Outbreak of Carbapenem-Resistant *Acinetobacter baumannii* Producing the Carbapenemase OXA-58 in Turkey

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Abstract: The use of carbapenems to eradicate multi-drug resistant (MDR) *Acinetobacter baumannii* may become compromised by the spread of carbapenem-hydrolyzing class D β-lactamase (CHDL) genes (OXA-23, OXA-40, or OXA-58). Here, we describe the phenotypical and genotypical characterization of MDR *A. baumannii* isolates, recovered between June and November 2003 in a tertiary-care hospital in Antalya, Turkey.

Hundred and sixteen MDR *A. baumannii* isolates were isolated from 23 patients, mostly from respiratory samples and from 11 environmental samples. These MDR isolates, belonged to a single clone and remained susceptible to colistin and rifampin only. They produced CHDL OXA-58. In addition, they were also positive for the $bla_{\text{OXA-51}}$, for a novel $bla_{\text{AMPC (ADC-43)}}$ gene and for IS*Aba1*. The $bla_{\text{OXA-58}}$ gene was located onto a non self-transferable 50-kb plasmid that could be electroporated to *A. baumannii* 7010 reference strain. One isogenic carbapenem-susceptible strain lost its plasmid carrying $bla_{\text{OXA-58}}$ gene. PCR mapping identified similar genetic structures surrounding the $bla_{\text{OXA-58}}$ gene as for the prototype $bla_{\text{OXA-58}}$ gene, e.g. two IS*Aba3*-like insertion sequences bracketing $bla_{\text{OXA-58}}$ gene.

This is the first molecular description of an outbreak of OXA-58- producing *A. baumannii* isolates in Turkey, further underlining the global spread of such carbapenemase- producing strains in the Mediterranean area.

Keywords: OXA-58, *A. baumannii*, outbreak, carbapenem resistance, Turkey.

INTRODUCTION

Imipenem and meropenem are among the drugs of choice for treating nosocomial infections due to multidrug-resistant *A. baumannii* [1, 2]. However, their efficacy is being increasingly compromised and carbapenem-resistant isolates are becoming widespread in several regions of the world [3-5]. A large variety of molecular mechanisms for resistance to carbapenems have been reported in *A. baumannii*, i.e. acquisition of carbapenem-hydrolyzing β-lactamases of molecular Ambler class B (metallo-enzymes) and mostly of class D enzymes (oxacillinases) but also rare mutations in genes coding for penicillin-binding proteins and decreased outer membrane permeability [5-8]. Whereas class B enzymes found are of IMP and VIM types in *A. baumannii* [6], the acquired carbapenem-hydrolyzing class D β-lactamases (CHDL) are members of three subgroups of enzymes: the OXA-23-, OXA-24/40- and OXA-58- enzymes [3, 6]. OXA-58 shares less than 50% amino acid identity with the two other CHDL groups, which share 60 % amino acid sequence identity [6, 9]. Recently, a fourth subgroup of OXA β-lactamases sharing less than 63% amino acid identity with subgroups 1 and 2 has been identified [10, 11]. These enzymes (OXA-51 or 69-like) correspond to naturally-occurring oxacillinases of *A. baumannii* and it is likely that their overexpression associated with a degree of outer membrane permeability defect may explain partially carbapenem-resistance in *A. baumannii* [11-13].

Outbreaks of OXA-23 or OXA-40-producing *A. baumannii* have been increasingly reported worldwide [3]. β-Lactamase OXA-58 was first identified in France from a carbapenem-resistant *A. baumannii* isolate recovered in 2003 that was at the origin of an outbreak [9, 14]. This $bla_{\text{OXA-58}}$ gene was plasmid-located. Since then, the $bla_{\text{OXA-58}}$ gene has been identified worldwide [15, 16] and several outbreaks have been documented [17-21]. Genetic studies showed that the gene was bracketed by insertion sequences likely at the origin of its acquisition and its expression [22].

The aim of this study was to analyze the molecular mechanisms of carbapenem resistance in *A. baumannii* isolates identified at the university hospital of Antalya, Turkey.

