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Dissemination of carbapenemase-producing Enterobacteriaceae in France, 2012

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Objectives: Carbapenem-resistant Enterobacteriaceae isolates ($n=1485$) were received at the French Associated National Reference Center for Antibiotic Resistance in 2012 and were characterized for their mechanism of resistance to carbapenems.

Methods: Carbapenemase production was detected using the biochemical-based Carba NP test, based on the detection of *in vitro* hydrolysis of imipenem. All isolates with a positive Carba NP test result were characterized by PCR and sequencing.

Results: Carbapenemase production was identified in 23.1% of the isolates. The main carbapenemase type identified was OXA-48 and derivatives (75.5%). An overseas source was clearly demonstrated for only 27.6% of the isolates.

Conclusions: OXA-48 and derivatives are now the most prevalent carbapenemases in France, with a possible spread of these producers in the community.

Keywords: *K. pneumoniae*, OXA-48, resistance, Carba NP test, NDM, antibiotics

Introduction

During the last decade, Gram-negative clinical isolates, including Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp. with decreased susceptibility to carbapenems, have been increasingly reported in Europe.[1,2] Carbapenem resistance in Enterobacteriaceae may be related either to the association of a decrease in bacterial outer-membrane permeability with overexpression of β-lactamases possessing a very weak carbapenemase activity, or to a β-lactamase with strong hydrolytic activity towards carbapenems, i.e. a carbapenemase.[2] Differentiation of these mechanisms may have consequences for the clinical management of patients and prevention of nosocomial outbreaks.[3–5] The main carbapenemases described in Enterobacteriaceae are the Ambler class A KPC enzymes,[6] Ambler class B β-lactamases (metallo-β-lactamases) of VIM, IMP and NDM types,[2,7] and Ambler class D carbapenemases of the OXA-48 type.[8] The carbapenemase-producing Enterobacteriaceae are usually resistant to many other β-lactam and non-β-lactam antibiotics, giving rise to multiresistant and pandrug-resistant isolates.

Methods

From January 2012 to December 2012, 1485 enterobacterial isolates were received from all areas of France by the French Associated National Reference Center for Antibiotic Resistance in order to test for carbapenemase activity leading to reduced susceptibility to carbapenems. All isolates were identified at the species level using a MALDI-TOF spectrometric identification system (bioMérieux, La Balme-les-Grottes, France). Most of them were *Enterobacter* spp. (42.2%), *Klebsiella pneumoniae* (35.2%) and *Escherichia coli* (12.7%). Susceptibilities to antibiotics, including carbapenems, aminoglycosides, quinolones, tigecycline, fosfomycin, co-trimoxazole, chloramphenicol and nitrofurantoin, were determined by disc diffusion on Mueller–Hinton agar (Bio-Rad, Marnes-la-Coquette, France). According to EUCAST breakpoints (www.eucast.org), most of the isolates were non-susceptible to at least one of the three carbapenems for which MICs were determined by Etest (bioMérieux) (imipenem, meropenem and ertapenem). Carbapenemase production was detected using the Carba NP test, based on the detection of *in vitro* hydrolysis of imipenem demonstrated by a chromogenic test.[9] All isolates giving a positive Carba NP test result were characterized by PCR aimed at identifying the main carbapenemase genes described in Enterobacteriaceae: bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{IMI}

Table 1. Geographical origins of carbapenemase-producing isolates

	Carbapenemase type														
	OXA-48	OXA-162	OXA-181	OXA-204	KPC-2	KPC-3	NDM-1	NDM-4	NDM-5	NDM-7	VIM-1	VIM-2	VIM-4	IMI-1	IMI-2
Morocco	17														
India			2				11	2							
Turkey	3	1													
Tunisia	11										1				
Algeria	14														
Lebanon	1														
Libya	5														
Egypt	1												1		
China					1										
Israel					2										
Italy						3					2				
Romania	1														
Russia	1														
Vietnam	1						1								
Senegal	1														
Kuwait					1								1		
Brazil					1										
Nepal							2								
Seychelles							1								
Middle East							1								
Mali								1							
Singapore									1						
Burma										1					
Mauritania													1		
Linkage with foreign country (%)	56 (22.6)	1 (33.3)	2 (100)	0 (0)	5 (20.8)	3 (20.0)	16 (76.2)	3 (75.0)	1 (100)	1 (100)	3 (42.9)	0 (0)	3 (100)	0 (0)	0 (0)
Unknown or no linkage with foreign country (%)	192 (77.4)	2 (66.7)	0 (0)	4 (100)	19 (79.2)	12 (80.0)	5 (23.8)	1 (25.0)	0 (0)	0 (0)	4 (57.1)	6 (100)	0 (0)	1 (100)	1 (100)
Total	248	3	2	4	24	15	21	4	1	1	7	6	3	1	1

