respectively (3). These three ESBLs, TEM-92, TEM-3, and TEM-66, have the same mutations, Glu-104→Lys and Gly-238→Ser, implicated in the extension of the spectrum of activity.

P. mirabilis CF 529 and *P. stuartii* 1606 differed from *P. mirabilis* strains CF 39 (TEM-3) and CF 669 (TEM-66) in that the first two strains had higher levels of resistance to cefotaxime (MICs for CF 529 and 1606, 64 and 32 μg/ml, respectively; MICs for CF 39 and CF 669, 2 and 8 μg/ml, respectively). The ceftazidime MIC was 8 μg/ml for all strains except *P. mirabilis* CF 39 (TEM-3), for which it was 0.5 μg/ml.

Aztreonam MICs (≤2 μg/ml) remained low. The four strains were susceptible to moxalactam (MICs, ≤0.25 μg/ml) and cefoxitin (MICs, ≤8 μg/ml), unlike *Klebsiella pneumoniae* NEM 865 producing TEM-52 (14). Clavulanate restored the impaired activity of cefotaxime. Analytical isoelectric focusing was performed with crude lysates from polyacrylamide gels containing ampholines with a pH range of 3.5 to 10.0, as described previously (15). Both of the clinical strains (*P. mirabilis* CF 529 and *P. stuartii* 1606) produced a beta-lactamase with a pI of 6.0.

Several transfer experiments were tried with mutants of *Escherichia coli* HB 101, *E. coli* C600, and *P. mirabilis* ATCC 29906 as recipient strains. Only one transconjugant strain was obtained by mating *P. mirabilis* CF 529 with rifampin-resistant *P. mirabilis* ATCC 29906. The phenotype of resistance to aminoglycosides (kanamycin, tobramycin, and gentamicin) observed by the diffusion method was cotransferred with the ESBL phenotype.

Plasmids from clinical isolates and the transconjugant were extracted by the method of Kado and Liu and electrophoresed at 250 V for 4 h in a 0.7% agarose gel. They were blotted onto Nytran filters.

Hybridization with a TEM probe obtained by PCR with primers TEM-A (5′-TAAAATTCTTGAAGACG-3′) and

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TABLE 1. ESBL-producing clinical strains used in this study

Strain	Reference	Beta-lactamase produced (location, yr of isolation)	Isoelectric point
P. mirabilis CF 529	This study	TEM-92 (Clermont-Ferrand, 1998)	6.0
P. stuartii 1606	This study	TEM-92 (Paris, 1998)	6.0
P. mirabilis TrCF529	This study	TEM-92 (transconjugant)	6.0
P. mirabilis CF 39	3	TEM-3 (Clermont-Ferrand, 1996)	6.3
P. mirabilis CF 669	3	TEM-66 (Clermont-Ferrand, 1997)	6.0
K. pneumoniae NEM 865	11	TEM-52 (Paris, 1996)	6.0

TEM-B (5′-TTACCAATGCTTAATCA-3′) (3) and labeled by random priming (DNP-DNA labeling kit; Appligen Oncor, Illkirch, France) showed that the TEM gene resided on a 50-kb plasmid of *P. mirabilis* CF 529 and *P. stuartii* 1606 (data not shown). In *K. pneumoniae* NEM 865 (TEM-52), β-lactam resistance was transferred alone, without aminoglycoside resistance genes, and was located on a 13.5-kb plasmid. Great variability in plasmid size in TEM-52-producing *E. coli* and *K. pneumoniae* strains (between 71 and 100 kb) was also observed in Korea (12). PCR amplification and DNA sequencing of the promoter and coding regions of the $bla_{\text{TEM-92}}$ gene were performed as described previously (3).

The nucleotide sequences of the two bla_{TEM} genes from *P. mirabilis* CF 529 and *P. stuartii* 1606 were identical. Analysis of the deduced protein sequence compared to that of $bla_{\text{TEM-1}}$ showed four amino acid substitutions, Gln-6→Lys, Glu-104→Lys, Met-182→Thr, and Gly-238→Ser. This protein sequence is identical to that of TEM-52 reported previously (12, 14) except for the substitution Gln-6→Lys in the signal peptide. We suggest that the enzyme be designated TEM-92.

In Table 3, which shows the positions known to allow discrimination of bla_{TEM} genes (10), the sequence of $bla_{\text{TEM-92}}$ is compared with those of $bla_{\text{TEM-52}}$ genes reported previously (12, 14) and with that of $bla_{\text{TEM-20}}$ (2). $bla_{\text{TEM-92}}$ and $bla_{\text{TEM-20}}$ have the same promoter with a 135-bp deletion between nucleotides 22 and 158 combined with the mutation G-162→T. This combination of the deletion and the mutation resulted in a promoter sequence that contained TTGAA for the −35 region and TACAAT for the −10 region and that is thereby of closer similarity to the consensus promoter sequence, which conferred a strong promoter (2). No deletion was observed in the promoters of $bla_{\text{TEM-52}}$ genes reported previously, in which strong promoters, P4 (G-162→T) for *K. pneumoniae* NEM 865 (14) and Pa + Pb (C-32→T) for *K. pneumoniae* KMK 107 (BLAST program, National Center for Biotechnology Information, accession number AF 027 199 [http://www.ncbi.nlm.nih.gov/BLAST/]), were observed.