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1876-5181/09

MATERIALS AND METHODOLOGY

Hospital Setting, Outbreak and Surveillance

Akdeniz University Hospital is a 746-bed tertiary-care teaching hospital with four ICUs totalizing 90 beds. The outbreak occurred on the fourth ICU with 12 beds and 4 single rooms. After the first isolation of MDR *A. baumannii* from the tracheal aspirate of a patient in the ICU in May 2003, a prospective surveillance study was initiated in order to determine the epidemiology of MDR *A. baumannii*, and infection control measures were strengthened according to the recommendations of Centres for Disease Control and Prevention by the Infection Control Committee of the hospital [23]. After the first isolation of MDR *A. baumannii*, a screening programme was initiated. Samples were taken once a week from all patients hospitalized in the ICU and for all newly admitted patients as well as the environmental cultures. Environmental sampling included: (i) swabs taken from the patient's direct environment including medical devices; (ii) swabs from handwashing sinks; and (iii) fintertips of healthcare workers. Samples were taken using cotton-tipped swabs moistened with sterile saline. Swabs, rinsing fluid and fingertips of healthcare workers were cultured on antibiotic-supplemented (imipenem 2μg/ml) and standard MacConkey agar plates and incubated aerobically at 37°C for up to 48 h. In addition to contact precautions, stringent compliance with standard precautions, especially the use of alcohol-based hand-rub solutions, was strongly recommended for all patients in the ward regardless of their bacteriological status. Active surveillance was continued until three months after the last colonized patient was discharged. Thus in March 2004, the epidemic was officially considered over and the surveillance programme returned to a normal operation level (Fig. **1**).

Bacterial Strains Antimicrobial Agents and MIC Determinations

A. baumannii isolates was identified by means of a commercial identification gallery (API 32GN system, BioMérieux, Marcy-l'Etoile, France). Identification of *A. baumannii* isolates was confirmed by detection and sequencing of the intrinsic $bla_{oxa\text{-}51/69}$-like gene [24]. *A. baumannii* MAD [9], *A. baumannii* Gr-1 [19], *A. baumannii* Ank-1 and *A. baumannii* Ist-1 [15] was used as a reference strains for PCR mappings and PFGE. *A. baumannii* CIP7010 and *Escherichia coli* DH10B were used for electroporation, and azide-resistant *E. coli* J53 or rifampin-resistant *A. baumannii* CIP7010 for conjugation. *E. coli* NCTC 50192 harbouring 154-, 66-, 38-, and 7-kb plasmids was used as a plasmid-containing reference strain [17].

Antimicrobial susceptibility testing was determined by disk diffusion method using interpretative criteria of the Clinical and Laboratory Standard Institute (CLSI) [25]. MICs of the different antibiotics were determined by E-test (AB Biodisk, Solna, Sweden) according to manufacturer's instructions and interpreted according to CLSI, and Eucast for colistin and tigecyclin.

PFGE

Pulsed-field gel electrophoresis (PFGE) was performed with *Apa*I-restricted whole-cell DNAs embedded in 1% agarose plugs and separated in a 1% pulsed field-certified agarose gel using a contour-clamped homogeneous electric field DRII system (Bio-Rad, Marnes-La-Coquette, France), as previously described [26]. PFGE results were analysed by eye according to the criteria of Tenover *et al.* [27].

Plasmid Content, Electroporation and Mating Out Experiments

Direct transfer of resistance into azide-resistant *E. coli* J53 or to rifampin-resistant *A. baumannii* CIP7010 (MIC >256 μg/mL) was attempted as previously reported [17]. Extraction of plasmid DNA from *A. baumannii* isolates was attempted using the Kieser extraction method [28]. Plasmid extracts were analyzed by electrophoresis on a % 0, 7 agarose gel [9]. Extracted plasmids were electroporated into *A. baumannii* CIP7010 and *E. coli* DH10B strains using a Gene Pulser II electroporator (Bio-Rad, Ivry-sur-Seine, France). Electroporants were selected on ticarcillin (50 μg/ml)-containing TSA plates.

Isoelectric Focusing (IEF) Analyses and Biochemical Properties

Crude β-lactamase extracts were obtained from 10-ml cultures of *A. baumannii* and analytical isoelectric focusing

