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OXA-48-like carbapenemases: the phantom menace

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OXA-48-type carbapenem-hydrolysing class D β-lactamases are increasingly reported in enterobacterial species. To date, six OXA-48-like variants have been identified, with OXA-48 being the most widespread. They differ by a few amino acid substitutions or deletions (one to five amino acids). The enzymes hydrolyse penicillins at a high level and carbapenems at a low level, sparing broad-spectrum cephalosporins, and are not susceptible to β-lactamase inhibitors. When combining permeability defects, OXA-48-like producers may exhibit a high level of resistance to carbapenems. OXA-163 is an exception, hydrolysing broad-spectrum cephalosporins but carbapenems at a very low level, and being susceptible to β-lactamase inhibitors. The bla_{OXA-48}-type genes are always plasmid-borne and have been identified in association with insertion sequences involved in their acquisition and expression. The current spread of the bla_{OXA-48} gene is mostly linked to the dissemination of a single IncL/M-type self-transferable plasmid of 62 kb that does not carry any additional resistance gene. OXA-48-type carbapenemases have been identified mainly from North African countries, the Middle East, Turkey and India, those areas constituting the most important reservoirs; however, occurrence of OXA-48 producers in European countries is now well documented, with some reported hospital outbreaks. Since many OXA-48-like producers do not exhibit resistance to broad-spectrum cephalosporins, or only decreased susceptibility to carbapenems, their recognition and detection can be challenging. Adequate screening and detection methods are therefore required to prevent and control their dissemination.

Keywords: oxacillinases, β-lactamases, carbapenems, class D

Introduction

In the last few years the emergence of carbapenem resistance in Gram-negatives has been observed worldwide, both in non-fermenters (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*) and in fermenters (Enterobacteriaceae). This phenomenon is mostly related to the spread of different types of β-lactamases. In Enterobacteriaceae, the main carbapenem-hydrolysing β-lactamases are the Ambler class A β-lactamases (e.g. KPC) and the Ambler class B β-lactamases/metallo-β-lactamases (e.g. IMP, VIM and NDM).[1] In addition, the class D β-lactamase OXA-48 (and its variants) possessing weak but significant carbapenemase activity is increasingly reported in Enterobacteriaceae. Recent studies and general epidemiological observations showed that OXA-48-like producers are being increasingly identified in many countries.[1]

This review aims to summarize the main characteristics of the OXA-48-type carbapenemases, including genetic, enzymatic, microbiological and epidemiological features.

General properties of OXA-48

Class D β-lactamases are not inhibited by clavulanic acid, tazobactam and sulbactam (apart from very few exceptions), whereas their activity may be *in vitro* inhibited by NaCl.[2] Some of those class D β-lactamases hydrolyse carbapenems and are therefore defined as carbapenem-hydrolysing class D β-lactamases (CHDLs).[2] This is true for OXA-48, which was first identified from a carbapenem-resistant *Klebsiella pneumoniae* isolate that had been recovered in Istanbul, Turkey, in 2001.[3] That very first OXA-48 producer isolate was multidrug resistant and exhibited a high level of resistance to all β-lactams, including broad-spectrum cephalosporins, cephamycins, monobactams and carbapenems. The identified bla_{OXA-48} gene was plasmid-located and encoded a β-lactamase weakly related to other class D β-lactamases, sharing only 46%, 36%, 32% and 21% amino acid identity with OXA-10, OXA-23, OXA-40 and OXA-1, respectively.[3]

Further investigations showed that this OXA-48-producing *K. pneumoniae* co-expressed several β-lactamases, including the class A extended-spectrum β-lactamase (ESBL) SHV-2a and the narrow-spectrum β-lactamases TEM-1 and OXA-47, and exhibited defects in several outer membrane proteins, leading to its high-level antibiotic resistance pattern.[3] Although OXA-48 hydrolyses penicillins at a high level, it hydrolyses carbapenems only at a low level. In addition, it shows very weak activity against expanded-spectrum cephalosporins.[3,4] In fact, it hydrolyses cefotaxime very poorly, but does not significantly

Table 1. OXA-48-producing enterobacterial species and their countries of isolation (other variants are not listed here)

