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Spread in an Italian Hospital of a Clonal *Acinetobacter baumannii* Strain Producing the TEM-92 Extended-Spectrum β-Lactamase▽

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Clinical isolates of *Acinetobacter baumannii* ($n = 470$) were collected during a 7-year period and investigated for the genetic determinants of resistance to expanded-spectrum β-lactams. Thirty-one isolates produced the TEM-92 extended-spectrum β-lactamase (ESBL) and were clonally related. This is the first report of *A. baumannii* producing a TEM-type ESBL.

Acinetobacter baumannii has emerged as a significant opportunistic pathogen responsible of nosocomial infections (11). Treatment of infections due to this organism is becoming a serious clinical concern, primarily because *A. baumannii* is frequently resistant to multiple classes of antibiotics (18, 25). Expression of chromosomal *Acinetobacter*-derived cephalosporinases (ADC) and production of other β-lactamases, especially of class D, can confer resistance to expanded-spectrum β-lactams and β-lactam β-lactamase inhibitor combinations (1, 9, 25). Isolates of *A. baumannii* producing different extended-spectrum β-lactamases (ESBLs) have been reported worldwide: PER-1 in Belgium, France, Turkey, and Korea (13, 20, 24, 26); PER-2 in Argentina (17); VEB-1 in Belgium, Argentina, and France (2, 13, 17, 21); SHV-12 in China (8); and CTX-M-type enzymes in Bolivia and Japan (3, 14). To our knowledge, TEM-type ESBLs have not yet been reported in *A. baumannii* (1, 25).

This study was initiated to investigate ESBL genes in clinical isolates of *A. baumannii* obtained at the Ospedale di Circolo (Varese, Italy) during a 7-year period (1999 to 2005). From 1999 to 2002, identification and antimicrobial susceptibility tests were performed with the Sceptor System (Becton Dickinson Diagnostic Systems, Sparks, MD); from 2003 onward, the Phoenix System (Becton Dickinson) was used. As shown in Table 1, 470 nonreplicated isolates of *A. baumannii* (286 from inpatients and 184 from outpatients) were collected. Isolates were frequently derived from the urinary (37.7%) and lower respiratory (30.0%) tracts. Hospital isolates were obtained from medical (50.7%), surgical (18.9%), and intensive care unit patients (30.4%). On the whole, 119/470 isolates (25.3%) exhibited a MIC of ≥16 μg/ml for ceftazidime and/or cefotaxime. The latter isolates were stored at −80°C, and their identifications were confirmed using ID32GN strips (bioMérieux, Marcy L'Étoile, France).

The following assays used to evaluate ESBL production in enterobacteria were performed: (i) the double-disk synergy test (10) on Mueller-Hinton agar plates with disks containing 30 μg of aztreonam, ceftazidime, cefotaxime, or cefepime and placed at a 20-mm distance (center to center) from a disk containing amoxicillin (20 μg) plus clavulanate (10 μg); (ii) the disk diffusion test on Mueller-Hinton agar plates with disks containing 30 μg of ceftazidime, cefotaxime, cefpodoxime, or cefpirome alone and in combination with clavulanate. A ≥5 mm increase of a zone diameter for either antimicrobial agent tested in combination with clavulanate versus its zone when tested alone was considered positive (4).

Synergistic activity between expanded-spectrum β-lactams and clavulanate was shown by both methods in 31/119 isolates. Of these, 26/31 were obtained from hospitalized patients (medical [$n = 14$], surgical [$n = 7$], and intensive care unit [$n = 5$] wards], and 5/31 were from outpatients who had been hospitalized at our institution during the preceding 12 months. The 31 investigated *A. baumannii* isolates showed a similar resistance phenotype (Etest; AB Biodisk, Solna, Sweden). MICs are shown in Table 2.

The 31 isolates demonstrating an ESBL phenotype were further studied by biochemical and molecular assays. Isoelectric focusing, performed as described previously (15), showed two β-lactamase bands common to all 31 isolates: the first at a pI of ~9.0, generally associated with the expression of the chromosomal ADC enzymes, the second at a pI of 5.9, consistent with the expression of a TEM-type enzyme. The presence of the bla_{ADC} gene was confirmed by PCR amplification (9). Genes encoding ESBLs (TEM, SHV, PER, VEB, and CTX-M type) were searched for by PCR as described previously (13, 16, 19). All isolates yielded a bla_{TEM} amplification product. Sequencing on both strands of the PCR product (6) revealed that all isolates carried a bla_{TEM} allele encoding the TEM-92 ESBL (5).

