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Spread of OXA-48-Positive Carbapenem-Resistant *Klebsiella pneumoniae* Isolates in Istanbul, Turkey▽

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The first outbreak of carbapenem-resistant *Klebsiella pneumoniae* isolates producing the plasmid-encoded carbapenem-hydrolyzing oxacillinase OXA-48 is reported. The 39 isolates belonged to two different clones and were collected at the University Hospital of Istanbul, Turkey, from May 2006 to February 2007, and they coproduced various β-lactamases (SHV-12, OXA-9, and TEM-1 for clone A and CTX-M-15, TEM-1, and OXA-1 for clone B).

Outbreaks of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* have been extensively reported worldwide (9, 17, 18) and carbapenems often remain the treatment of last resort. However, carbapenem resistance in *K. pneumoniae* has occasionally been reported and may be due to plasmid-mediated AmpC cephalosporinases associated with porin modifications (3, 14) or carbapenemases (23). Ambler class A β-lactamases of the GES type and the KPC type have been reported for *K. pneumoniae* isolates from several parts of the world, including for GES Greece (26) and Korea (13) and for KPC the United States (4), Israel (24), and China (6). *K. pneumoniae* isolates producing metallo-β-lactamase VIM-1 have been identified in Greece (11) and recently in Spain (25) and in Italy (5). The class D β-lactamase OXA-48, possessing significant carbapenemase activity, was identified from sporadic carbapenem-resistant *K. pneumoniae* isolates from Turkey (1, 10, 22). The bla_{OXA-48} gene was part of the Tn*1999* composite transposon made of two copies of the insertion sequence IS*1999* (2).

Here we describe a nosocomial outbreak of carbapenem-resistant *K. pneumoniae* strains expressing OXA-48.

Thirty-nine nonrepetitive carbapenem-resistant *K. pneumoniae* isolates were collected from patients hospitalized at the University Hospital of Istanbul, Turkey, from May 2006 to January 2007. Among them, 27 were isolated from clinical samples (4 endotracheal aspirate, 8 pus, 1 ascite, 7 blood culture, 2 wound, 1 urine, and 4 catheter samples). The remaining 12 isolates were grown from routine rectal screening swabs. Most of the patients were hospitalized in intensive care units (ICUs) or for emergency surgery (Table 1). Sixteen patients were infected, and 13 of them were being treated with carbapenems prior to and also after the determination of isolate susceptibility (Table 1). The three untreated patients were suffering from infections which did not require systemic administration of antibiotic. Despite treatment, 9 out of the 16 infected patients died.

Antibiotic susceptibility was determined by the disk diffusion method according to Clinical and Laboratory Standards Institute guidelines (7). Additionally, the MICs of several antibiotics were determined by using E-test strips (AB Biodisk, Solna, Sweden). Thirty-seven isolates were resistant to all penicillins and expanded cephalosporins. They exhibited heterogeneous decreased susceptibility to carbapenems, with meropenem and ertapenem being resistant, whereas MICs of imipenem ranged from 4 to 32 μg/ml (Table 2). Isolates 11 and 21 were resistant to carbapenems but remained susceptible to cefotaxime, ceftazidime, and aztreonam. Taking resistance to β-lactam and non-β-lactam antibiotics into account, the following three distinct resistance patterns were defined: group 1 included 23 isolates susceptible to ciprofloxacin but resistant to aminoglycosides; group 2 included 14 isolates resistant to ciprofloxacin but susceptible to gentamicin; and group 3 included isolates 11 and 21, susceptible to ceftazidime and ciprofloxacin but resistant to aminoglycosides. Double-disk synergy tests were performed for ESBL detection as described previously (12) and positive results were obtained for isolates of groups 1 and 2 but not for the two isolates of group 3. These various resistance patterns may explain the treatment failure observed for most of the infected patients.

