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Nosocomial outbreak of carbapenem-resistant *Enterobacter cloacae* highlighting the interspecies transferability of the *bla*_{OXA-48} gene in the gut flora

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Sir,

The emergence and dissemination of carbapenemases (KPC, VIM, IMP, NDM or OXA-48) among Enterobacteriaceae is a serious concern worldwide as it raises the problem of the lack of therapeutic options linked to frequent co-resistance.¹ In November 2010, French guidelines were published to control the spread of carbapenemase-producing Enterobacteriaceae from patients repatriated and travellers hospitalized in French hospitals.¹ However, we report the *in vivo* interspecies transferability of the OXA-48 carbapenemase by the investigation and management of a nosocomial outbreak in France.

In April 2011, an elderly patient (Patient A) was transferred from Agadir (Morocco) to the internal medicine unit at Nantes University Hospital, France, for the treatment of a hip prosthetic joint infection. Upon admission, contact precautions were immediately adopted. A rectal swab inoculated on CHROMagar™ KPC medium (CHROMagar, Paris, France) revealed the gastrointestinal carriage of *Enterobacter cloacae* and *Escherichia coli*, both resistant to ertapenem and positive for *bla*_{OXA-48} by PCR.² Therefore, a weekly colonization surveillance was performed on all patients hospitalized in the unit, and led to the discovery of OXA-48-producing *E. cloacae* in 3/54 patients (B, C and D) without recent history of travel. Furthermore, rectal swabs performed for Patients A and B found two OXA-48-producing *Klebsiella pneumoniae* isolates (Figure 1). The time between admission to the unit and the first positive culture varied between 3 and 16 days for the three secondary patients. However, Patient D, with a first negative screening, was transferred to the intensive care unit, and was detected as a carrier 3 days after re-admission to the internal medicine unit. We cannot exclude the possibility that this patient was colonized during the first stay in the internal medicine unit. OXA-48-producing *E. cloacae* isolates were detected intermittently in this patient (Figure 1). None of

the four carriers developed infection. Active surveillance was continued until the last colonized patient was discharged.

All isolates were resistant to ertapenem (range of MICs, 2 to ≥ 32 mg/L) and exhibited intermediate susceptibility or susceptibility to imipenem (range of MICs, 0.38–6 mg/L) and meropenem (range of MICs, 0.25–0.5 mg/L) according to the EUCAST guidelines 2011.³ Molecular testing⁴ showed that all *E. cloacae* isolates harboured the *bla*_{CTX-M-15} ESBL gene, while both *E. coli* and *K. pneumoniae* isolates were susceptible to third-generation cephalosporins and did not present any of the additional β -lactamases searched for (*bla*_{TEM}, *bla*_{SHV} apart from *bla*_{SHV-1}, and *bla*_{CTX-M}).

The *E. coli* and *K. pneumoniae* isolates did not yield subcultures when plated on a CHROMagar™ ESBL medium (CHROMagar, Paris, France). Although other authors⁵ reported poor growth of *E. coli* strains, and underlined difficulties in differentiating colonies of *E. cloacae* and *K. pneumoniae*, in our experience the CHROMagar™ KPC medium was useful. The OXA-48 producing *E. coli* isolate from Patient A yielded a few small pink colonies, whereas the OXA-48-producing *K. pneumoniae* isolate showed better growth, with large navy blue colonies easily distinguishable from the steel blue colonies of the OXA-48-producing *E. cloacae* isolate.

All *E. cloacae* isolates showed indistinguishable PFGE patterns.⁴ According to PFGE and multilocus sequence typing (MLST; <http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>) analyses, *K. pneumoniae* isolates were not clonally related [one new sequence type (ST) and one ST152]. The *E. coli* isolate belonged to ST38 (MLST, <http://mlst.ucc.ie/mlst/dbs/Ecoli>).