Country of isolation	Species	References
Turkey	*K. pneumoniae* *E. coli* *C. freundii* *E. cloacae* *S. marcescens* *Providencia rettgeri*	3, 5, 7–9, 18, 47, 75, 76
Morocco	*K. pneumoniae* *S. marcescens* *E. cloacae* *Klebsiella oxytoca*	17, 29, 45, 77
Senegal	*K. pneumoniae* *E. coli* *E. cloacae* *Enterobacter sakazakii*	16
France	*K. pneumoniae* *E. cloacae* *E. coli*	15, 18, 19, 23, 47, 56, 78
Belgium	*K. pneumoniae* *E. coli* *E. cloacae*	24, 79
The Netherlands	*K. pneumoniae*	27, 28
Italy	*E. coli*	48
Lebanon	*K. pneumoniae* *E. coli*	11, 12
Germany	*K. pneumoniae* *E. coli* *E. cloacae*	30, 32
Egypt	*K. pneumoniae*	47, L. Poirel (personal data)
Libya	*K. pneumoniae*	80
Tunisia	*K. pneumoniae*	20–22
South Africa	*K. pneumoniae*	L. Poirel (personal data)
Israel	*E. coli* *K. pneumoniae* *K. oxytoca*	25, 31
Sultanate of Oman	*K. pneumoniae*	13, 81
Saudi Arabia	*K. pneumoniae*	14
Ireland	*K. pneumoniae*	82, 83
Spain	*K. pneumoniae* *E. coli* *E. cloacae*	84–86
UK	*K. pneumoniae* *E. coli* *E. cloacae*	87
Russia	*K. pneumoniae*	26
Slovenia	*K. pneumoniae*	88
India	*K. pneumoniae* *E. cloacae*	89, 90
Switzerland	*K. pneumoniae* *E. coli*	L. Poirel (personal data)

hydrolyse ceftazidime and cefepime. Interestingly, OXA-48 is the class D β-lactamase with the highest known catalytic efficiency (k_{cat} value of 2 s^{-1}) for imipenem.[3,4]

The analysis of the crystal structure of OXA-48 revealed that this β-lactamase possessed a structure similar to that of the narrow-spectrum class D β-lactamase OXA-10.[4] Interestingly, the carbapenemase activity of OXA-48 is likely resulting from subtle changes in the active site region compared with other class D β-lactamases. The efficient hydrolysis of carbapenems might rely on the rotation of the substrate α-hydroxyethyl group promoted by the nature and conformation of residues located in or close to the β5–β6 loop, which allows the movement of the deacylating water molecule toward the acylated serine residue.[4]

Epidemiology of OXA-48 producers

After the first identification of an OXA-48-producing *K. pneumoniae* from Istanbul, an outbreak of OXA-48-producing *K. pneumoniae* isolates was reported in Istanbul from May 2006 to January 2007.[5] Two distinct clones (differing from the index OXA-48 producer) were identified in the same hospital, both producing different ESBL determinants (SHV-12 and CTX-M-15, respectively).[5] In addition, the bla_{OXA-48} gene has been identified in *Escherichia coli* and *Citrobacter freundii*, again first in Turkey.[6,7] For many years, almost all the reports of OXA-48 producers remained from patients hospitalized in Turkey or from patients with a link to Turkey.[8,9]

More recently, the identification of the bla_{OXA-48} gene has been reported in many countries, most often from *K. pneumoniae* isolates. It appears that OXA-48 producers have spread in Turkey, the Middle East and North African countries (Table 1 and Figure 1). All those countries can now be considered to be important reservoirs of OXA-48 producers. In Turkey, a series of sporadic cases, but also outbreaks, have been reported during the last 8 years since the first description of OXA-48 in 2004.[5,10] In the Middle East, sporadic cases have been recently reported, some of them actually corresponding to identification of the OXA-181 variant (see below). However, there have been OXA-48-producing isolates reported in Lebanon,[11,12] Sultanate of Oman,[13] Saudi Arabia[14] and Kuwait[15] (Table 1). In Africa, most of the data are from the northern countries (Morocco, Tunisia, Egypt and Libya), and occurrence of OXA-48 producers has also been reported in Senegal[16] and South Africa (Table 1). In Morocco, a nosocomial dissemination of OXA-48-producing *K. pneumoniae*, *Klebsiella oxytoca* and *Enterobacter cloacae* has been reported.[17] Notably, the occurrence of some OXA-48-producing *E. cloacae* isolates in France was demonstrated to originate from Morocco.[18,19] In Tunisia, OXA-48-producing *K. pneumoniae* isolates have been reported in different hospitals located in different cities.[20–22] There are no published data yet from Algeria, but our own observations with patients transferred from that country strongly suggests that it is also a country where OXA-48 producers might be endemic (Figure 1).

Additionally, OXA-48 producers have been identified sporadically in several European countries, including France, Germany, The Netherlands, Italy, Belgium, the UK, Ireland, Slovenia, Switzerland and Spain (Table 1). In countries such as France,

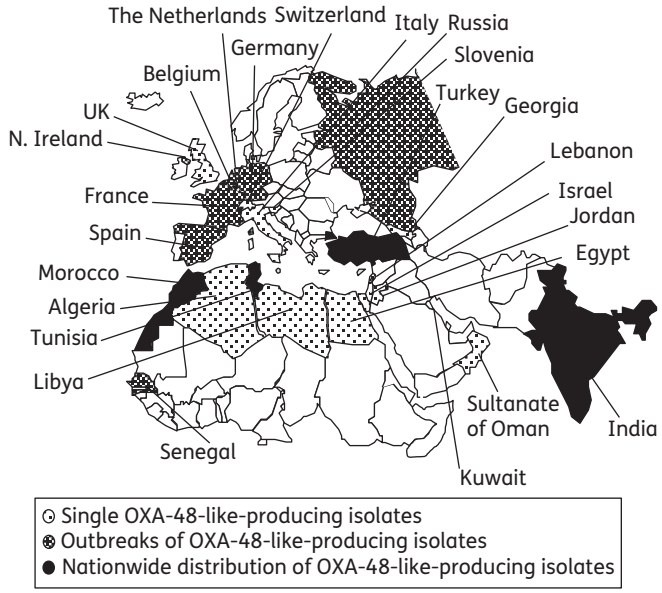

Figure 1. Geographical distribution of OXA-48-like-producing enterobacterial isolates. The country corresponds to that where the isolate has been recovered or was originating from. Data are referring to our own published and unpublished data, together with those of other studies. Argentina, New Zealand and South Africa are countries where OXA-48-like producers have been detected, but are not included on the map.