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TABLE 1. Epidemiology of *Acinetobacter baumannii* isolates from 1999 to 2005 from both inpatients and outpatients

Source or characteristic of isolates	No. of isolates (%) from yr:																	
	1999		2000		2001		2002		2003		2004		2005		Total 7-yr period			
	INP[a]	OUTP[b]	INP	OUTP	INP	OUTP	INP	OUTP	INP	OUTP	INP	OUTP	INP	OUTP	INP	OUTP	Total
A. baumannii isolates	60	16	35	20	49	35	50	33	35	38	30	29	27	13	286	184	470
Urinary tract infection	25 (41.7)	10 (62.5)	9 (25.7)	10 (50.0)	18 (36.7)	19 (54.3)	12 (24.0)	16 (48.5)	6 (17.1)	21 (55.3)	4 (13.3)	13 (44.8)	6 (22.2)	8 (61.5)	80 (30.0)	97 (52.7)	177 (37.7)
Lower respiratory tract infection	22 (36.7)	1 (6.3)	17 (48.6)	6 (30.0)	16 (32.7)	4 (11.4)	24 (48.0)	2 (6.1)	17 (48.6)	4 (10.5)	9 (30.0)	3 (10.3)	15 (55.6)	1 (7.7)	120 (42.0)	21 (11.4)	141 (30.0)
Soft tissue infection	4 (6.7)	3 (18.8)	1 (2.9)	1 (5.0)	3 (6.1)	9 (25.7)	3 (6.0)	10 (30.3)	2 (5.7)	11 (28.9)	5 (16.7)	11 (37.9)	1 (3.7)	4 (30.8)	19 (6.6)	49 (26.6)	68 (14.5)
Wound infection	1 (1.7)	0 (0.0)	4 (11.4)	0 (0.0)	8 (16.3)	0 (0.0)	4 (8.0)	0 (0.0)	6 (17.1)	0 (0.0)	4 (13.3)	0 (0.0)	1 (3.7)	0 (0.0)	28 (9.8)	0 (0.0)	28 (6.0)
Bloodstream infection	2 (3.3)	0 (0.0)	1 (2.9)	0 (0.0)	1 (2.0)	0 (0.0)	3 (6.0)	0 (0.0)	2 (5.7)	0 (0.0)	1 (3.3)	0 (0.0)	1 (3.7)	0 (0.0)	11 (3.8)	0 (0.0)	11 (2.3)
Vascular catheter colonization	4 (6.7)	0 (0.0)	1 (2.9)	0 (0.0)	1 (2.0)	0 (0.0)	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (2.4)	0 (0.0)	7 (1.5)
Other	4 (6.7)	2 (12.5)	2 (5.7)	3 (15.0)	2 (2.0)	3 (8.6)	3 (6.0)	5 (15.2)	2 (5.7)	2 (5.3)	7 (23.3)	2 (6.9)	3 (11.1)	0 (0.0)	21 (7.3)	17 (9.2)	38 (8.1)
CAZ- and/or CTX-resistant[c] isolates	19 (31.7)	1 (6.3)	10 (28.6)	1 (5.0)	13 (26.5)	1 (2.9)	19 (38.0)	7 (21.2)	12 (34.3)	6 (15.8)	4 (13.3)	5 (17.2)	17 (63.0)	4 (30.8)	94 (32.9)	25 (13.6)	119 (25.3)
ESBL-TEM-92-positive isolates	8 (13.3)	0 (0.0)	2 (5.7)	0 (0.0)	6 (12.2)	1 (2.9)	9 (18.0)	2 (6.1)	1 (2.9)	1 (2.6)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	26 (9.1)	5 (2.7)	31 (6.6)
Imipenem-resistant[d] isolates	1 (1.7)	0 (0.0)	2 (5.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (20.0)	0 (0.0)	0 (0.0)	1 (3.4)	6 (22.2)	0 (0.0)	16 (5.6)	1 (0.5)	17 (3.6)

[a] INP, inpatients.
[b] OUTP, outpatients.
[c] CAZ, ceftazidime; CTX, cefotaxime. MIC ≥ 16 μg/ml according to CLSI methods (4).
[d] MIC ≥ 8 μg/ml according to CLSI methods (4). Imipenem resistance was not detected among TEM-92-positive isolates.