If we consider the silent mutations at positions 226, 346, 436, 604, 682, and 925 known to allow the discrimination of bla_{TEM} genes, $bla_{\text{TEM-92}}$ is related to $bla_{\text{TEM-2}}$ (10). In comparison, $bla_{\text{TEM-52}}$ from *K. pneumoniae* NEM 865 was identical to $bla_{\text{TEM-1a}}$, and $bla_{\text{TEM-52}}$ from *K. pneumoniae* KMK 107 was identical to $bla_{\text{TEM-1b}}$. Compared to the $bla_{\text{TEM-15}}$ gene (10), we could designate the gene from *K. pneumoniae* NEM 865 $bla_{\text{TEM-52a}}$ and the gene from *K. pneumoniae* KMK 107 $bla_{\text{TEM-52b}}$. If we consider the sequences of the promoter and coding regions, $bla_{\text{TEM-92}}$ was identical to $bla_{\text{TEM-20}}$ (2) except at two positions, 218 and 512 (Glu-6→Lys and Glu-104→Lys, respectively), and both genes belong to the $bla_{\text{TEM-2}}$-like group (4).

Among ESBLs, TEM-52 was hitherto not frequent except in Korea, where it was observed in an epidemic (12). It was first reported in a *K. pneumoniae* strain isolated from a girl originating from Athens, Greece (14), and a strain was observed in France (8). These TEM-52 and TEM-92 enzymes, which harbor the same critical substitutions involved in the extension of the beta-lactamase spectrum at positions 104, 182, and 238, differed by their geographical locations, the species implicated, the sizes of the plasmids carrying the bla_{TEM} gene, and their nucleotide sequences. The occurrence of these enzymes could be due to a convergent evolution from different bla_{TEM} genes (11).

A wide variety of TEM-type ESBLs were observed in *P. mirabilis*. Some of them (TEM-8, TEM-10, TEM-24, TEM-26 [3], and TEM-87) confer a ceftazidimase resistance phenotype, whereas others (TEM-3, TEM-21, TEM-66 [3] and TEM-72 [13]) confer a cefotaximase resistance phenotype. The latest, TEM-87, has the same mutation (Gln-6→Lys) in the peptide signal as TEM-92. This diversity is perhaps related to the variety of the ecological niches of this species, which is rarely implicated in nosocomial infections (7). In *P. stuartii*, unlike in *P. mirabilis*, ESBLs were rarely reported (TEM-24 in our hospital [unpublished data] and TEM-60 [9]). That could be due to the level of expression in this species. A lower level of expression of TEM-92 was observed in *P. stuartii* 1606 than in *P. mirabilis* CF 529. For both species we could suspect the

TABLE 2. MICs for the clinical isolates of ESBL-producing *P. mirabilis* and *P. stuartii*

Strain (beta-lactamase)	MIC (μg/ml)[a]							
	ATM	FOX	CTX	CTX + CLA	CAZ	FEP	CPO	MOX
P. mirabilis CF529 (TEM-92)	0.5	8	64	0.12	8	32	16	0.25
P. stuartii 1606 (TEM-92)	2	2	32	≤0.06	8	2	2	0.25
P. mirabilis CF 39 (TEM-3)	0.06	4	2	0.06	0.5	0.25	2	0.25
P. mirabilis CF 669 (TEM-66)	0.25	8	8	0.06	8	0.12	16	0.25

[a] ATM, aztreonam; FOX, cefoxitin; CTX, cefotaxime; CLA, clavulanic acid (2 μg/ml); CAZ, ceftazidime, FEP, cefepime; CPO, cefpirome; MOX, moxalactam.

TABLE 3. Nucleotide and amino acid substitutions of bla_{TEM-92} and related bla_{TEM} genes

Region and nucleotide no.[a] (amino acid no.[b])	Nucleotide (amino acid) in the following genes						
	bla_{TEM-1a}[d]	$bla_{TEM-52a}$[e]	bla_{TEM-1b}[d]	$bla_{TEM-52b}$[f]	bla_{TEM-2}[d]	bla_{TEM-20}[g]	bla_{TEM-92} (this study)
Promoter							
32	C	C	C	T	T	Deleted	Deleted
147	T	T	T	T	T	Deleted	Deleted
162	G	T	G	G	G	T	T
175	A	A	G	G	A	A	A
Gene							
218 (6)	C (Gln)	C	C	C	C	C	A (Lys)
226[c]	C	C	T	T	C	C	C
317 (39)	C (Gln)	C	C	C	A (Lys)	C	C
346[c]	A	A	A	A	G	G	G
436[c]	C	C	T	T	T	T	T
512 (104)	G (Glu-104)	A (Lys)	G	A (Lys)	G	G	A (Lys)
604[c]	G	G	T	T	G	G	G
682[c]	T	T	T	T	C	C	C
747 (182)	T (Met)	C (Thr)	T	C (Thr)	T	C (Thr)	C (Thr)
914 (238)	G (Gly)	A (Ser)	G	A (Ser)	G	A (Ser)	A (Ser)
925[c]	G	G	G	G	A	A	A

[a] Numbering is according to Sutcliffe (17).
[b] Numbering is according to Ambler et al. (1).
[c] Positions at which only silent mutations occur.
[d] Goussard and Courvalin (10).
[e] Poyart et al. (14).
[f] Cho et al. (GenBank accession no. AF 027199).
[g] Arlet et al. (2).

existence of a factor that leads to weak expression of β-lactam resistance, despite the presence of a strong promoter (3).

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