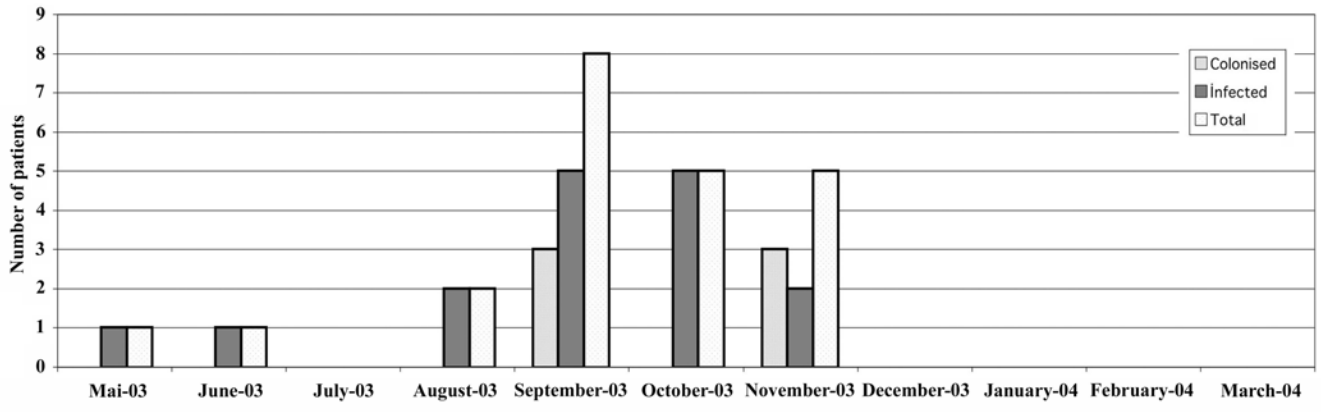

Fig. (1). Epidemic curve.

(IEF) was performed with an ampholine polyacrylamide gel, as described previously [17]. The specific carbapenem hydrolysis was measured as described [17].

PCR Analyses and Sequencing

Genomic DNA extraction and PCR experiments were performed as previously described [17, 26]. The PCR amplification and the primers used to search for known β-lactamase genes of Ambler class B and D carbapenemases were sought by PCR with primers specific for the bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{OXA-23}-like, bla_{OXA-40}-like, and bla_{OXA-58} genes and for Ambler class A bla_{TEM}, bla_{SHV}, $bla_{PER-1/2}$, bla_{VEB-1}, bla_{GES-1} and bla_{CTX-M} in *A. baumannii* isolates have been described previously [17, 26]. Detection of the bla_{AmpC}-type and bla_{OXA-69} genes of *A. baumannii* was performed with primers listed in Table **1** which hybridized to internal parts but also to outer parts of these β-lactamase genes [17, 26]. The presence of IS*Aba1* inserted upstream of a bla_{AmpC} and bla_{OXA-69}-like β-lactamase gene were also sought by PCR [12, 29].

For PCR-mapping experiments of the genetic environment of bla_{OXA-58}, 500 ng of total DNA of *A. baumannii* isolates were used in standard PCR reaction mixtures [9, 17]. The PCR primers are listed in Table **1** and Fig. (**2A**). The following amplification program was used: 10 min, 94ºC; 35 cycles of 1 min, 94ºC, 1 min, 55ºC, 3-5 min, 72ºC; followed by a final extension of 10 min at 72ºC.

PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Les Ulis France) and subsequently sequenced on both strands on an Applied Biosystem sequencer (ABI 3100, Les Ulis, France). Nucleotide sequence analysis was performed at the National Centre of Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

The nucleotide sequences reported in this paper have been assigned to the GenBank nucleotide database.

RESULTS

Epidemiology and Surveillance Study of the Carbapenem-Resistant *A. baumannii* Isolates

The first MDR *A. baumannii* was isolated from the tracheal aspirate of a patient hospitalized in ICU in May 2003 (Fig. **1**). From May until December 2003, at least one colonized patient was always present in the ward. Patient-care equipment and environmental surfaces were also screened for *A. baumannii* colonization. From January until March 2004 (end of surveillance), no new cases of MDR *A. baumannii* were diagnosed throughout the ICU (Fig. **1**). Between May 2003 and March 2004, a total of 116 *A. baumannii* isolates were recovered during this outbreak, being 11 environmental samples and 105 clinical samples from 23 patients. Clinical data were analyzed for all 23 patients (Table **2**). The ages of the patients ranged from 3 to 75 years (mean age, 36, 5 years). Seventeen patients were infected (74%) and 6 (26%) were colonized according to guidelines from Centre for Diseases Control and Prevention [23]. Most of the *A. baumannii*

