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 oxacillinases (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 Antibigram Committee of the French Microbiology Society. All ceftazidime-resistant (Caz^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 Caz^R *P. aeruginosa* isolates were obtained from 48 patients.

An ESBL phenotypic screening test was performed for all Caz^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, isoelectric 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 integron (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 Caz^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.

Fig. 1. Pulsed-field electrophoresis banding patterns after *DraI* digestion of non-duplicate *P. aeruginosa* isolates.

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

Fig. 2. Spatio-temporal distribution of the PFGE pattern of OXA-19-producing isolates. Numbers indicate patient number according to the date of first isolation; circles represent PFGE pattern 8; squares represent PFGE pattern 2; and the triangle represents PFGE pattern 6. Arrows indicate the transfer of the patient between units.

screening tests using conventional distance (≥ 20 mm) between third-generation cephalosporins and clavulanate. Extended-spectrum oxacillinase activity is also inhibited by cloxacillin, a compound used in phenotypic screening tests to unmask ESBLs in an AmpC overproduction background (Aubert *et al.*, 2004). OXA-19-producing *P. aeruginosa* is detected only when ceftazidime- 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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