The *bla*_{OXA-48} gene was transferred by conjugation⁴ to a rifampicin-resistant *E. coli* J53-2 from the *E. cloacae*, *K. pneumoniae* and *E. coli* isolates, while transfer of the *bla*_{CTX-M-15} gene from the *E. cloacae* isolates failed. Extraction of plasmids⁴ revealed that *E. cloacae* isolates carried two plasmids (60 and 165 kb), whereas *E. coli*, both *K. pneumoniae* isolates and all *bla*_{OXA-48}-positive transconjugants carried a single plasmid that co-migrated with the 60 kb plasmid of *E. cloacae* isolates. The *bla*_{OXA-48} gene was part of the plasmid-borne Tn1999.2 transposon, since an insertion sequence IS1999 interrupted by an IS1R was detected by PCR mapping upstream of the *bla*_{OXA-48} gene.²

This is the first report of a patient colonized with three enterobacterial isolates (*E. cloacae*, *E. coli* and *K. pneumoniae*) harbouring the *bla*_{OXA-48} gene. The emergence of this gene has been linked to the spread of a peculiar Tn1999-type transposon, but also to the dissemination of specific clones. Poirel *et al.*⁶ indicated that the same strain of OXA-48-producing *E. coli*, belonging to ST38, had been imported from Egypt and Turkey into France. In our study, Patient A carried an ST38-type *E. coli*, but the strain did not display an ESBL phenotype, as previously described.⁶ The discovery of the OXA-48 carbapenemase in several enterobacteria of the index case's gastrointestinal flora rather suggested the possibility of an *in vivo* transfer of the OXA-48-encoding plasmid. This was confirmed by the isolation of another OXA-48-producing *K. pneumoniae* isolate in Patient B. In the gut, selection of resistant strains has been associated with a biological fitness cost and often reflects the impact of antimicrobial selection pressure. Previous exposure to fluoroquinolones or antipseudomonal penicillins has been described as a risk factor for acquisition of