the UK, Germany and Belgium, recent studies revealed an emergence of OXA-48-producing enterobacterial isolates, at least in hospital settings (Table 1 and Figure 1). However, the spread of the bla_{OXA-48} gene might be much more important than thought. In fact, the detection of OXA-48-like producers is difficult since the level of acquired resistance to carbapenems may remain quite low (see below).

Interestingly, an emergence of outbreaks related to OXA-48-producing *K. pneumoniae* is currently observed, involving strains always exhibiting multidrug resistance patterns, being in particular highly resistant to carbapenems. That phenomenon is no longer observed in Turkey only, since hospital outbreaks have been reported in France,[23] Belgium,[24] Israel,[25] Russia[26] and The Netherlands.[27] The same OXA-48-producing *K. pneumoniae* of sequence type (ST) 395 was actually identified in Morocco, France and Amsterdam, indicating a clonal dissemination.[28]

One of the main sources of concern corresponds to the occurrence OXA-48 producers in the community, often as a consequence of importations from endemic countries, but not systematically. In North Africa, since those countries are probably facing endemic situations, it is likely that OXA-48 producers have spread in the community. This has been exemplified with cases reported in Morocco.[29] In Europe, this may be particularly true in France and Belgium, and probably in Germany, where OXA-48-producing isolates have been established already (Figure 1).[30] Notably, the recently identified occurrence of OXA-48 producers in Israel was demonstrated to be linked with medical tourism involving patients who had been transferred from Georgia or in Jordan.[31]

Variants of OXA-48

Since its discovery in 2004, and until very recently, prospective and retrospective studies were always identifying the exact OXA-48 variant. Then the OXA-162 variant (differing by a single amino acid substitution) was identified from *K. pneumoniae* isolates in Turkey (GenBank ACZ73269) (Figure 2). OXA-162 shares identical hydrolytic activity against β-lactams in general, and carbapenems in particular (L. Poirel, personal data). Recently it has been identified in Germany in various species (i.e. *E. coli*, *C. freundii* and *Raoultella ornithinolitica*).[32]

Then the OXA-163 variant, which exhibits very specific enzymatic characteristics, was identified from Argentinean isolates (see below).

The OXA-181 variant, differing from OXA-48 by four amino acid substitutions, has been identified quite concomitantly by different groups (Figure 2). Interestingly, it has been found to be associated with other carbapenemase genes, such as the bla_{NDM-1} and bla_{VIM-5} genes, in particular in isolates for which a link with the Indian subcontinent could be traced.[33–35] The $bla_{OXA-181}$ gene has been identified in multiple clonally unrelated *K. pneumoniae* isolates in India;[34] in *K. pneumoniae* isolates in The Netherlands,[36] New Zealand[37] and the Sultanate of Oman;[35] in *C. freundii*[33] and *Providencia rettgeri* (L. Poirel, personal data) in France; and in one *E. coli* in India.[34] OXA-181 shares the same hydrolytic properties as OXA-48.[35] Since the main reservoir of OXA-181 producers seems to correspond to the Indian subcontinent, we believe that their spread worldwide will mirror that of the NDM-1 producers.[38]

OXA-204 was recently identified from a series of *K. pneumoniae* isolates recovered from patients having a link with Algeria or Tunisia. OXA-204 exhibits two amino acid substitutions compared with OXA-48, and preliminary data indicate a substrate profile very similar to that of OXA-48 (L. Poirel, unpublished data) (Figure 2).

OXA-232 has been recently identified from *K. pneumoniae* isolates in France, from patients who had been transferred from Mauritius or India (L. Poirel, A. Potron and P. Nordmann, unpublished data). It exhibits five amino acid substitutions compared with OXA-48, but is just a point mutant derivative of OXA-181 (Figure 2). Again, preliminary data indicate a very similar hydrolysis spectrum for that variant.