Specific primers were used for the detection of β-lactamase-encoding genes that had been previously identified in *K. pneumoniae* 11978, namely, bla_{TEM}, bla_{SHV}, bla_{OXA-1}, bla_{OXA-9}, and bla_{OXA-48} (22). In addition, PCR experiments were performed to identify other ESBL-encoding genes by use of specific primers for bla_{CTX-M}, bla_{VEB}, bla_{PER}, and bla_{GES}, which had been designed previously (9, 15, 19). All isolates from group 1 were positive for the bla_{OXA-48}, bla_{TEM}, bla_{SHV}, and bla_{OXA-9} genes; all isolates from group 2 were positive for the bla_{OXA-48}, bla_{TEM}, bla_{CTX-M}, and bla_{OXA-1} genes; and isolates from group 3 were positive for the bla_{OXA-48}, bla_{OXA-9}, and bla_{TEM} genes only. Sequence analysis of the entire genes

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TABLE 1. Clinical features of the 39 isolates of carbapenem-resistant *K. pneumoniae* isolates

K. pneumoniae isolate	Clone	Date (mo/day/yr) of:		Hospitalization unit	Source	Infected or colonized status[b]	Treatment	Outcome
		Isolation	Hospitalization					
1	B	05/24/06	05/19/06	ICU	Catheter[a]	C	None	Cured
2	A	05/26/06	05/03/06	ICU	Catheter	C	None	Cured
3	B	05/27/06	05/08/06	Urology	Wound	C	None	Cured
4	A	05/28/06	04/09/06	ICU	Blood	I	Imipenem	Dead
5	B	05/28/06	04/24/06	ICU	Blood	I	Imipenem + ciprofloxacin	Dead
6	A	06/13/06	05/27/06	ICU	Catheter	C	None	Cured
7	B	06/13/06	05/31/06	ICU	Ascites	I	Imipenem + netilmicin	Dead
8	A	06/14/06	05/23/06	ICU	Catheter	C	None	Cured
9	A	06/14/06	05/31/06	ICU/Emergency Surgery	Pus	I	None	Cured
10	A	06/25/06	05/05/06	Transplantation unit	Pus	I	Imipenem	Dead
11	A	07/14/06	10/30/06	General Surgery	Rectal swab	C	None	Dead
12	A	08/12/06	11/17/06	ICU/Emergency Surgery	Rectal swab	C	None	Cured
13	A	09/30/06	08/15/06	ICU/Emergency Surgery	Rectal swab	C	None	Cured
14	B	10/23/06	10/17/06	ICU	Endotracheal	C	None	Cured
15	A	11/13/06	11/05/06	ICU/Emergency Surgery	Blood	I	Imipenem	Dead
16	B	11/16/06	10/02/06	ICU	Endotracheal	I	Imipenem	Dead
17	B	11/18/06	10/27/06	ICU/Neurosurgery	Urine	C	None	Cured
18	A	11/22/06	10/03/06	ICU/Emergency Surgery	Pus	I	None	Dead
19	B	11/25/06	10/22/06	Chest	Endotracheal	C	None	Dead
20	A	12/01/06	11/22/06	ICU/Emergency Surgery	Pus	I	Imipenem + amikacin	Cured
21	A	12/01/06	11/13/06	ICU/Emergency Surgery	Pus	C	None	Cured
22	B	12/02/06	11/10/06	ICU/Emergency Surgery	Rectal swab	C	None	Cured
23	A	12/06/06	10/23/06	ICU/Emergency Surgery	Rectal swab	C	None	Cured
24	B	12/07/06	12/01/06	ICU/Emergency Surgery	Blood	I	Imipenem + ciprofloxacin	Cured
25	A	12/07/06	12/04/06	ICU	Pus	I	Imipenem + netilmicin	Dead
26	A	12/08/06	10/04/06	ICU	Rectal swab	C	None	Cured
27	A	12/12/06	11/22/06	General Surgery	Blood	I	Piperacillin + tazobactam + ciprofloxacin	Cured
28	A	12/17/06	11/13/06	Urology	Pus	I	None	Cured
29	B	12/19/06	12/01/06	ICU	Endotracheal	C	None	Cured
30	A	12/19/06	12/30/06	General Surgery	Rectal swab	C	None	Dead
31	A	12/30/06	12/26/06	ICU/Emergency Surgery	Rectal swab	C	None	Dead
32	A	12/30/06	12/22/06	ICU	Rectal swab	C	None	Cured
33	A	01/04/07	01/31/07	ICU/Emergency Surgery	Wound	C	None	Cured
34	B	01/05/07	12/31/06	ICU/Emergency Surgery	Rectal swab	C	None	Cured
35	A	01/13/07	12/20/06	ICU/Emergency Surgery	Rectal swab	C	None	Cured
36	B	01/16/07	01/04/06	ICU/Emergency Surgery	Rectal swab	C	None	Cured
37	A	01/29/07	07/09/06	ICU	Blood	I	Imipenem + ciprofloxacin	Dead
38	A	02/02/07	01/31/07	ICU/Emergency Surgery	Pus	I	Ciprofloxacin + metronidazole	Cured
39	B	02/10/07	01/15/07	ICU/Emergency Surgery	Blood	I	Imipenem	Dead

