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Outbreak of OXA-48-Positive Carbapenem-Resistant *Klebsiella pneumoniae* Isolates in France[▽]

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Seventeen *Klebsiella pneumoniae* isolates producing the OXA-48 carbapenemase, obtained from 10 patients hospitalized from April to June 2010, mostly in the medical intensive care unit of the Villeneuve-Saint-Georges Hospital in a suburb of Paris, France, were analyzed. Seven patients were infected, of whom five were treated at least with a carbapenem, and five patients died. Molecular analysis showed that the isolates belonged to a single clone that harbored a 70-kb plasmid carrying the bla_{OXA-48} gene and coproduced CTX-M-15 and TEM-1 β-lactamases. This is the first reported outbreak of OXA-48-producing *K. pneumoniae* isolates in France.

Carbapenems possess the most consistent *in vitro* activity against extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* bacteria. Resistance to carbapenems, while still rare in *Enterobacteriaceae*, is increasing and represents a significant threat in the management of multidrug-resistant isolates (22, 25). It is mediated mostly by two main mechanisms. The first involves the production of a β-lactamase (a cephalosporinase or an ESBL) with a very low level of carbapenem-hydrolyzing activity combined with decreased permeability due to porin loss or alteration (13, 21). The second mechanism is related to carbapenem-hydrolyzing β-lactamases. The carbapenemases identified in *Klebsiella pneumoniae* isolates are metallo-β-lactamases (IMP, VIM, NDM) (6), plasmid-mediated clavulanic acid-inhibited β-lactamases (NmcA, IMI, SME, GES, and KPC) (22, 25), and the expanded-spectrum oxacillinase OXA-48 (23, 24).

The Ambler class D β-lactamase OXA-48, initially identified from a carbapenem-resistant *K. pneumoniae* isolate from Istanbul, Turkey, hydrolyzes penicillins and imipenem, sparing expanded-spectrum cephalosporins (23). The bla_{OXA-48} gene is located on Tn*1999*, a composite transposon made of two copies of the insertion sequence IS*1999* (2). Outbreaks of OXA-48-producing *K. pneumoniae* and other enterobacterial isolates have been described in several cities in Turkey (1, 8, 9, 17) and once in the United Kingdom (27). Subsequently, single isolates of OXA-48-producing *K. pneumoniae* have been reported from Lebanon (20), Belgium (11), the United Kingdom (19), Tunisia (14), Israel (16), Morocco (4), Argentina (5), and India (3).

We describe here a nosocomial outbreak of carbapenem-resistant *K. pneumoniae* strains expressing OXA-48 associated with a CTX-M-15 ESBL in France.

In April 2010, three patients hospitalized in the medical intensive care unit (ICU) at the hospital of Villeneuve-Saint-Georges (VSG), a suburb of Paris, France, were infected by a multidrug-resistant *K. pneumoniae* with resistance to expanded-spectrum cephalosporins and carbapenems. During the subsequent period of time, all patients who had contact with these three patients were screened for fecal carriage of multidrug-resistant bacteria, using a medium designed to select for ESBL producers, BLSE agar (AES, Bruz, France) (26). From April 2010 to June 2010, a total of 260 patients hospitalized in the ICU and the internal medicine unit were screened. Of those 260 patients, 7 were infected and 3 were only colonized by multidrug-resistant *K. pneumoniae* isolates with the same susceptibility pattern (Table 1), indicating probable nosocomial transmission. Five patients had nosocomial pneumonia and three patients developed a catheter-related bloodstream infection, one of them having both types of infection. Infected patients were treated with carbapenems prior to and/or after the determination of antibiotic susceptibility. Colistin and/or amikacin were added in most of the infection cases. Despite treatment, five of the seven infected patients died; two of them improved. Until now, OXA-48 producers in France have been identified from patients transferred from a country in the Mediterranean area (12, 14). None of the VSG patients had a recent history of travel to a country known for having widespread OXA-48 producers, such as Turkey. The first patient infected with the OXA-48-producing *K. pneumoniae* bacteria had never traveled abroad and was retrospectively found to have been colonized at the end of March 2010, suggesting a possible lack of detection of the true index case and subsequent nosocomial transmission. Cohorting of patients and reinforced hygiene measures have been implemented, but several patients were already infected or colonized and may have contributed to the spread of OXA-48 producers.