OXA-163, an expanded-spectrum class D β-lactamase

Even though OXA-48 exhibits a substrate profile including penicillins and carbapenems, but not expanded-spectrum cephalosporins, OXA-163 hydrolyses expanded-spectrum cephalosporins, but very weakly carbapenems.[39] Its peculiar substrate profile enables that enzyme to be classified into the group named extended-spectrum OXAs (ES-OXAs),[2] some ES-OXAs being just point-mutant derivatives of narrow-spectrum class D β-lactamases that have been mainly identified in *P. aeruginosa*. OXA-163 is also peculiar in the way that its activity is partially inhibited by clavulanic acid and tazobactam, in contrast to the other class D β-lactamases.[2] It actually exhibits a substrate profile that is similar to that of OXA-18 identified in *P. aeruginosa*.[40] Sequence analysis of the amino acid sequence

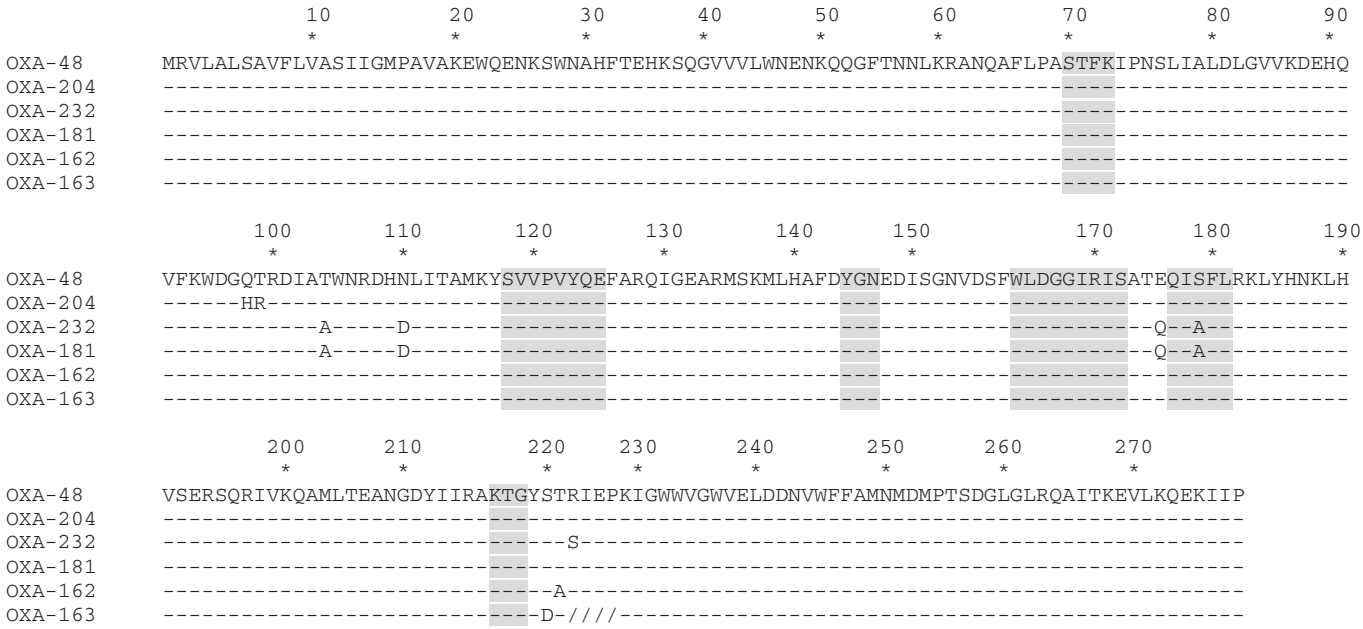

Figure 2. Amino acid alignment of OXA-48 and the five other variants. Dashes indicate identical residues among all the amino acid sequences. Slashes indicate absent amino acids. Amino acid motifs that are well conserved among class D β-lactamases are indicated by boxes. Numbering is according to DBL.[41]

of OXA-163 revealed that it differed from OXA-48 by a four amino-acid deletion [Arg214, Ile215, Glu216 and Pro217, class D β-lactamase (DBL) numbering[41]], and a substitution corresponding to a Ser to Asp change at DBL position 220 (Figure 2). The $bla_{OXA-163}$ gene was initially identified in two isolates recovered from two patients in Argentina: one *K. pneumoniae* and one *E. cloacae*.[39] Both isolates were resistant to expanded-spectrum cephalosporins, but were still susceptible to carbapenems, except for ertapenem, with an MIC of 2 mg/L for the *E. cloacae* isolate. Despite its low level of carbapenemase activity, OXA-163 was able to confer high-level resistance to those molecules when produced by a carbapenem-susceptible *E. coli* strain lacking two porins (L. Poirel, personal data). Then the $bla_{OXA-163}$ gene was identified in multiple enterobacterial species recovered from different hospitals in Argentina.[42]

Origin of the OXA-48-like β-lactamases

Even if the progenitors of the widespread bla_{KPC}, bla_{IMP}, bla_{VIM} and bla_{NDM} carbapenemase genes remain unknown, the origin of some CHDLs has been identified.[2] The waterborne species *Shewanella oneidensis* does possess an intrinsic bla_{OXA-54} gene encoding a β-lactamase that shares 92% amino acid identity with OXA-48.[43] Recently, *Shewanella xiamenensis* was identified as the progenitor of the $bla_{OXA-181}$ gene, with the exact same sequence identified on the chromosome of this waterborne species.[44] More generally, the *Shewanella* spp. constitute reservoirs of CHDL-encoding genes.[2] If mobilized on mobile genetic structures, they might lead to acquisition of carbapenem resistance in Enterobacteriaceae.