[a] All catheters corresponded to central venous catheters.
[b] C, colonized; I, infected.

revealed perfect identity with the $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-9}}$, $bla_{\text{OXA-48}}$, $bla_{\text{SHV-12}}$, and $bla_{\text{TEM-1}}$ genes.

Isoelectrofocusing analysis performed as described elsewhere (20) confirmed gene identification for each group, with pI values of 7.4, 7.2, 8.2, 8.9, and 5.4, corresponding to OXA-48, OXA-1, SHV-12, CTX-M-15, and TEM-1, respectively (data not shown). As shown for *K. pneumoniae* 11978, OXA-9 was not expressed, and no isoelectric focusing band at the corresponding pI was found. No additional β-lactamase signal was observed for isolates from group 3.

The genetic relationship between the different isolates studied by pulsed-field gel electrophoresis (8) revealed only two distinct profiles, defining clone A as grouping 25 isolates from groups 1 and 3 and clone B as corresponding to the 14 isolates of group 2 (Fig. 1 and data not shown). It is noteworthy that clones A and B were genetically distinct from *K. pneumoniae* 11978, which is known to express another ESBL clavulanic acid-inhibited determinant, SHV-2a (22) (data not shown).

Conjugation experiments were performed with one isolate belonging to clone A and with one isolate belonging to clone B by use of amoxicillin, ceftazidime, or cefotaxime as the selective agent as described previously (22). Plasmid DNA extraction according to the Kieser technique (16) showed that the $bla_{\text{OXA-48}}$ gene was carried by a 70-kb plasmid present in each clone (named pAb and pBb for clones A and B, respectively),

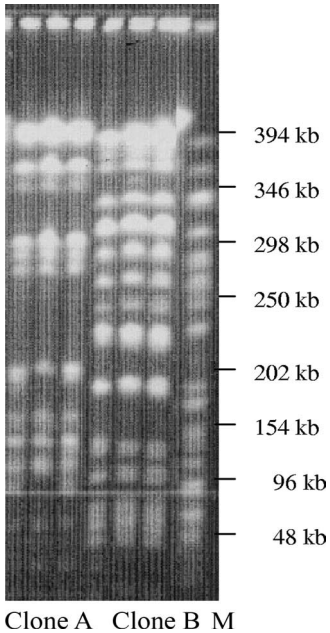

FIG. 1. Pulsed-field gel electrophoresis patterns of OXA-48-producing *K. pneumoniae* isolates. Lanes: 1 to 3, three isolates of clone A harboring the $bla_{\text{OXA-48}}$, $bla_{\text{OXA-9}}$, $bla_{\text{SHV-12}}$, and $bla_{\text{TEM-1}}$ genes; 4 to 6, three isolates of clone B harboring the $bla_{\text{OXA-48}}$, $bla_{\text{OXA-1}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{TEM-1}}$ genes; M, molecular mass markers.

TABLE 2. MICs of β-lactams and non-β-lactams[a]