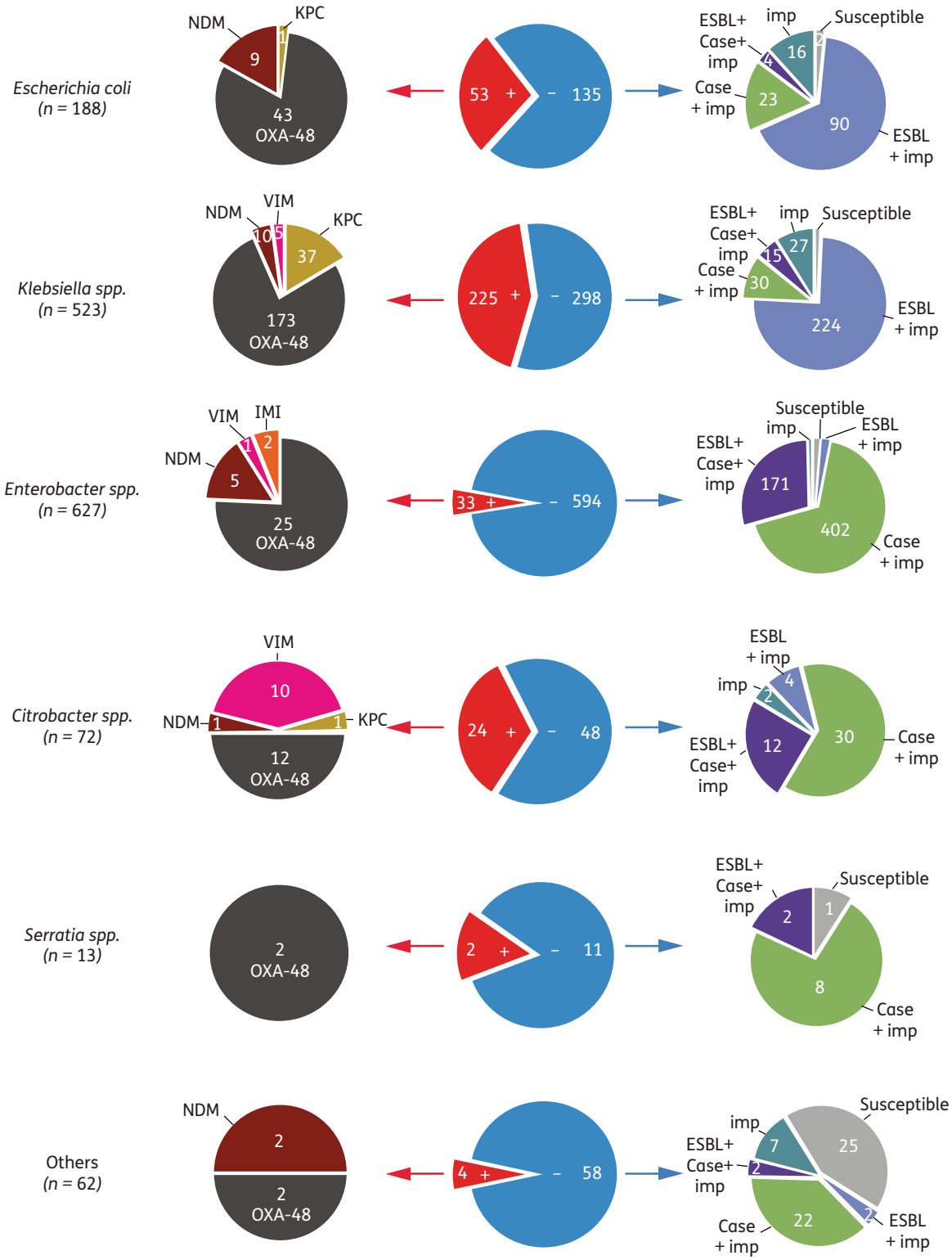

Figure 1. Distribution of enterobacterial species of carbapenemase-producing (+) and non-carbapenemase-producing (−) isolates. (Left) Distribution of OXA-48-like, NDM, KPC, VIM and IMI carbapenemases for each group of enterobacterial species. (Middle) Distribution of carbapenemase-producing (+) and non-carbapenemase-producing (−) isolates in enterobacterial species. (Right) Distribution of non-carbapenemase mechanisms responsible for decreased carbapenem susceptibility in enterobacterial species: impermeability (imp), ESBL+impermeability, cephalosporinase (Case)+impermeability and ESBL+cephalosporinase+impermeability. Carbapenem-susceptible isolates (after MIC confirmation) are also shown. Numbers correspond to the numbers of isolates. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

and bla_{OXA-48}. PCR amplicons were subsequently sequenced.[3] Decreased carbapenem susceptibility due to overexpression of a cephalosporinase (chromosome encoded or plasmid acquired) was suspected for isolates (i) having carbapenem susceptibility restored on cloxacillin-supplemented (200 mg/L) Mueller–Hinton agar and (ii) being negative in the Carba NP test. Additionally, growth on cloxacillin-supplemented medium led to the detection of extended-spectrum β-lactamase (ESBL)-producing isolates that also produce a cephalosporinase at a high level. ESBL production was demonstrated by double disc synergy testing between extended-spectrum cephalosporins (cefotaxime, ceftazidime, cefepime and aztreonam) and clavulanate (ticarcillin/clavulanic acid), performed on Mueller–Hinton agar and cloxacillin-supplemented Mueller–Hinton agar.