Therefore, considering that the waterborne, environmental and non-human-pathogenic *Shewanella* spp. are the progenitors of the bla_{OXA-48}-like genes, it might be speculated that those genes have been mobilized in the environment. The main hypothesis is that the involved genetic tools, being insertion sequences (see later), have mobilized the chromosomal genes onto plasmids that have subsequently disseminated in clinically relevant species such as in *K. pneumoniae* or *E. coli*. That link between the donor (*Shewanella*) and the recipient might have been direct, or indirect through intermediate reservoirs. This latter hypothesis is exemplified by the recent identification of a plasmid-borne bla_{OXA-48} gene in a *Serratia marcescens* strain recovered from an aquatic environment in Morocco,[45] suggesting that *S. marcescens*, being an environmental and opportunistic enterobacterial pathogen, could have played the role of intermediate reservoir, being in close contact with the donor in the environment, but also with clinically relevant enterobacterial species in the human gut.

Genetic platforms of OXA-48-like β-lactamase-encoding genes

The bla_{OXA-48} gene has been originally identified in association with insertion sequence IS*1999* in *K. pneumoniae*, providing promoter sequences responsible for its expression.[3] It has been demonstrated that bla_{OXA-48} was part of a composite transposon named Tn*1999* and made of two copies of IS*1999* bracketing this gene.[46] *In vitro* experiments confirmed that Tn*1999* was functional, even though transposition occurred at very low frequency in *E. coli* ($<10^{-7}$). Then transposon Tn*1999.2* was identified from *K. pneumoniae* isolates from Istanbul, differing by the insertion of IS*1R*.[47] In fact, IS*1R* had targeted the region upstream of bla_{OXA-48}, thus enhancing its expression by providing strong promoter sequences. Interestingly, the isolates harbouring the Tn*1999.2* structure exhibited higher MICs of carbapenems

compared with those possessing Tn*1999*.[47] Very recently a third isoform of Tn*1999* has been identified in an *E. coli* isolate from Italy, with a second copy of IS*1R* located downstream of bla_{OXA-48}.[48]

Interestingly, the $bla_{OXA-181}$ gene has been identified in a totally different genetic environment, without any IS*1999* feature in the surrounding sequences. Insertion sequence IS*Ecp1* was identified upstream of the $bla_{OXA-181}$ gene.[35] That insertion sequence is known to be widely responsible for the acquisition of the broad-spectrum β-lactamase bla_{CTX-M} and bla_{CMY} genes.[49,50] The transposition process mediated by IS*Ecp1* is peculiar, since only a single copy can mobilize sequences located at its right-end extremity by recognizing imperfect right inverted repeat sequences (one-ended transposition).[50] In *K. pneumoniae* KP3 from Oman, the $bla_{OXA-181}$ gene was identified inside a 3139 bp long IS*Ecp1*-made transposon named Tn*2013*, flanked by a 5 bp duplicated sequence being the signature of the transposition.[35] The same association between IS*Ecp1* and $bla_{OXA-181}$ was found in the other OXA-181 producers we have identified (L. Poirel, A. Potron and P. Nordmann, personal data).

Plasmids involved in the spread of OXA-48-like-encoding genes

So far, acquisitions of bla_{OXA-48}-like genes have been identified only in Enterobacteriaceae, but have never been found in other Gram-negatives such as *A. baumannii* or *P. aeruginosa*, even though other CHDL-encoding genes are identified in those species.[2] This absence of transfer to non-enterobacterial species might be explained by the narrow host range of plasmids bearing the bla_{OXA-48}-like genes. Indeed, the acquired bla_{OXA-48} gene has always been identified on plasmids. This is also true for the $bla_{OXA-163}$ and $bla_{OXA-181}$ genes. Several studies initially reported that the bla_{OXA-48} gene was located on ~70 kb plasmids that were self-transferable and did not carry additional resistance determinants.[47] All those observations led us to speculate on the epidemicity of a single plasmid carrying bla_{OXA-48}. That hypothesis has been recently confirmed through the complete sequencing of the bla_{OXA-48}-carrying plasmid pOXA-48a recovered from a *K. pneumoniae* isolate in 2001 in Istanbul, Turkey.[51] The whole sequence of pOXA-48a was obtained and subsequent analysis revealed that it was a 62.3 kb IncL/M-type plasmid backbone on which the Tn*1999* composite transposon had inserted.[51] IncL/M-type plasmids are common in Enterobacteriaceae, and have been identified at the origin of the acquisition of a variety of antibiotic resistance genes.[52] They are broad host range plasmids, being identified in *Erwinia* spp., *Ralstonia* spp. and *Pseudomonas* spp. A single replication module was identified on pOXA-48a, as for other IncL/M-type plasmids, and the transfer operon identified was also very similar to those of that plasmid group. The conjugation rate of plasmid pOXA-48a among enterobacterial species was found to be quite high, at 3.3×10^{-5}. Molecular investigations showed that plasmid pOXA-48a was identified in all OXA-48-producing isolates recovered from many countries.[51] This observation is noteworthy since it indicates that the current spread of OXA-48 producers is related to the spread of a single plasmid among different enterobacterial isolates.