Drug	MIC (μg/ml) against indicated isolate (genotype)							
	K. pneumoniae clone A (bla_{OXA-48} bla_{TEM-1} bla_{SHV-12} bla_{OXA-9})	*K. pneumoniae* clone B (bla_{OXA-48} bla_{TEM-1} $bla_{CTX-M-15}$ bla_{OXA-1})	*E. coli* J53(pAa) ($bla_{CTX-M-15}$ bla_{TEM-1} bla_{OXA-1})	*E. coli* J53(pAb) or -(pBb) (bla_{OXA-48})	*E. coli* J53(pBa) (bla_{SHV-12} bla_{TEM-1} bla_{OXA-9})	*E. coli* J53	*K. pneumoniae* isolate 11 (bla_{OXA-48} bla_{TEM-1} bla_{OXA-1})	*K. pneumoniae* isolate 21 (bla_{OXA-48} bla_{TEM-1} bla_{OXA-9})
Amoxicillin	>512	>512	>512	>512	>512	1	>512	>512
Amoxicillin + clavulanic acid	>512	>512	>513	>512	>512	1	>512	>512
Ticarcillin	>512	>512	>514	>512	>512	4	>512	>512
Ticarcillin + clavulanic acid	>512	>512	>515	>512	>512	4	>512	>512
Piperacillin	>512	>512	>512	>512	>512	4	>512	>512
Piperacillin + tazobactam	>512	>512	>4–8	64	>4–8	4	>512	>512
Cefotaxime	>512	>512	>32	0,5	4	0.5	4	4
Ceftazidime	>512	>512	128	1	64	1	2	2
Cefepime	>512	>512	4	<0.5	0.5	0.125	4	4
Aztreonam	>512	>512	8	<0.125	64	0.5	0.5	0.5
Cefoxitin	16–512	32–>512	2–4	4–8	2–4	4	16	>32
Moxalactam	16–>512	16–>512	<0.5	2	<0.5	1		
Imipenem	4–32	4–16	<1	0.5	<1	2	8–16	8–16
Meropenem	>32	6–32	<1	0.12	<1	<1	16	16
Ertapenem	>32	>32	<1	0.25	<1	<1	>32	>32
Kanamycin	>256	128						
Tobramycin	>64	>64						
Netilmicin	>128	3						
Amikacin	16	2						
Gentamicin	>64	1						
Sulfamides	>256	>256						
Trimethoprim	>32	>32						
Tetracycline	>256	>256						
Nalidixic acid	>64	>64						
Norfloxacin	8	>256						
Ofloxacin	2	>64						
Ciprofloxacin	1	>128						

[a] MICs are for *K. pneumoniae* isolates of clones A and B, *E. coli* J53 harboring natural plasmids pAa or pAb from clone A, *E. coli* J53 harboring natural plasmids pBa or pBb from clone B, *K. pneumoniae* isolates 11 and 21, and *E. coli* J53, as indicated.

while the other β-lactamases genes were carried on plasmids of more than 150 kb (pAa and pBa) and were identified by PCR (Table 2 and data not shown).

The genetic environment of the bla_{OXA-48} gene was determined by PCR using specific primers for the insertion sequence IS*1999*, located upstream and downstream of the bla_{OXA-48} gene in Tn*1999* (2). A structure identical to Tn*1999*, as found in *K. pneumoniae* 11978, was identified in clone A isolates, whereas in clone B isolates, the IS*1999* element located upstream of the bla_{OXA-48} gene was truncated by IS*1R*, which had targeted the transposase gene of IS*1999* (Fig. 2). Whereas IS*1999* was shown to provide −35 and −10 promoter sequences for the expression of the bla_{OXA-48} gene in *K. pneumoniae* 11978 and thus acts similarly in isolates belonging to the clone A (Fig. 3A), it is likely that the promoter driving the expression in clone B isolates was made of a −35 box located inside IS*1R* together with the −10 box originally located in IS*1999* (Fig. 3B). This hybrid promoter possesses the features of a strong promoter for bla_{OXA-48} gene expression, with a more efficient −35 sequence and an optimal 17-bp spacing between the −35 and −10 boxes. Since the genetic structures identified upstream of the bla_{OXA-48} gene varied, their role in bla_{OXA-48} gene expression was studied by measuring hydrolytic activities as described previously (21). Hydrolysis of imipenem (100 μM) obtained from *Escherichia coli*(pAb) (23 [± 0.002] mU/mg of protein^{-1}) was twofold lower than that obtained from *E. coli*(pBb) (48 [± 0.013] mU/mg of protein^{-1}). This result indicated a higher expression of the bla_{OXA-48} gene in clone B.