TABLE 2. MICs of 31 *Acinetobacter baumannii* isolates producing the TEM-92 ESBL

Drug	Susceptibility[a]	MIC (μg/ml) (range)
Amikacin	S	≤8 (4–8)
Gentamicin	R	>128
Tobramycin	R	>32
Ciprofloxacin	R	>32
Levofloxacin	R	>32
Piperacillin	R	>128
Piperacillin-tazobactam	S	≤2/4 (1/4–2/4)
Ampicillin-sulbactam	S	≤2/1 (1/0.5–2/1)
Ceftriaxone	R	>128
Ceftazidime	R	>128
Cefotaxime	R	>128
Cefepime	R	>128
Aztreonam	R	>128
Imipenem	S	≤1 (0.5–1)
Meropenem	S	≤2 (1–2)

[a] S, susceptible; R, resistant (according to CLSI methods [4]).

By pulsed-field gel electrophoresis analysis using the ApaI restriction enzyme (23), the profiles of all TEM-92-positive isolates were identical. Investigated strains were different from the TEM-92-negative *Acinetobacter* strains isolated at our hospital during the same period (Fig. 1 and data not shown). The genetic context of the $bla_{\text{TEM-92}}$ gene was investigated by PCR mapping experiments, using the primers designed on the Tn*3* transposon sequence (Table 3). Results of experiments carried out with the index isolate (VA-239/99) and with additional isolates collected at later stages of the investigated period (VA-234/02 and VA-157/03) showed that the $bla_{\text{TEM-92}}$ gene was always associated with a Tn*3*-like transposon. Interestingly, an IS*26* sequence was inserted into the *tnpR* gene of the transposon (named Tn*6004*) from the index isolate. This IS*26*

FIG. 1. Macrorestriction profile of ApaI-digested chromosomal DNA of the *Acinetobacter baumannii* index isolate VA-239/99 (lane 4). Profiles of TEM-92-negative *A. baumannii* isolates obtained during the investigated period are shown for comparison (lane 2, VA-804/03; lane 3, VA-817/05; lane 5, VA-834/03).

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TABLE 3. Oligonucleotide primers used for PCR mapping and sequencing of the Tn*3*-like elements carrying the bla_{TEM-92} gene

Primer no.	Primer name	Sequence (5′–3′)
1	IR tn1-3	TGACGCTCAGTGGAACGAA
2	tnpA-tn3/F	GCATGTTCGTACCTGCTGA
3	tnpA-tn3/R	TCAGCAGGTACGAACATGC
4	tnpR-tn3/F	CCAGTCAGCAGTCTCTTGA
5	tnpR-tn3/R	GGCAATACTGAGCTGATGAG
6	IS15/26	CCACCATCAAAGGTATTGAG
7	pFOR-TEM	ATAAAATTCTTGAAGACGAA
8	Rev-TEM	ATATGAGTAAGCTTGGTCTGACAG

was absent from the transposons collected at a later stage (Fig. 2). Based on these findings, it is speculated that bla_{TEM-92} was initially acquired by the *Acinetobacter* strain as part of Tn*6004*, from which IS*26* could have subsequently been excised. However, an independent acquisition of the two types of Tn*3*-like elements carrying bla_{TEM-92} cannot be excluded.

Matings between *A. baumannii* VA-239/99 and *Escherichia coli* J53-2 (Rif^r) were carried out overnight at 37°C in LB broth (22). The conjugation experiments failed to result in the transfer of the ESBL determinant when ceftazidime (10 μg/ml) was used for selection. The negative transfer result suggests that the TEM-92 ESBL gene carried by the *A. baumannii* strain was either inserted in the chromosome or carried by a nonconjugative plasmid.

This is the first report of *A. baumannii* isolates producing a TEM-type ESBL. The TEM-92 determinant was possibly acquired from TEM-92-producing *Enterobacteriaceae* which has been shown to be prevalent at our institution since the late 1990s (6, 12).