Few studies have identified OXA-181-producing isolates so far. However, it has been shown that the $bla_{OXA-181}$ gene might be found on different plasmid scaffolds. In *K. pneumoniae* KP3 from Oman, the $bla_{OXA-181}$ gene was found on a small (7605 bp) ColE2-type plasmid, which was not self-transferable (but mobilizable), did not carry additional resistance determinants and was of broad host range.[35] In a *C. freundii* isolate from France co-harbouring a bla_{NDM-1}-positive plasmid, the $bla_{OXA-181}$ gene was located onto a 83557 bp IncT-type plasmid that was self-conjugative, though at a low frequency, and mobilizable.[53]

Detection of OXA-48-like enzymes

One of the major concerns for controlling the spread of OXA-48-like producers is the absence of phenotypical tests that could contribute to their easy recognition. In particular, a search for carbapenemase production in any enterobacterial isolates with a slight decrease of susceptibility to carbapenems is important, because there is currently a paucity of clinical experience for treating infections due to carbapenemase producers, and also very limited knowledge about the possibility to select *in vivo* mutants with increased levels of resistance to carbapenems through additional mechanisms.

There are actually two main concerns: (i) recognizing the production of OXA-48 in those isolates recovered from infections; and (ii) isolating the OXA-48 producers by using a reliable screening process. First, based on the literature and our own experience, we propose that production of a carbapenemase should be suspected only for enterobacterial isolates with MIC values of ertapenem ≥0.5 mg/L, or imipenem or meropenem ≥1 mg/L.[54] What is noteworthy with OXA-48 producers is that very different β-lactam resistance patterns can be observed, with some isolates being still susceptible to broad-spectrum cephalosporins and carbapenems, some being still susceptible to broad-spectrum cephalosporins but resistant to carbapenems, and some being resistant to broad-spectrum cephalosporins and carbapenems (Figure 3). That means that suspicion of OXA-48 production is very often challenging.

Recently, several countries adopted new guidelines recommending the screening of patients transferred from foreign hospitals or patients returning from travels in foreign countries known to be endemic for multiresistant bacteria. Indeed, the prevention of spread of carbapenemase producers relies on the early and accurate detection of carriers. Those recommendations included screening procedures for colonization, by using, for instance, commercially available ESBL-targeting media or carbapenem-supplemented media. Several studies report using media containing imipenem at 1–2 mg/L, which may be too high for efficient detection of carbapenemase producers with low-level resistance, which is often the case for OXA-48/OXA-181 producers. In addition, the ESBL-targeting media approach fails to detect OXA-48-like producers (except OXA-163 producers), which do not produce any ESBL determinants, since they remain susceptible to broad-spectrum cephalosporins.[55] An enrichment procedure including an overnight culture in broth supplemented with ertapenem before plating on Drigalski agar medium with ertapenem and imipenem Etest strips has