Table 1. Primers Used in this Study

Primers	Primer as in Fig. (2)	Sequences (5'---->3')	References
OXA-58A	1	CGATCAGAATGTTCAAGCGC	[14]
OXA-58B	2	ACGATTCTCCCCTCTGCGC	[14]
ISAba2A	3	AATCCGAGATAGAGCGGTTC	[22]
ISAba3B	4	CGTTTACCCCAAACATAAGC	[22]
ISAba3C	5	AGCAATATCTCGTATACCGC	[22]
MAD-Th	6	AACAGCAATAGCCATCAAC	[22]
SM2	7	AAGTGTCTATATTCTCACC	[22]
Re27-2	8	AACATAATGGCTGTTATACG	[22]
Re27-1	9	TTCGTATAACCGCCATTATG	[22]
PreEt	10	CTATTTGGTTTTAAGGGGC	[22]
PreTh	11	ATCCAACCATTCATCAAACTCTGGC	[22]
IS Aba-1 A	12	ATGCAGCGCTTCTTTGCAGG	[17, 22]
IS Aba-1 B	13	AATGATTGGTGACAATGAAG	[17, 22]
pre-ABprom+	14	GACCTGCAAAGAAGCGCTGC	[17]
pre-AB 1	15	ACAGAGGAGCTAATCATGCG	[17]
pre-AB2	16	GTTCTTTTAAACCATATACC	[17]
oxa-ABint1	17	CGACCGAGTATGTACCTGCTT	[17]
oxa-ABint2	18	CTAAGTTAAGGGAGAACGC	[17]
pre-oxa-69A	19	CTAATAATTGATCTACTCAAG	[17]
pre-oxa-69B	20	CCAGTGGATGGATAGATTATC	[17]

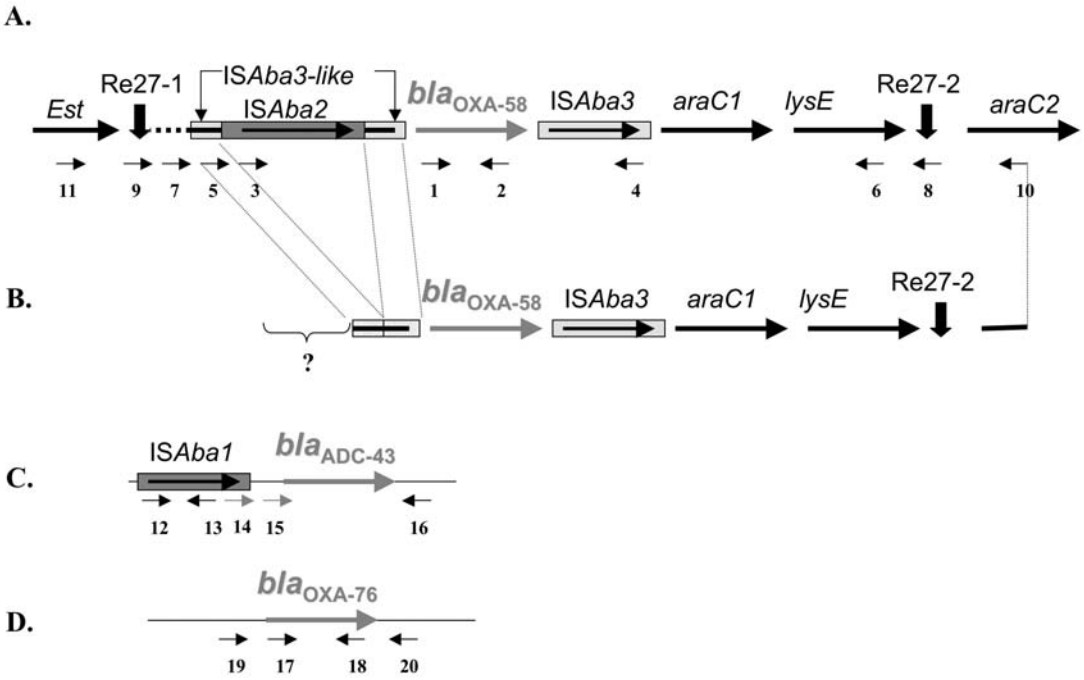

Fig. (2). Schematic map of the genetic structure (**A**) containing the *bla*OXA-58 genes found in (A. baumannii MAD [9, 15], (**B**) that found in *A. baumannii* 7a (this study), (**C**) of IS*Aba*1 insertied in front of bla_{ADC-43}, and (**D**) of bla_{OXA-76} gene. Genes and their corresponding transcription orientations are indicated by horizontal arrows. The transcription regulator genes (*araC1* and *araC2*), the threonine efflux protein gene (*lysE*), the esterase gene (*Est*), the putative regulatory gene (*reg*), the gene encoding an ORF of unknown function (*orf*), the putative transposase gene (*tnpA*), and the putative resolvase gene (*res*) are indicated. Vertical arrows are for the Re27-like sequences. The genetic structure which is highly similar between [1] and (2) is indicated with the dotted lines. The primers are indicated under arrows and the number correspond to the table.