As reported with regard to clonally related PER-1- and VEB-1-positive *A. baumannii* isolates (2, 21, 24, 26), the investigated TEM-92-producing strains were shown to persist at our institution over a long period of time (1999 to 2004). The results obtained with genotyping methods suggested the genetic stability of these *A. baumannii* isolates that showed a classical multidrug-resistant pattern (18). Only amikacin, ampicillin-sulbactam, piperacillin-tazobactam, and carbapenems maintained their in vitro activity. Though ESBL-positive isolates were not observed during 2005 (Table 1), detection of ceftazidime- and/or cefotaxime-resistant *A. baumannii* strains increased during the study period (from 31.7% in 1999 to 63% in 2005 [inpatients]). Imipenem-resistant isolates also increased from 1.7% in 1999 to 22.2% in 2005.

The detection of *A. baumannii* possessing TEM-92 in the hospital setting is of interest due to the increasing relevance of this multidrug-resistant pathogen (7, 11). It will be important to determine if ESBL production in *A. baumannii* has an impact on clinical outcome.

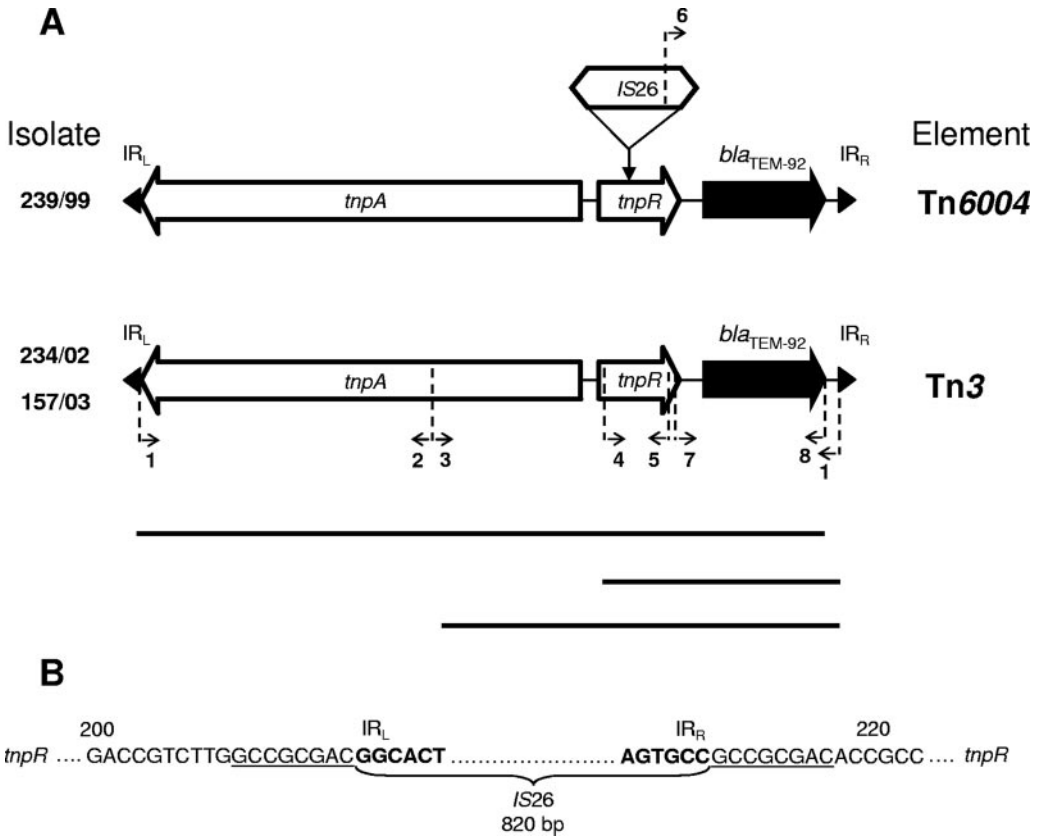

FIG. 2. (A) Structure of the Tn*3*-like elements carrying the bla_{TEM-92} ESBL gene and PCR mapping strategy. The locations of primers (numbered as reported in Table 3) are shown by arrows. PCR products generated to map the elements are indicated by thin lines. (B) Detail of the insertion site of IS*26* into the *tnpR* gene in Tn*6004*. Numbering refers to *tnpR*. The 8-bp target site duplication is underlined.

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