Results

Among the 1485 tested isolates, 341 (22.9%) produced a carbapenemase. These carbapenemases were OXA-48 like (75.4%), KPC (11.4%), NDM (7.9%), VIM (4.7%) and IMI (0.6%) (Table 1). Most of the carbapenemase producers were *K. pneumoniae* isolates (65.9%), which are mostly nosocomial pathogens. Additionally, another group of nosocomial pathogens, *Enterobacter* species, represented 9.7% of the carbapenemase producers. Therefore, the spread of carbapenemase-producing Enterobacteriaceae was mainly associated with hospital dissemination in France. However, several *E. coli* isolates were identified among the

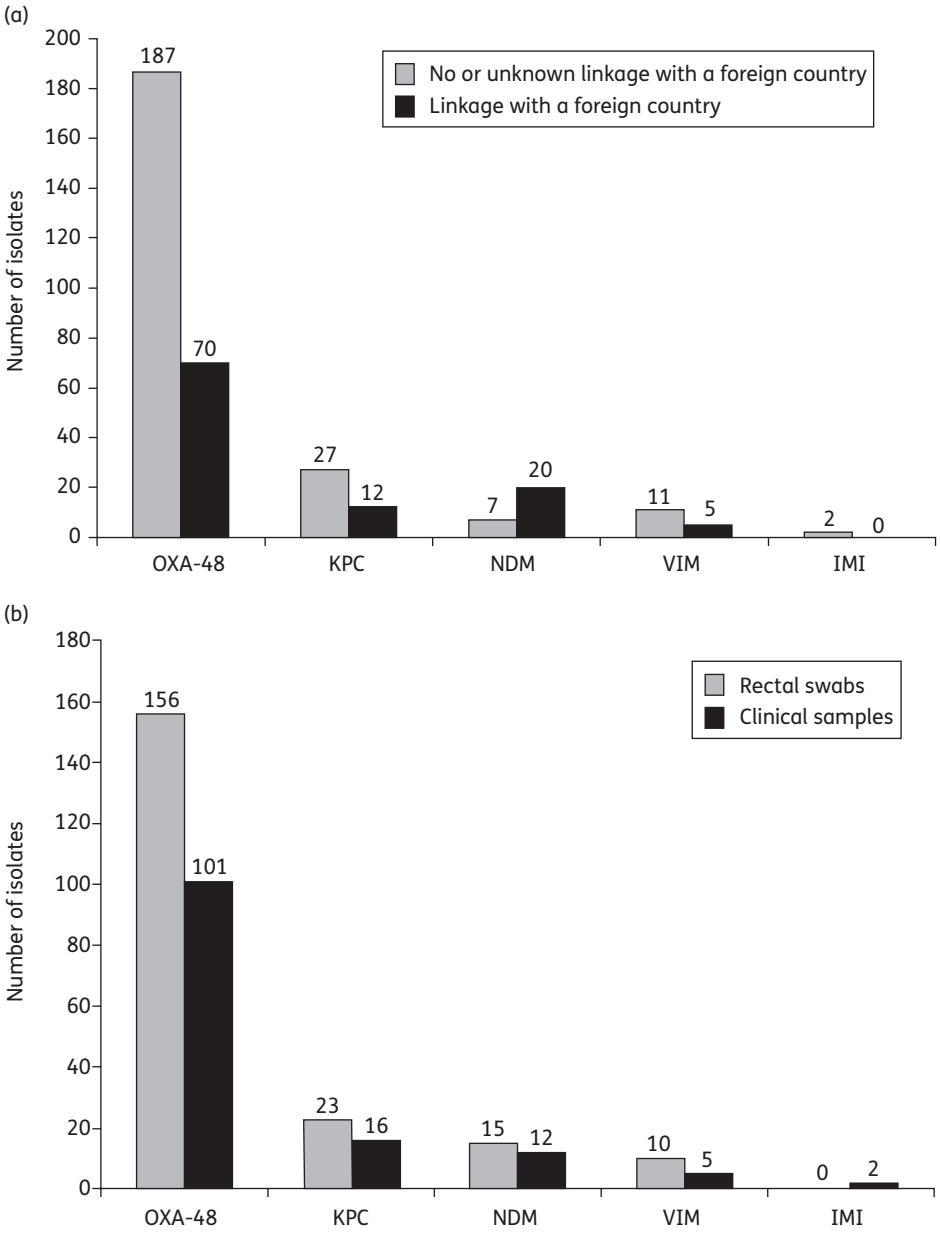

Figure 2. (a) Determination of linkage with a cross-border country for patients infected or colonized with carbapenemase-producing Enterobacteriaceae. (b) Distribution of specimens from which carbapenemase producers were recovered.