were isolated from the respiratory tract (42 respiratory samples from 20 patients) in ICU patients. Environmental samples that were positive for *A. baumannii* were taken from the ICU unit, consisted of 5 hand cultures from staff, 2 from respiratory devices, 3 from respiratory monitors and 1 from dressing medical devices (Table **3**). *A. baumannii* isolates were resistant to all β-lactams, including imipenem (MIC: ≥ 32 μg/mL) and meropenem (MIC: 4 to >32 μg/mL), to ciprofloxacin (MIC: >32 μg/mL), and to amikacin (MIC: > 256 μg/mL). Variable susceptibility was found for rifampin (MIC: 4 to >256 μg/mL). The isolates were resistant to tigecyclin (MIC: 12 μg/mL) and borderline susceptible to colistin (MIC: 3 μg/mL). Strain 1 isolated from patient 1 (index patient) on the day of his admission showed higher MICs for sulbactam (32 μg/mL), meropenem (>32 μg/mL) and imipenem (>32 μg/mL). Several strains had variable sulbactam: 8-32 μg/mL, imipenem: 4-32 μg/mL meropenem: 8-32 μg/mL and tazobactam: 32-256 μg/mL susceptibility suggesting that resistant strains even though clonally-related might exhibit different pattern of resistance. While not yet fully understood, this phenomenon was already reported in *A. baumannii* [17]. It is tempting to suggest that rearrangements in plasmids and/or modification in outer membrane proteins (OMPs) could account for the different MIC values. By contrast two strains (7a and 7b) were isolated the same day from patient 7, differing only by the imipenem-resistance; strain 7b being susceptible both to imipenem (MIC: 4 μg/mL) and to meropenem (MIC: 4 μg/mL) (Table **2**).

PCR Analysis, IEF and Carbapenem Hydrolysis

One *A. baumannii* isolate per patient, except for patient 7 where two strains were retained, and 2 environmental isolates were further investigated. These *A. baumannii* isolates were positive for bla_{OXA-58}-like and the chromosomally-located bla_{OXA-51}-like and bla_{AMPC} genes. The carbapenem-susceptible strain 7b was positive only for bla_{OXA-51}-like and for bla_{AMPC} genes. Significant carbapenem-hydrolysis was found for all isolates except for strain 7b. Isoelectric-focusing confirmed the expression of OXA-58 (pI 7.0) and AmpC (pI > 8.5) (data not shown). Sequencing of the amplified fragments confirmed the presence of these genes and showed that the bla_{OXA-58} gene was identical to that of the prototype OXA-58 β-lactamase described in Toulouse [9] and of other southern European regions [15, 19, 20], thus underscoring the wide geographical spread of this gene. Sequencing of bla_{OXA-51}-like gene revealed the presence of the bla_{OXA-76} gene previously described in *A. baumannii* isolates from different regions (Spain, Turkey, Hong Kong and Singapore) [10].