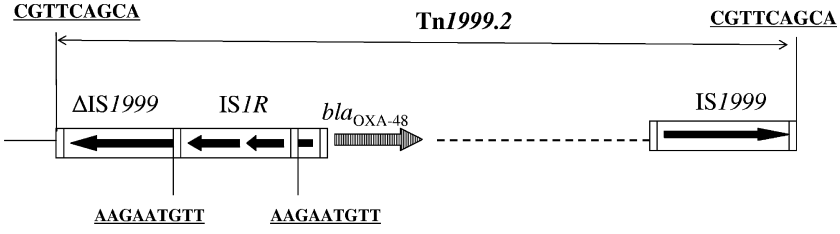

FIG. 2. Schematic representation of Tn*1999.2* described for clone B. The coding regions are represented as boxes, with arrows indicating their transcription orientations.

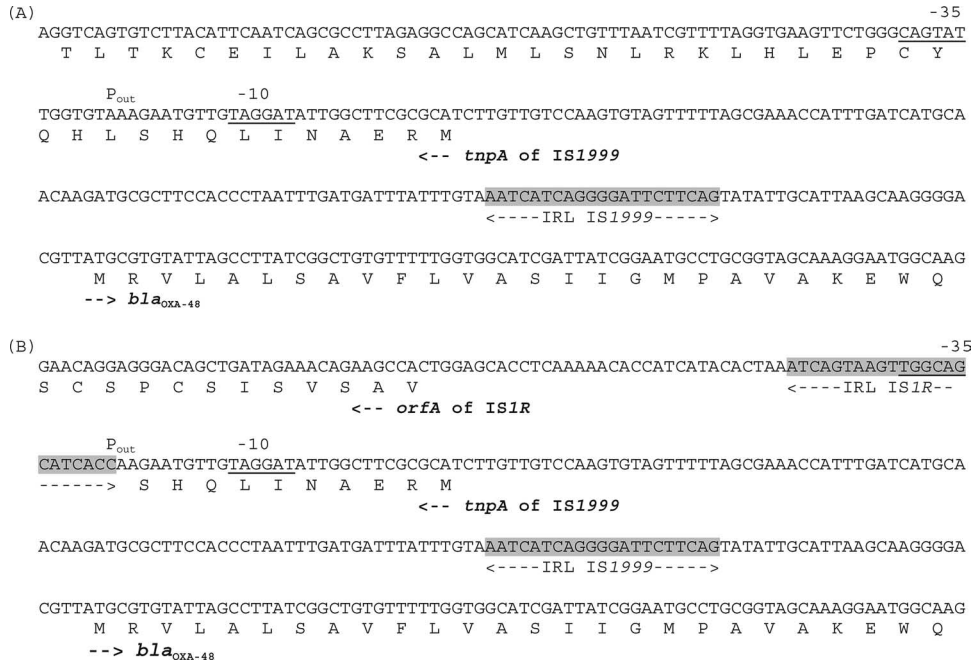

FIG. 3. Nucleotide sequence of the −35 and −10 putative promoter regions within IS*1999* described for clone A (A) and clone B (B). The left inverted repeats (IRL) of IS*1999* and of IS*1R* are shaded in gray, and the −35 and −10 putative sequences are underlined. The *tnpA* transposase gene is indicated.

Our study identified the first large outbreak of OXA-48-positive carbapenem-resistant *K. pneumoniae* isolates. Most isolates produced multiple β-lactamases, including ESBLs, narrow-spectrum oxacillinases, and penicillinases, therefore leading to resistance to all β-lactams. Several OXA-48-producing isolates also expressed ESBL SHV-12, whereas others expressed ESBL CTX-M-15. This association was not due to the dissemination of single plasmids, since the ESBL and OXA-48 genes were not located on the same plasmids. The OXA-48 producers identified here were not clonally related to the previously identified OXA-48-positive *K. pneumoniae* 11978 isolate from Turkey, indicating the concomitant spread of several OXA-48-producing clones in Istanbul. This identification of *K. pneumoniae* isolates harboring the worldwide-spread CTX-M-15 determinant but also the OXA-48 carbapenemase is worrying, since carbapenems are often the last resort for treating infections due to ESBL-producing strains. In addition, we showed here that a higher level of OXA-48 expression was likely due to a particular genetic structure providing a stronger promoter.

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