Hospital outbreak of *Pseudomonas aeruginosa* producing extended-spectrum oxacillinase OXA-19

*Pseudomonas aeruginosa* is one of the leading causes of bacteraemia and pneumonia in patients hospitalized in intensive care units (Bertrand et al., 2001). In addition to being intrinsically resistant to several antimicrobial agents, *P. aeruginosa* can acquire resistance to conventional anti-pseudomonal antibiotics including anti-pseudomonal penicillins, ceftazidime, carbapenems, aminoglycosides and ciprofloxacin (Carmeli et al., 1999). The mechanisms of resistance to β-lactams mostly involve stable upregulation of the intrinsic cephalosporinase AmpC, acquisition of transferable β-lactamases (with narrow or extended spectrum), outer-membrane impermeability (alteration or underproduction of porin OprD) or MexAB–OprF efflux pump overproduction (Livermore, 2002).

Clinical *P. aeruginosa* isolates producing class A extended-spectrum β-lactamases (ESBLs) such as TEM, SHV, PER, GES, VEB,BEL and CTX-M are frequently described (Weldhagen et al., 2003). Ambler class B enzymes (metallo-β-lactamases, such as IMP, VIM, SPM and GIM) also spread among clinical *P. aeruginosa* isolates, especially in Asia and Europe (Rossolini et al., 2007). *P. aeruginosa* strains producing extended-spectrum oxacillinas (from the Ambler class D) have sporadically been described but knowledge of their spread in the clinical setting remains poor (Poirel et al., 2010).

Within the framework of a hospital clinical research project, we found evidence of an outbreak of *P. aeruginosa* producing the extended-spectrum oxacillinase OXA-19. This report describes the microbiological investigation of this outbreak.

In the bacteriology laboratory of Nancy University Hospital (Lorraine, France), the susceptibility of all *P. aeruginosa* isolates to a panel of anti-pseudomonal agents was systematically determined by the disc diffusion method on Mueller–Hinton agar (bioMérieux) according to the guidelines of the Antibiotic Committee of the French Microbiology Society. All ceftazidime-resistant (*Ca*R) (MIC >8 mg l⁻¹) *P. aeruginosa* strains, excluding cystic fibrosis isolates, were collected over 1 year (from October 2007 to September 2008). Overall, 92 *Ca*R *P. aeruginosa* isolates were obtained from 48 patients.

An ESBL phenotyping screening test was performed for all *Ca*R isolates by using the double disc susceptibility test (DDST), where discs of ceftazidime, aztreonam and cefepime (30 μg each) were positioned at a variable distance from a disc containing amoxicillin (20 μg) and clavulanate (10 μg) (Jiang et al., 2006). A slight synergy was observed for 48 isolates between ceftazidime- and clavulanate-containing discs only when the distance between discs (centre to centre) was 12 mm (data not shown).

For all of these isolates, isolectric focusing (Hocquet et al., 2003) revealed the presence of the AmpC cephalosporinase (pI 8.1), as well as a secondary β-lactamase exhibiting a pI of 7.6. The identification of the secondary β-lactamase was carried out by PCR on whole-cell DNA using primers specific for the class D β-lactamases (OXA-10, OXA-2 and OXA-1 derivatives) because of the basic pI of this enzyme and the poor synergy provided by clavulanate toward ceftazidime (Bert et al., 2002). Only PCR using primers targeting the *bla*OXA-10 group was positive. As *bla*OXA genes are most frequently found in class 1 integrons (Fluit & Schmitz, 2004), we also performed PCR experiments using consensus primers 5’-CS and 3’-CS specific for class 1 integrons (Levesque et al., 1995). The PCR amplicons (1673 bp) were sequenced on both strands, resulting in the identification of a class 1 integrin (GenBank accession no. FJ906752) containing two resistance gene cassettes, namely *aacA4*, which determines an aminoglycoside 6’-N-acetyltransferase conferring high resistance to gentamicin and tobramycin, and *bla*OXA-19, which encodes the extended-spectrum OXA-19 (Mugnier et al., 1998).

PFGE typing was performed on all *Ca*R isolates as previously described (Bertrand et al., 2000). We used the GelCompar software (Applied Maths) to establish a DNA similarity matrix. A dendrogram was constructed using the unweighted pair-group method with arithmetic averages clustering method and the Dice coefficient. We ensured that the gels were comparable by including *Staphylococcus aureus* NCTC 8325 as a reference. PFGE results were interpreted according to international recommendations (Tenover et al., 1995). PFGE analysis showed that the 92 isolates clustered in 10 different PFGE patterns: two large clusters comprising 23 and 14 isolates, respectively; three smaller clusters comprising three, two and three isolates, respectively; and four unique patterns from four isolates (Fig. 1). A single PFGE pattern was detected in 42 patients, and at least two different PFGE patterns were identified in six patients. The 48 isolates producing OXA-19 recovered from 27 patients were clustered into three PFGE patterns: no. 2 (three patients), no. 6 (one patient) and no. 8 (23 patients). One intensive care unit was the epicentre of the main OXA-19-producing clone (no. 8), which further spread to other units, likely as a consequence of patient transfers.

Moreover, considering the epidemic curve, it is likely that the *bla*OXA-19 gene was transferred from PFGE pattern 8 to PFGE patterns 2 and 6 (Fig. 2). Among the 27 non-replicate OXA-19-producing isolates (one per patient), 22 were resistant to all anti-pseudomonal antibiotics tested (ticarcillin, piperacillin–tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, tobramycin, amikacin and ciprofloxacin) with the exception of colistin.

The GenBank/EMBL/DDBJ accession number for the sequence of the class 1 integron is FJ906752.
To our knowledge, this is the first report describing an outbreak involving *P. aeruginosa* isolates producing a clavulanate-resistant extended-spectrum oxacillinase. During the same period, we also evidenced an outbreak of *P. aeruginosa* producing OXA-28 in another French hospital (Hocquet et al., 2008). The emergence of *P. aeruginosa* producing such β-lactamases may be related to a recent spread of extended-spectrum oxacillinases among *P. aeruginosa* clinical isolates. This phenomenon may be underestimated because of the difficulty in detecting such strains in clinical laboratories. Indeed, most of these oxacillinases (with the exception of OXA-18) are poorly inhibited by clavulanate. This undermines DDST
In conclusion, the emergence and spread of extended-spectrum oxacillinases among P. aeruginosa is detected only when cefazidime- and clavulanate-containing discs are in close approximation to each other.

In conclusion, the emergence and spread of extended-spectrum oxacillinases among P. aeruginosa clinical strains is a matter of concern. These multidrug-resistant bacteria caused infections that are difficult to treat, as colistin often remains the unique therapeutic option. Ongoing surveillance of these micro-organisms, with the use of a specific screening test, is warranted as well as the implementation of infection control measures to prevent their cross-transmission between patients.

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