The antibiotic susceptibilities of the isolates was first determined with the Vitek 2 system (bioMérieux, Marcy l'Etoile, France) (AST-N103 card, software version 04.02), which identified resistance to carbapenems. As routinely performed, the antibiogram was confirmed by the disk diffusion method ac-

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TABLE 1. Clinical features and MICs associated with isolation of carbapenem-resistant *K. pneumonia* isolates

Patient	Date of hospitalization (mo/day/yr)	Hospital unit	Underlying disease	Isolate	Date of isolation (mo/day/yr)	Site of isolation	Infection(s)/colonization	Treatment	Patient outcome	MIC (μg/ml) of[a]:						
										IP	ETP	MP	DP	AN	CS	TGC
1	03/30/10	ICU	Legionnaire disease	VSG1	03/30/10	Rectal swab	Colonization	None								
				VSG2	04/16/10	Catheter blood culture	Catheter-related bloodstream infection + thrombophlebitis	Doripenem + amikacin + colistin	Improved	0.75	3	0.75	0.50	<4	0.25	3
				VSG3	04/17/10	Endotracheal aspirate	Nosocomial pneumonia									
2	04/14/10	ICU	Severe pneumonia	VSG4	04/25/10	Endotracheal aspirate	Nosocomial pneumonia	Imipenem	Deceased	2	3	0.50	0.50	<4	0.75	3
3	04/20/10	ICU	Chronic bronchopneumonia	VSG5	05/12/10	Endotracheal aspirate	Nosocomial pneumonia	Imipenem + amikacin + colistin (aerosol)	Deceased	4	3	0.75	0.50	<4	0.50	3
				VSG6	05/24/10	Catheter	Colonization	None								
4	05/10/10	ICU	Pyelonephritis	VSG7	05/30/10	Endotracheal aspirate	Nosocomial pneumonia	Meropenem + colistin	Improved	1	4	1	0.75	<4	0.75	2
5	05/14/10	ICU	Chronic bronchopneumonia	VSG8	05/17/10	Rectal swab	Colonization	None								
				VSG9	05/27/10	Catheter blood culture	Catheter-related bloodstream infection	Colistin + ciprofloxacin + ceftazidime	Deceased	0.75	4	0.75	0.50	<4	0.25	2
6	05/14/10	ICU	Lung cancer	VSG10	05/26/10	Rectal swab	Colonization	None								
				VSG11	06/04/10	Endotracheal aspirate	Nosocomial pneumonia	None	Deceased	0.75	4	1	0.75	<4	0.19	2
7	05/21/10	ICU	Biliary duct adenocarcinoma													
	06/01/10	Internal medicine		VSG12	06/07/10	Rectal swab	Colonization	None	Improved	0.75	3	0.75	0.75	<4	0.25	3
8	05/31/10	Internal medicine	Acute pulmonary edema													
	06/03/10	ICU		VSG13	06/03/10	Rectal swab	Colonization	None	Deceased	1	4	0.50	0.50	<4	0.75	2
9	06/07/10	Internal medicine	Severe pneumonia													
	06/11/10	ICU		VSG14	06/21/10	Rectal swab	Colonization	None								
				VSG15	06/24/10	Catheter	Catheter-related bloodstream infection	Meropenem + colistin	Deceased	1	4	1	0.50	<4	0.75	2
10	06/14/10	ICU	Brain stroke	VSG16	06/21/10	Rectal swab	Colonization	None	Improved	1	3	0.75	0.25	<4	0.50	3

[a] IP, imipenem; ETP, ertapenem; MP, meropenem; DP, doripenem; AN, amikacin; CS, colistin; TGC, tigecycline.

