Molecular epidemiology of OXA-48-producing Klebsiella pneumoniae in France

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Abstract

We characterized 53 OXA-48-producing Klebsiella pneumoniae (OXA-48-Kp) isolated between 2011 and 2013 in 21 French hospitals. All the isolates were genotyped using MLST and PFGE and the population structure of the species was determined by a nucleotide-based analysis of the entire K. pneumoniae MLST database. Most of the OXA-48-Kp isolates also produced CTX-M-15 and remained susceptible to imipenem and meropenem. The isolates were distributed into 20 STs, of which five were dominant (ST15, ST101, ST147, ST395 and ST405). All the OXA-48-Kp clustered in the major clade of K. pneumoniae Kpl.

Keywords: Multidrug resistance, multilocus sequence typing, phylogenetic analysis

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The emergence of carbapenemase-producing Enterobacteriaceae (CPE) has become a major public health concern [1,2]. In France, OXA-48-producing Klebsiella pneumoniae (OXA-48-Kp) represented two-thirds of the 627 cases of CPE reported between January 2004 and September 2013 to the mandatory national surveillance system [3]. Here, we assess the genetic population structure of a significant sample of the OXA-48-Kp recovered in French hospitals.

We selected 53 OXA-48-Kp isolated between 2011 and 2013 in 21 French hospitals located in 10 of the 22 regions of France. We kept only the index isolate of each outbreak to get a better picture of the diversity of the strains. This represents approximately one-sixth of all the OXA-48-Kp identified in France during the study period. All the isolates were recovered from human samples (rectal screening or clinical samples). Twenty-eight (53%) of the patients travelled or were hospitalized in a foreign country in the previous 12 months and 15 (28%) did not travel abroad. The antibiotic susceptibility was determined by the disk diffusion method and the production of an extended-spectrum β-lactamase (ESBL) was detected by a synergy test [4]. We also determined the MICs of imipenem, meropenem and ertapenem using the strip-test (bioMérieux, La Balme-les-Grottes, France). The presence of blaoxa-48 was controlled in all isolates and bloesbl genes were identified in ESBL-producers by sequencing [5]. Pulsed-field gel electrophoresis (PFGE) was performing the XbaI and multilocus sequence typing (MLST) was performed as previously described [6]. In order to build a dendrogram with the 1392 STs available at the time of the study, we concatenated the sequences of seven MLST genes to form a 3078-bp sequence alignment and define 930 polymorphic positions. The best-fit nucleotide substitution model for these data was GTR + G + I, as determined with ModelTest 0.1.1 [7]. We used Escherichia coli K12 as the outgroup strain. A maximum likelihood tree was constructed with RAxML 7.2.8 and visualized with Dendroscope [8].

According to the EUCAST guidelines, 93% of the 53 OXA-48-Kp isolates were non-susceptible to ertapenem, 23% non-susceptible to imipenem and 17% non-susceptible to...
meropenem. Most of the OXA-48-Kp isolates (42/53; 79%) produced an ESBL, with a large predominance of CTX-M-15 (40/42; 95%). The production of an ESBL was not linked to previous travel abroad. ESBL-producing OXA-48-Kp isolates were more frequently resistant to ertapenem, extended-spectrum cephalosporins, tobramycin and fluoroquinolones than non-ESBL-producers ($p < 0.01$).

The 53 OXA-48-Kp isolates were distributed into 20 STs. ST15 was the most frequent ST (14 isolates; 26%), followed by ST147 and ST395 (six isolates each; 11%) and ST101 and ST405 (five isolates each; 9%). ST17, ST48 and ST70 were represented by two isolates each. Other STs were represented by one isolate (ST37, ST45, ST231, ST307, ST336, ST589, ST663, ST1299, ST1300, ST1382 and ST1383). Isolates belonging to dominant STs (ST15, ST101, ST147, ST395, and ST405) were more likely to produce an ESBL (92% vs. 53%; $p = 0.001$) and more likely to be resistant to extended-spectrum cephalosporins, tobramycin and fluoroquinolones ($p < 0.01$) than isolates from non-dominant STs. Of interest, isolates from patients with a prior history of international travel were found in both dominant and non-dominant STs.

The phylogenetic tree built from the sequence data of the seven MLST genes of the 1392 STs of K. pneumoniae clearly showed three major clades, which correspond to the three major phylogenetic groups (KpI, KpII and KpIII) (Fig. 1). These three clades have been previously defined by the comparison of gyra and parC sequences [9]. The majority (75%) of the 1392 defined STs clustered into the clade KpI, which encompassed all the 53 OXA-48-Kp of the present study (Fig. 1).

Here we found that 79% of the emerging OXA-48-Kp in France additionally produced an ESBL, mostly of CTX-M-15-type. Such a proportion and the large predominance of CTX-M-15 have also been observed by Potron et al. [10] in an international collection. In our collection of OXA-48-Kp, the ESBL production was associated with a higher resistance to extended-spectrum cephalosporins and to fluoroquinolones. It is worth mentioning that 77% and 83% of our OXA-48-Kp remain susceptible to imipenem and meropenem, respectively. The analysis of MLST data revealed that a quarter of OXA-48-Kp belonged to ST15, while four other clones (ST101, ST147, ST395 and ST405) were represented each by five or six isolates. These STs are recognized as established ‘international’ clones that predominantly harboured blaESBL genes and less frequently carbapenemase-encoding genes. However, these clones have not yet had the epidemiological success of ST258, which drives the worldwide dissemination of blaKPC [2]. Overall, this suggests that the spread of OXA-48-Kp combines the clonal expansion of successful STs and the dissemination of plasmid-borne blaOXA-48 among various strains of K. pneumoniae. Indeed, a

![FIG. 1. Comparative analysis based on concatenated sequences of the seven housekeeping genes of the Klebsiella pneumonia MLST scheme. (A) Clonal assignment of the STs in the entire K. pneumonia MLST database ($n = 1392$ STs). (B) Close-up of the KpI group. The STs found in the present collection are represented with red spots.](image-url)
62-kb self-conjugative IncL/M-type plasmid has been shown to contribute to the diffusion of bla\textsubscript{OXA-48} in Europe [11]. Also, the genetic association of the bla\textsubscript{CTX-M-15} and bla\textsubscript{OXA-48} genes on mobile genetic elements, which may easily spread among Enterobacteriaceae, is a matter of concern [12].

The phylogenetic tree built with the whole MLST database reveals that all the STs recovered in this study clustered in the KpI group, the clade that includes all the multidrug-resistant clinical isolates [13]. The scattering of our OXA-48 Kp isolates throughout the Kp group suggests that the acquisition of bla\textsubscript{OXA-48} is not associated with a subset of KpI strains.

Interestingly, our study, together with the French national survey [14], shows that approximately one-quarter of OXA-48-Kp could be autochthonous (i.e. with no history of travel or hospitalization abroad) and independent from a previous hospitalization in a French hospital.

In conclusion, emergence and spread of OXA-48-Kp is nowadays a public health concern, because of therapeutic dead-ends and risk of transmission of the bla\textsubscript{OXA-48} gene to commensal E. coli clones. The ‘search and isolate’ French policy regarding CPE in hospitals has been demonstrated to be an efficient tool until now because most of the reported CPE episodes remained sporadic. However, such infection control measures will be difficult to maintain if the amount of bla\textsubscript{OXA-48} continues to increase.

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Transparency Declaration

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