Table 2. Case History of ICU Patients with OXA-58-Producing *A. baumannii.*

Patient #	Date of Hospitalization (dd/mm/yy)	Date of Isolation	Age (Sex[a])	Underlying Disease[b]	Site of Isolation[c]	Treatment[d]	Outcome	PFGE Type[f]	Oxa-58 PCR	TZP	IPM	MP	PM	TZ	CSF	TM	AK
1	27/05/03	06/2003	42(M)	Subarachnoid bleeding	TA, CSF, ID	CSP		A	+	256	32	32	64	64	16	24	96
2	09/06/03	06/2003	17(M)	Trauma	CSF, ID	GM, MP	Deceased[e]	A	+	256	32	32	16	256	32	256	256
3	25/08/03	08/2003	3 (F)	Multiple trauma	TA, CSF, ID, blood culture	MP, GM		A_1	+	256	32	8	64	32	16	64	256
4	25/08/03	08/2003	34 (F)	Multiple trauma	TA	CSP		A	+	256	32	32	24	48	8	256	256
5	06/09/03	09/2003	27(M)	Burn	ID	CSP		A	+	128	32	12	32	64	8	24	48
6	08/09/03	09/2003	26(M)	Multiple trauma	TA, CSF, ID, blood culture	CSP		A_1	+	256	32	32	48	256	12	256	256
7a	09/09/03	09/2003	53(M)	Subarachnoid bleeding	CSF, blood culture	GM, CSP	Deceased	A_1	+ *	256	32	8	24	96	32	48	256
7b	09/09/03	09/2003		Subaracnoid bleeding	CSF, blood culture	CSP		A_1	- *	128	6	4	24	48	8	64	256
8	18/09/03	09/2003	56(M)	Aneurism	TA, ID, blood culture	MP, GM	Deceased	A_1	+	256	32	32	32	256	32	24	64
9	17/09/03	09/2003	36(M)	Subaracnoid bleeding	TA	CSP		A	+	256	8	4	256	256	6	24	32
10	20/09/03	09/2003	3(F)	Suffocation	TA, ID	CSP		A_1	+	256	32	32	32	48	4	256	256
11	22/09/03	09/2003	48(M)	Subarachnoid bleeding	CSF, blood culture	CSP, GM	Deceased	A_1	+	256	32	32	64	96	32	128	256
12	29/09/03	10/2003	75(F)	Trauma	TA, absess culture	CSP		A_1	+	256	32	32	32	256	16	64	256
13	30/09/03	10/2003	4(M)	Burn	TA, blood culture	CSP, GM		A_1	+	256	32	8	16	64	24	32	256
14	4/10/03	10/2003	48(M)	Subarachnoid bleeding	CSF, abscess culture	CSP, MP	Deceased	A_1	+	256	32	32	256	24	12	256	256
15	8/10/03	10/2003	24(M)	Trauma	TA, ID	GM, MP		A_1	+	256	32	32	64	64	16	24	96
16	12/10/03	10/2003	45(M)	Trauma	TA, catheter	CSP		A_1	+	256	32	24	16	24	6	256	256
17	20/10/03	10/2003	61(M)	Femur fracture	TA, blood culture	CSP		A_1	+	256	32	32	16	64	8	128	256
18	30/10/03	11/2003	73(F)	Trauma	TA, blood culture.	CSP, MP	Deceased	A_1	+	256	32	32	256	256	32	256	256
19	2/11/03	11/2003	20(F)	Organophosphate intoxication	TA, absess culture	CSP, MP		A_1	+	256	32	32	48	256	32	8	48
20	4/11/03	11/2003	42(F)	Intracranial bleeding	ID, blood culture	CSP		A_1	+	256	32	32	64	64	12	256	256
21	8/11/03	11/2003	60(F)	COPD	ID, blood culture	MP, CSP	Deceased	A_1	+	256	32	32	24	256	16	256	64
22	17/11/03	11/2003	22(F)	Epilepsia	TA, ID, blood culture	MP, GM		A_1	+	256	32	32	256	32	24	48	256
23	19/11/03	11/2003	21(F)	Arteirio-venose malformation	TA	CSP	Deceased	A_1	+	256	32	32	64	64	12	128	256
E1	28/09/03	09/2003		Environment	Hand culture			A_1	+	256	32	24	16	24	6	256	256
E2	26/09/03	09/2003		Environment	Respiratory monitor of patient 10			A_1	+	128	6	4	16	48	6	128	256
R1	MAD			[9]													
R2	Greece			[15]													
R3	Turkey			[15]													
R4	Turkey			[15]													
R5	Ab7010			[17]													
T1	Ab7010			Transformant 1	Parental strain 7a												
T2	Ab7010			Transformant 2	Parental strain E2												

* imipenem suspectible and bla_{oxa58} gene negative strains. [a] M: Male; F: Female. [b] COPD : Chronic obstructive pulmonary disease. [c] TA: Tracheal aspirate, CSF: Cerebro-spinal fluid, ID: indweling device. [d] Only patients that received antibiotic treatment for their *A. baumannii* infections are shown. The treatment consisted of Meropenem (MP), gentamicin (GM) and cefaperazone-sulbactam (CSP). [e]Patient 2 died after 30 days, and patients 7, 8, 11, 14, 18, 21, and 23 died within 25 days.. [f] Pulsotypes are according to Tenover *et al.* [27]. All isolates were imipenem-resistant and positive for bla_{OXA-58} by PCR (except strain 7b and E1). ICU : intensive care unit.

Sequencing of bla_{AmpC} gene and deduction of its amino acid sequence revealed two changes (E322V; N341S) with the mobilized version of *A. baumannii* AMPC, *aba-1* found in *Oligella urethalis* AMPC [30]. The sequence was assigned theADC number ADC-43. The closest bla_{AmpC} was bla_{ADC-7} gene, with 98% similarity (16 nucleotide leading to 5 amino acid changes). IS*Aba1* was found in *A. baumannii* inserted upstream of bla_{AMPC} genes [31].