TABLE 2. Structure of Tn*1999*, other β-lactamases, plasmid analysis, pulsotypes, and ST types of different OXA-48-producing *Klebsiella pneumoniae* isolates from various geographic origins

Isolate	Origin	Reference	PCR and sequencing characterization of:							Characterization of:			
			Tn*1999*			Other β-lactamases				bla_{OXA-48}-carrying plasmid		Bacterial isolate	
			OXA-48	IS*1999*	IS*1*	SHV	TEM	CTX-M	OXA	Size (kb)	Inc[a]	PFGE type	ST type
VSG1-16	France	This study	+	+	+	SHV-11	TEM-1	CTX-M-15	OXA-1	70	P	A	ST-395
UCL-1	Belgium	11	+	+	+	SHV-11	TEM-1			70	P	B	ST-147
KpE	Egypt	12	+	+	+	SHV-11	TEM-1	CTX-M-15	OXA-1	70	P	C	ST-152
HPA-1	Tunisia	14	+	+	+	SHV-11	TEM-1	CTX-M-15	OXA-1	70	P	D	ST-101
KpL	Lebanon	20	+	+	+	SHV-11	TEM-1	CTX-M-15	OXA-1	70	P	D	ST-496
11978	Turkey	23	+	+	−	SHV-11 SHV-2a	TEM-1		OXA-1 OXA-47	70	P	E	ST-14

[a] Inc, plasmid incompatibility group.

cording to Clinical and Laboratory Standards Institute guidelines (10). Additionally, the MICs of several antibiotics were determined using Etest strips (bioMérieux) and the Vitek 2 system (Table 1). All isolates were resistant to all penicillins and expanded-spectrum cephalosporins, and they exhibited heterogeneous decreased susceptibilities to carbapenems, with ertapenem resistance, imipenem being in the intermediate or susceptible range, and meropenem and doripenem being in the susceptible range according to Clinical and Laboratory Standards Institute guidelines updated in June 2010 (10). Moreover, these isolates were resistant to aminoglycosides, except for amikacin, fluoroquinolones, cotrimoxazole, and tigecycline, and remained susceptible to colistin, according to guidelines from the Committé Antibiogramme—Société Française de Microbiologie (CA-SFM) (http://www.sfm-microbiologie.org/UserFiles/file/casfm_2010.pdf).

Specific primers were used for the detection of carbapenemases genes and for β-lactamase-encoding genes that had been previously identified in *K. pneumoniae* isolates that produce OXA-48, namely, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA-1}, and bla_{OXA-47} (9). All isolates were positive for the bla_{OXA-48}, $bla_{CTX-M-15}$, bla_{SHV-1}, bla_{TEM-1}, and bla_{OXA-1} genes (Table 2).

The genetic relationship between the different isolates studied by pulsed-field gel electrophoresis (PFGE) revealed that the isolates were clonally related. These strains were compared with *K. pneumoniae* 11978 from Turkey (23) and with isolates identified in Lebanon (20), Belgium (11), Tunisia (14), and Egypt (12). This epidemic clone was genetically distinct from all the other isolates (Table 2). Multilocus sequence typing (MLST) with seven housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, and *tonB*) was performed according to the method of Diancourt et al. (15). Allele sequences and sequence types (STs) were verified by using the PubMLST *Klebsiella pneumoniae* MLST Database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/kpneumoniae.html). All isolates from VSG displayed ST-395 (allelic profile, 3-1-2-4-1-1-4) (Table 2). The analysis of STs using eBURST (http://pubmlst.org) showed that ST-395 is a single-locus variant of ST-134. The ST types of isolates from other geographical origins were also determined and were different, confirming the results of PFGE (Table 2).