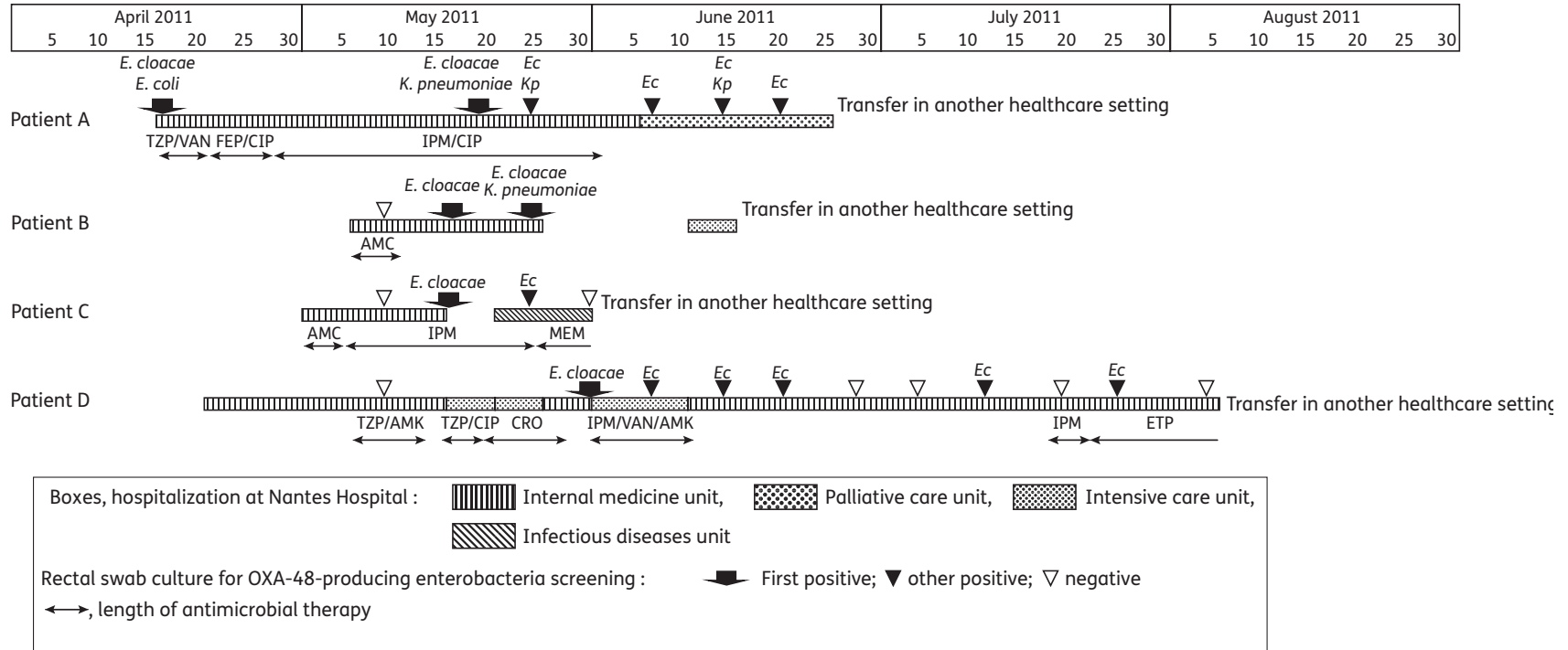


Figure 1. Synoptic picture providing a summary overview of the isolation of OXA-48-producing isolates at Nantes Hospital. *Ec*, *E. cloacae*; *Kp*, *K. pneumoniae*; AMK, amikacin; AMC, amoxicillin/clavulanate; CIP, ciprofloxacin; CRO, ceftriaxone; ETP, ertapenem; FEP, cefepime; IPM, imipenem; MEM, meropenem; TZP, piperacillin/tazobactam; VAN, vancomycin.

carbapenem-resistant *K. pneumoniae*.⁷ Here, during the hospital stay, Patient A received successively piperacillin/tazobactam with vancomycin, cefepime with ciprofloxacin, and imipenem with ciprofloxacin, but Patient B only received amoxicillin/clavulanate (Figure 1). It is well established that *K. pneumoniae* isolates are an important reservoir of β -lactamases. Thus, the dissemination of the KPC carbapenemase has been linked to the dispersion of a clonal ST258-type *K. pneumoniae* strain. Nevertheless, it appears that the rapid emergence of *bla*_{OXA-48} in *K. pneumoniae* would be explained by the horizontal transmission of an OXA-48-encoding plasmid within strains belonging to different STs.⁸

Our experience raises concern about a possible rapid rise in carbapenem resistance in enteric bacteria through the spread of *bla*_{OXA-48}-positive plasmids and/or strains. Outbreaks involving different OXA-48-producing species have already been described.¹ Early detection by sensitive screening methods is needed, with targeted surveillance and consideration given to infection control measures, to prevent this spread.

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Transparency declarations

None to declare.

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Emergence of metallo- β -lactamases GIM-1 and VIM in multidrug-resistant *Pseudomonas aeruginosa* in North Rhine–Westphalia, Germany

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Sir,

Little is known about the emergence and dissemination of metallo- β -lactamase (MBL)-producing *Pseudomonas aeruginosa* in Germany. In the last decade only a few sporadic cases have been reported. In 2004 the detection of the unique MBL-type GIM-1 in five identical *P. aeruginosa* isolates from a German hospital was described.¹ Several reports on *P. aeruginosa* possessing the MBLs VIM-1, VIM-2 and VIM-16 were published in 2005 and 2008.^{2,3} Here we report on the molecular characterization of GIM-1 and VIM MBLs in clinical isolates of *P. aeruginosa*.

Between March 2009 and August 2010 we isolated 3000 clinical strains of *P. aeruginosa* from 37 hospitals in the federal state of North Rhine–Westphalia, Germany. Included in the present study were all multidrug-resistant *P. aeruginosa* isolates showing resistance to penicillins (piperacillin/tazobactam), cephalosporins (ceftazidime) and fluoroquinolones (ciprofloxacin) and being additionally resistant to carbapenems (imipenem and meropenem). Among the 3000 isolates, 18 (0.6%) consecutive non-duplicate strains were found fulfilling the criteria. Furthermore, these isolates were resistant to gentamicin but remained susceptible to polymyxin B. MBL production was confirmed for 8 of the 18 *P. aeruginosa* isolates by Etest MBL (bioMérieux, Nürtingen, Germany).

Molecular screening was performed for the common MBL genes *bla*_{IMP}, *bla*_{VIM} and *bla*_{NDM-1} and for the locally occurring genes *bla*_{GIM}, *bla*_{SPM} and *bla*_{SIM}.⁴ Since most MBL genes are integrated at specific sites in class 1 integrons (int1), the gene sequence of the variable region was determined by PCR mapping.

PCR and sequencing of relevant MBL genes revealed the presence of *bla*_{GIM-1} in five isolates, *bla*_{VIM-2} in two isolates and