Genetic Environment

PCR mapping showed that upstream of bla_{OXA-58}, an IS*Aba3*-like element was found, that was not truncated by IS*26*, IS*Aba1* or IS*Aba2* elements as already described. In fact, PCR experiments failed to amplify the entire IS element, suggesting that the element might be truncated at its 5'-extremity, as already described [22] (Fig. **2**). Downstream of the bla_{OXA-58} gene, an entire copy of IS*Aba3* was identified, followed by the *araC1* and *lysE* genes, as observed in natural plasmid pMAD [22]. Downstream of the *lysE* gene, a sequence similar to what has been defined as the Re27-2 structure in *A. baumannii* MAD was identified. This structure has been proposed to be at the origin of the acquisition of the bla_{OXA-58} gene in that latter strain [22]. Then, an *araC2*-like gene was identified as found in *A. baumannii* MAD (Fig. **2**). Downstream of *araC2*, a gene encoding a putative regulator was found, followed by an *orf* corresponding to a putative 160 amino-acid long protein, followed by a gene encoding a putative transposase.

Table 3. Environmental Sampling

Environmental Samples	Total Sample	*A. baumannii*	Oxa 58 PCR
Hand culture	19	5	+*
Monitor	4	3	+
Dressing medical device	3	1	+
Respiratory devices	4	2	+
Stethoscope	2	-	-
Infusion pump	1	-	-
Sinks (Handwashing)	2	-	-

* One hand culture was found to be blaoxa 58 (+) and imipenem suspectible.

Genetic Location

In line with previous findings [9, 22], the bla_{OXA-58} gene was plasmid mediated. In all carbapenem-resistant isolates, a 50-kb plasmid was present but it was not self-transferable to rifampin-resistant *A. baumannii* strain. Electroporation with isolated plasmid into *A. baumannii* CIP7010 revealed that the bla_{OXA-58} gene was identified in those electroporants, but not in *E. coli*. Heritier *et al*. [32] demonstrated that OXA-58 has a weak carbapenemase activity and plays a role in carbapenem resistance in *A. baumannii*, particularly when highly expressed. MICs of transconjugants were identical to those already obtained with natural plasmid pMAD expressing OXA-58 (data not shown).

PFGE Analysis

The carbapenem-resistant *A. baumannii* isolates gave similar PFGE patterns (Fig. **3**), differing by only three bands for some isolates. The imipenem-susceptible 7b isolate from patient 7 showed the same PFGE pattern as the carbapenem-resistant 7a strains (Fig. **3**), but was lacking bla_{OXA-58}. This observation confirms, as recently suggested by Bogearts *et al.* [17], that OXA-58-producing *A. baumannii* isolates may occasionally loose the bla_{OXA-58} gene and its surrounding genetic structure in the absence of carbapenem selection pressure. No plasmid could be evidenced in that isolate, suggesting that the entire plasmid containing bla_{OXA-58} gene was lost. The bla_{OXA-76} gene was however detected by PCR in that strain, further suggesting that these β-lactamase genes may be present in carbapenem-susceptible strains as shown by Héritier *et al*. [10].

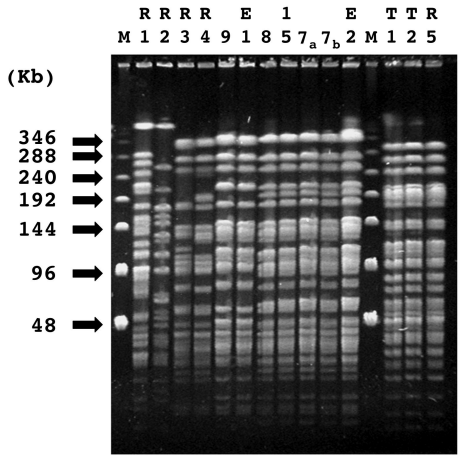

Fig. (3). PFGE patterns of *A. baumannii* isolates. The assigned numbers of *A. baumannii* isolates are shown over the lanes of the gel and correspond to those of Table **2**. The positions of molecular size markers in kilobases (M) are shown on the left side of the gel.

Very recently, *A. baumannii* OXA-58 isolates have been described in Greece and in Turkey [15, 22]. When the isolates of Antalya were compared to these strains, a genetic relationship could be established with the Turkish isolates but not with the Greek strain, suggesting that a single clone can be found in several regions of Turkey (Fig. **3**) [22].