Plasmid DNA extraction according to the Kieser technique (18) showed that in the VSG isolates, the bla_{OXA-48} gene was carried by a self-conjugative 70-kb plasmid, whereas the other β-lactamases genes were carried on larger plasmids (data not shown). Using specific primers as described by Carrër et al. (9), we identified the *repP* gene as the plasmid incompatibility group. Therefore, the plasmid content of the OXA-48-producing *K. pneumoniae* clone identified here was identical to that previously reported (9).

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 similar to Tn*1999*, Tn*1999.2*, was identified for all isolates. Tn*1999.2* differs from Tn*1999* by the presence of an IS*1R* element inserted in the IS*1999* element located upstream of the bla_{OXA-48} gene (Fig. 1) (9).

Here, we identify the first outbreak of OXA-48-positive, carbapenem-resistant *K. pneumoniae* isolates in France. During 2 months, 10 patients were found to be infected or colonized by similar multidrug-resistant isolates. Seven patients developed infection and, because of limited treatment options and comorbidities, five died. Two of the deceased patients were treated at least with imipenem (500 mg every 6 h), imipenem being reported in the intermediate susceptibility range according to the June 2010 updated guidelines of the CLSI. Although a valid comparison is difficult to establish, the death rate is comparable to that observed in another OXA-48-associated outbreak from Istanbul, where 10 of 15 infected patients died despite imipenem-containing treatment. Clinical laboratory detection of OXA-48-producing strains may be difficult because of a low level of resistance to carbapenems and, sometimes, lack of additional ESBLs (9, 11). In this study, detection of patients having gut carriage was facilitated by the additional resistance to cephalosporins due to the presence of the $bla_{CTX-M-15}$ gene (7). The OXA-48 producers identified here were not clonally related to the previously identified OXA-48-positive *K. pneumoniae* isolates, but a very similar 70-kb plasmid harboring bla_{OXA-48} was identified (9). Therefore, spread of the bla_{OXA-48} gene may be very much associated with the spread of a single plasmid type. This study underlines that *K. pneumoniae* is an enterobacterial species prone to cause hospital-based outbreaks of strains carrying multidrug-resis-

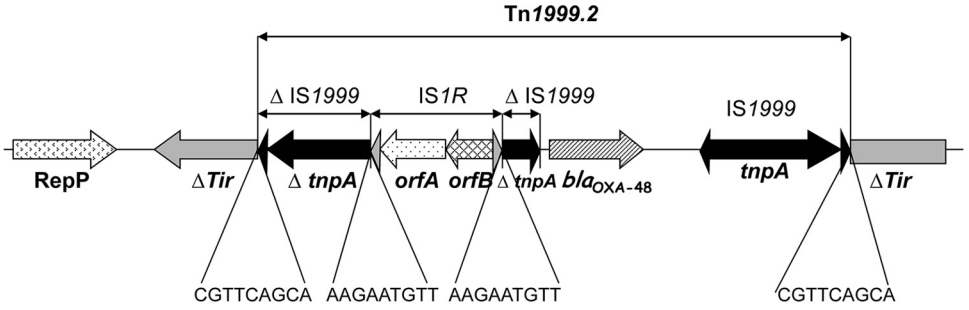

FIG. 1. Schematic representation of Tn*1999.2*, identified with the bla_{OXA-48} gene in the epidemic clone. Genes and their transcription orientations are represented as horizontal arrows. Black triangles represent inverted repeats of insertion sequence IS*1999*, and gray triangles represent inverted repeats of insertion sequence IS*1R*. Target site duplications are indicated below the sequence. Δ indicates that the gene/element is interrupted by an insertion element.

tant genes, such as ESBL and, now, carbapenemase genes. Outbreaks of OXA-48 *K. pneumoniae* producers are now identified in Western Europe after those producing other types of carbapenemases, in particular KPC-2 and VIM-1.

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