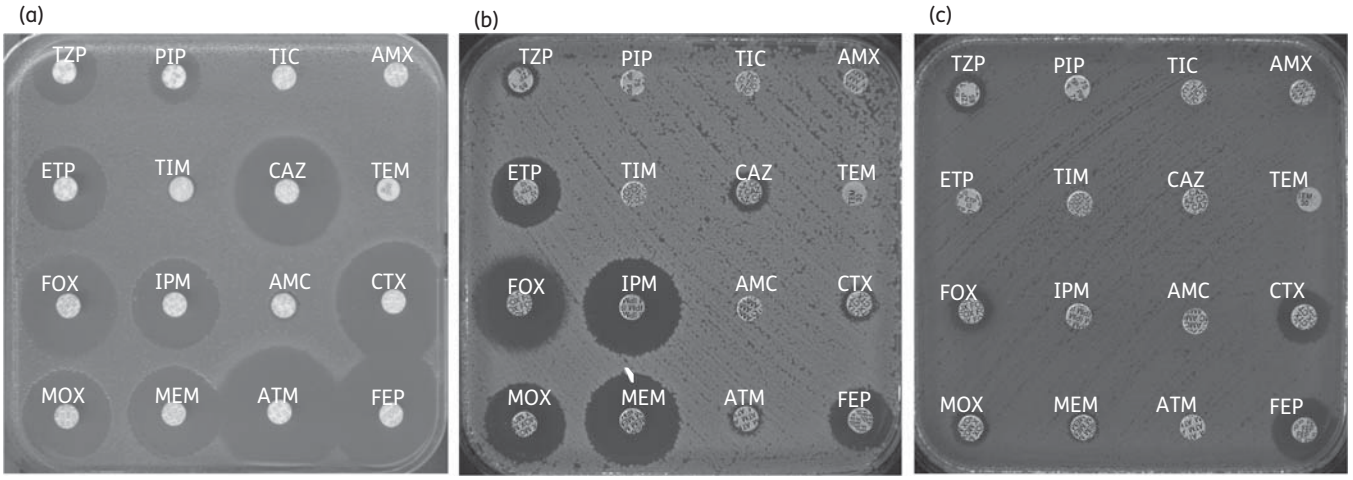

Figure 3. Antibiograms of β-lactam molecules performed for three representative OXA-48-producing *K. pneumoniae* isolates. (a) Isolate that does not produce any ESBL. (b) Isolate producing an ESBL and exhibiting low MICs of carbapenems. (c) Isolate producing an ESBL and exhibiting high MICs of carbapenems. AMC, amoxicillin/clavulanate; AMX, amoxicillin; ATM, aztreonam; CAZ, ceftazidime; CTX, cefotaxime; ETP, ertapenem; FEP, cefepime; FOX, cefoxitin; IPM, imipenem; MEM, meropenem; MOX, moxalactam; PIP, piperacillin; TIM, ticarcillin/clavulanate; TEM, temocillin; TIC, ticarcillin; TZP, piperacillin/tazobactam.

been suggested in one case.[56] The main disadvantage of this culture step is that it delays results by 18–24 h.

Once the isolate is obtained, either recovered from an infected source or as a result of a screening procedure, the first step is to confirm the carbapenemase activity. We recently showed that the modified Hodge test possessed a good sensitivity for detecting enterobacterial isolates producing OXA-48-like carbapenemases, and could therefore be used in clinical microbiology routine laboratories.[57] Reference laboratories may perform spectrophotometric determination of carbapenemase activity. Using crude protein extracts and UV spectrophotometry, hydrolysis of carbapenems such as imipenem can be measured at a wavelength of 262 nm by comparing the slope obtained with a non-carbapenemase producer (L. Poirel, personal data). In addition, recent studies showed that matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrophotometry might be used for detection of β-lactamase activity, and in particular production of carbapenemases, even though those studies did not yet evaluate the reliability for OXA-48 producers.[58–60] In contrast to other carbapenemase genes whose expression can be inhibited *in vitro* with clavulanic acid, EDTA, boronic acid or dipicolinic acid combined tests, no phenotypic test can detect class D β-lactamase producers in general, and CHDL producers in particular. Recent data have shown that OXA-48 hydrolytic activity could be inhibited by the NXL104 β-lactamase inhibitor, but not specifically, since that molecule inhibits a wide range of β-lactamases.[61,62] An interesting approach was recently highlighted by Glupczynski *et al.*,[24] who suggested that a high level of resistance of OXA-48 producers to the β-lactam temocillin might be presumptive of OXA-48 production.

Finally, the molecular-based techniques (essentially PCR) remain the gold standard techniques for the identification of isolates carrying bla_{OXA-48}-like genes.[63] Primers OXA-48A (5′-TTGGT GGCATCGATTATCGG-3′) and OXA-48B (5′-GAGCACTTCTTTTGTGATG GC-3′)[2] allow the amplification of all known bla_{OXA-48}-like genes identified so far. In order to detect the clinically relevant carbapenemase genes identified in Enterobacteriaceae, a multiplex PCR system in which screening of the bla_{OXA-48}-like genes was included has been developed.[64] A bla_{OXA-48}-specific real-time TaqMan PCR method has also been developed in order to speed the process of its molecular identification.[65] Finally, a microarray method has been developed that includes the detection of the bla_{OXA-48}-like genes.[66] As underlined, sequencing of the bla_{OXA-48}-like genes is necessary to differentiate between the different variants, and this may impact the interpretation of the positivity when considering that some of those variants do not possess a significant carbapenemase activity.

Clinical consequences

The clinical consequences related to the spread of OXA-48-type producers may be quite important, because many of those producers are classified as susceptible to carbapenems according to the EUCAST or CLSI guidelines.[67,68] The current recommendations proposed by those guidelines are to report susceptibility to carbapenems as found, whether or not the isolates produce a carbapenemase. It has been recently reported that imipenem could be successfully used to treat a bacteraemia due to an imipenem-susceptible but OXA-48-producing *K. pneumoniae*.[69] However, the efficacy of carbapenems for treating infections due to carbapenemase producers with low-level resistance or susceptibility to several carbapenems remains debatable since imipenem-containing therapy failed to treat several OXA-48 infections.[5,23] For those OXA-48-like-producing isolates in which there is no ESBL association, broad-spectrum cephalosporins such as ceftazidime may be theoretically used since those molecules are weakly or not hydrolysed by those enzymes (except OXA-163). However, very few clinical data are available to support their use, except one study in which we showed success in treating a newborn infection caused by an ESBL-negative and OXA-48-producing *K. pneumoniae* with a combination of cefotaxime and amikacin.[18]