DISCUSSION

Infections with *A. baumannii* are of great concern for hospitalized patients, showing the highest individual rate of incidence, particularly, in the intensive care units [3]. MDR *A. baumannii* clones are spreading throughout many different geographic areas [20] and treatment options for MDR *A. baumannii* infections are limited in most cases to carbapenems. The emergence of imipenem resistance means that

sulbactam, colistin and perhaps tigecyclin may be the only therapeutic options for treating infections caused by these strains. The high level of resistance to imipenem in *A. baumannii* clinical isolates and the clinical risk factors favouring the acquisition of imipenem-resistant *A. baumannii* have been reported previously [3, 4]. Although carbapenem resistance may be caused, in part by impaired permeability, resulting from decreased expression of porins, or by modifications in penicillin-binding proteins [3, 6], most recent reports have indicated that carbapenem-hydrolysing β-lactamases especially the OXA-types play a major role [32].

After the initial report in Toulouse, France [9], OXA-58-producing *A. baumannii* isolates have been reported in several countries around the world suggesting a wide distribution [15, 16]. In Europe, they have been reported in Greece, Italy, Turkey, Spain, and Belgium [15, 16, 22]. Hospitals outbreaks have been so far documented in France, Belgium, Italy, Greece and in Tunisia [17-21]. In Belgium, the isolation was subsequent of a patient transfer from Greece [17]. This is the first description of an outbreak of OXA-58 producing *A. baumannii* isolates in Turkey. Importantly, the fact that a large outbreak arose in the hospital despite early implementation of appropriate barrier precautions underlines the epidemic potential of such resistant strains.

This bacterial species naturally produces a chromosomally-encoded cephalosporinase that may be overexpressed due to insertion of IS*Aba1*, which brings promoter sequences necessary for high-level expression of this β-lactamase [29]. As observed for the other carbapenem-hydrolyzing oxacillinase genes, $bla_{\text{OXA-58}}$ was not present in the form of a gene cassette in a class 1 integron, a situation that contrasts to that found for most of the oxacillinase genes. The genetic structures surrounding the $bla_{\text{OXA-58}}$ gene were similar to those identified in association with the $bla_{\text{OXA-58}}$ gene in a series of isolates identified from France, Italy or Greece [22], indicating that a common structure has been at the origin of the dissemination of these genes. In addition, the IS element located upstream of $bla_{\text{OXA-58}}$ gene has been suggested to be responsible of high level expression by providing strong promoter sequences [22]. Furthermore, our study evidenced that $bla_{\text{OXA-58}}$ -positive plasmids might be lost suggesting a genetic instability of these *A baumannii* specific plasmids.

Contaminated hands and gloves of the ward staff seem to have an important role in patient-to patient transmission of *A. baumannii* [1, 2, 3, 17]. In our study, 25% of the samples collected from hands of ICU personnel were positive for imipenem-resistant *A. baumannii*. In addition, the detection of a common clone among environmental and clinical imipenem-resistant *A. baumannii* indicates that environmental contamination contributes to the difficulty in restricting spread of this organism in the hospital. This outbreak was restricted to only one ICU because the four ICUs are independent and geographically separated from each other, with dedicated nursing and medical staffs. Furthermore, patient transfer between the ICUs were rare and during the epidemic period were totally restricted. Surveillance cultures and strict antiseptic techniques possibly reduced the further spread of these bacteria.

This report constitutes the first report of a nosocomial dissemination of an OXA-58 producing *A. baumannii* strain in Turkey, after identification single OXA-58-producing *A. baumannii* isolates from this country [15, 33]. The current worldwide emergence of multiresistant *A. baumannii* isolates is mostly associated to acquisition of carbapenem-resistance determinants. Therefore, carbapenemases seem to be the main cause of carbapenem-resistance in *A. baumannii* and thus have to be considered as the main target for development of inhibitors. Prospective surveillance programs by characterization of antibiotic resistance, genotyping and plasmid analysis will be useful to follow the recurrence of the described *A. baumannii* clone as well as to rapidly detect genotype and resistance pattern changes in new emerging clones in our country.

ACKNOWLEDGEMENTS

NO and AE contributed equally to this work. We are grateful to Nicolas Fortineau for helpful discussions. ADC number was kindly assigned by Miriam Barlow. This work was funded by a grant from the Ministère de l'Education Nationale et de la Recherche (UPRES-EA3539), Université Paris Sud, and by the European Community (6th PCRD, LSHM-CT-2005-018705).

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Received: October 13, 2008 Revised: December 12, 2008 Accepted: December 14, 2008