carbapenemase producers (15.5%). Since their nosocomial acquisition could not be clearly established, dissemination of these strains in the community remains possible. All carbapenemase producers expressed a single carbapenemase type except one isolate of *K. pneumoniae* that was positive for NDM-1 and OXA-181 (an OXA-48 variant). Regardless of the enterobacterial species, OXA-48-like carbapenemases were predominant (76.8% of carbapenemase-producing *K. pneumoniae*, 81.1% of carbapenemase-producing *E. coli*, 75.8% of carbapenemase-producing *Enterobacter* spp., 53.3% of carbapenemase producers among the other enterobacterial species) (Figure 1). Like OXA-48 producers, NDM producers were equally distributed among the groups of bacterial species ($P > 0.05$, χ^2 test) (Figure 1). Conversely, KPC producers (95%, $P < 0.001$, χ^2 test) were significantly more represented in *K. pneumoniae* compared with the other enterobacterial species (37 K. *pneumoniae*, 1 *E. coli* and 1 *Citrobacter freundii*). The main species associated with VIM carbapenemases was *C. freundii* (62.5%), but this result is biased by the clonal dissemination of a single VIM-positive isolate in the same hospital. The IMI producers ($n = 2$) were *Enterobacter* spp. (1 *Enterobacter asburiae* and 1 *Enterobacter cloacae*).

In the absence of carbapenemase production, the decreased susceptibility to carbapenems was explained by decreased outer membrane permeability associated with the expression of a β-lactamase with weak hydrolytic activity against carbapenems. ESBL production was mostly detected in *K. pneumoniae* (75.2%) and *E. coli* (66.7%), whereas, as expected, overexpression of the chromosome-encoded cephalosporinase was mainly involved in *Enterobacter* spp. (68.2%) (Figure 1).

A probable link with a foreign country was established for 27.6% (94/341) of patients infected or colonized with a carbapenemase producer (Table 1). This link with a cross-border country was clearly identified for NDM producers (Table 1). Indeed, most of the NDM-producing isolates (77.8%) were related to the Indian subcontinent, which is known to be the main reservoir of metallo-β-lactamase.[7] Of note, several strains were isolated on Réunion island, indicating that this French territory (near to Madagascar) is the most exposed for NDM dissemination. The KPC-producing strains were often recovered from patients previously hospitalized in Italy and Israel, two countries where KPC producers are widely disseminated.[6] Curiously, despite the economic and social relationships between Greece and France, no KPC-producing isolate was recovered from patients having links with Greece, where KPC producers are endemic. Of the 56 OXA-48 producers with an established link with a foreign country, 48 (85.7%) were related to North African countries, and all OXA-181 producers were related to India. These results are in agreement with the epidemiological data known about OXA-48 and its variants.[8,10] The fact that OXA-48 and derivatives are now the most prevalent carbapenemases in France is related to the large population exchange with North African countries. However, a clear linkage with a cross-border country has been reported for only 29.9% of OXA-48-like producers, indicating a possible spread of OXA-48 producers in the community in France.

Among the 341 carbapenemase-producing isolates, 204 (59.8%) were isolated from rectal swabs and 137 (40.2%) were from clinical samples (Figure 2). These percentages were similar for all carbapenemase types. Of note, 43.1% (97/225) of the carbapenemase-producing *K. pneumoniae* were from clinical samples. This frequency was significantly higher ($P < 0.05$, Student's *t*-test) than that observed for carbapenemase-producing *E. coli* (28.3%).

Discussion

Carbapenem resistance in Enterobacteriaceae isolates from France has been increasing since 2005 (data not shown). Analysis of 1485 carbapenem-resistant isolates isolated in 2012 highlighted several concerns. (i) The two main organisms with decreased susceptibility to carbapenems are *K. pneumoniae* and *Enterobacter* spp. However, a major difference was observed between these two groups: 43% of *K. pneumoniae* isolates produced a carbapenemase, whereas only 5.3% of *Enterobacter* spp. did so. (ii) The main carbapenemase identified was OXA-48, which could be linked in part to dissemination in the community. The Carba NP test has been shown to be a powerful technique to discriminate rapidly between carbapenemase and non-carbapenemase producers.

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Transparency declarations

An international patent form for the Carba NP test has been filed on behalf of INSERM Transfert (Paris, France).

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