The β-lactamase inhibitor NXL104 should represent an interesting therapeutic option, since it possesses potent activity against class A, B and D β-lactamases,[58] meaning that it may inhibit both ESBL and OXA-48 in those isolates combining both mechanisms.[70] NXL104 has been shown to inhibit OXA-48 β-lactamase by forming a stable covalent complex.[70] A recent study reported that NXL104 is able to significantly reduce the MIC values of imipenem, cefepime and ceftazidime for OXA-48 producers co-producing an ESBL, leading to susceptibility for all the tested strains.[59] The same results were reported for the combination with cefotaxime or ceftaroline and NXL104.[58,71]

Recently, an experimental model of induced peritonitis has been developed in mice, using an ESBL-negative and OXA-48-producing *K. pneumoniae* strain.[72] That strain exhibited decreased susceptibility to carbapenems and susceptibility to broad-spectrum cephalosporins. Ceftazidime was shown to be an efficient therapy, whereas ertapenem and imipenem were not. That observation raises serious concerns about the efficacy of carbapenems for treating patients infected with OXA-48-type-producing Enterobacteriaceae, regardless of the observed level of resistance to carbapenems.

Colistin and tigecycline are most likely to be active *in vitro* against OXA-48 producers, but resistance to these molecules has been reported among OXA-48-producing isolates.[73] Fosfomycin might be useful as a last-resort option as part of association regimens because of the high potential for emergence of resistance.[74] Given that OXA-48 producers exhibit variable resistance profiles, selection of the appropriate therapy should be made on a case-by-case basis. Interestingly, by comparing ESBL-positive and -negative OXA-48 producers, we observed that the ESBL-negative OXA-48 producers were significantly more susceptible to aminoglycosides and fluoroquinolones than the ESBL-positive OXA-48 producers (P. Nordmann, personal data).

Conclusions

Since the beginning of the 2000s, carbapenemases of the Ambler class A KPC type or class B type, including IMP-, VIM- and NDM-like enzymes, were considered to be the most important carbapenemases in Enterobacteriaceae, since (i) their hydrolytic activity included not only carbapenems, but also broad-spectrum cephalosporins; (ii) their carbapenemase activities were significant; and (iii) their corresponding genes have been identified worldwide. In contrast, OXA-48 was not really considered so much problematic, considering that (i) its hydrolytic spectrum does not include broad-spectrum cephalosporins; (ii) its carbapenemase activity is somewhat low compared with class A and class B enzymes; and (iii) the spread of the bla_{OXA-48} gene was supposed to be limited to Turkey only for many years.

The difficulties linked to the detection of OXA-48 producers have played a significant role in their spread, which has been somehow silent. Indeed, the fact that expression of the bla_{OXA-48} gene only confers reduced susceptibility to carbapenems does not facilitate recognition of the OXA-48 producers. Also, since a significant proportion of OXA-48 producers does not co-express ESBLs, the observed susceptibility to broad-spectrum cephalosporins does not favour their recognition or even suspicion.

Is the current emergence of OXA-48 producers resulting from antibiotic selective pressure, and in particular to the overuse or misuse of carbapenems? That is an issue that is difficult to speculate about. Interestingly, the current spread of the bla_{OXA-48} gene is largely the consequence of the spread of a single epidemic plasmid that does not carry other resistance determinants. The absence of other resistance determinants on this plasmid suggests that other antibiotic families likely did not play a role in co-selection, as opposed to what can be hypothesized for many other carbapenemase genes, which are often physically associated with other resistance genes, and in particular, genes encoding resistance to aminoglycosides.

Despite the fact that bla_{OXA-48} is part of a functional transposon, its dissemination actually corresponds to the dissemination of its plasmid support and not of the transposon itself. Further investigations are required to better understand the reasons for such a successful spread. Another interesting observation in relation to the genetic context of the bla_{OXA-48}-like genes corresponds to the fact that all have been identified in association with insertion sequences, whereas most of the class D β-lactamase genes are very often identified as gene cassettes located in class 1 integron structures. Despite being closely related in terms of nucleotide sequence, the bla_{OXA-48} and $bla_{OXA-181}$ genes have always been identified in distinct genetic contexts (an IS*1999*- or an IS*Ecp1*-made transposon), suggesting that those two genes did not evolve from each other through mutations, but rather occurred through two separate events originally corresponding to mobilizations of two different genes from two *Shewanella* donor strains.

From a clinical point of view, further studies need to be performed in order to evaluate whether carbapenem-based treatments could be used for treating infections caused by OXA-48 producers remaining *in vitro* in the susceptibility ranges, and also whether treatments based on broad-spectrum cephalosporins could be safely used when facing infections caused by ESBL-negative OXA-48 producers. Currently, OXA-48-like enzymes represent a potential source of clinical failure for many β-lactams in Enterobacteriaceae. The lack of their detection may enhance their hidden and rapid spread among clinical isolates. In addition, since OXA-48 producers are mostly *E. coli* and *K. pneumoniae*, it is possible that non-specialized laboratories or infectious disease practitioners may let the outbreak continue because of lack of recognition and thus adequate management of those particular isolates. Unfortunately we foresee further and extensive spread (likely uncontrollable), at least in European countries as a consequence of a tight relationship with many North African and Middle East countries and Turkey.

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Transparency declarations

None to declare.

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