Nosocomial outbreak of carbapenem-resistant Enterobacter cloacae highlighting the interspecies transferability of the bla\textsubscript{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.\textsuperscript{1} In November 2010, French guidelines were published to control the spread of carbapenemase-producing Enterobacteriaceae from patients repatriated and travelers hospitalized in foreign hospitals.\textsuperscript{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\textsuperscript{TM} KPC medium (CHROMagar, Paris, France) revealed the gastrointestinal carriage of Enterobacter cloacae and Escherichia coli, both resistant to ertapenem and positive for bla\textsubscript{OXA-48} by PCR.\textsuperscript{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 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.\textsuperscript{3} Molecular testing\textsuperscript{4} showed that all E. cloacae isolates harboured the \(bla\textsubscript{CTX-M-15}\) ESBL gene, while both E. coli and K. pneumoniae isolates were susceptible to third-generation cephalosporins and did not present any of the additional \(\beta\)-lactamases searched for (\(bla\textsubscript{TEM}, \text{bla}\textsubscript{SHV}\) apart from \text{bla}\textsubscript{SHV-1}, and \text{bla}_{\text{CTX-M}}). The E. coli and K. pneumoniae isolates did not yield subcultures when plated on a CHROMagar\textsuperscript{TM} ESBL medium (CHROMagar, Paris, France). Although other authors\textsuperscript{5} reported poor growth of E. coli strains, and underlined difficulties in differentiating colonies of E. cloacae and K. pneumoniae, in our experience the CHROMagar\textsuperscript{TM} 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 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.\textsuperscript{6} According to PFGE and multilocus sequence typing (MLST, http://www.pasteur.fr/recherche/genopole/PO8/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 \(bla\textsubscript{OXA-48}\) gene was transferred by conjugation\textsuperscript{4} to a rifampicin-resistant E. coli J53-2 from the E. cloacae, K. pneumoniae and E. coli isolates, while transfer of the \(bla\textsubscript{CTX-M-15}\) gene from the E. cloacae isolates failed. Extraction of plasmids\textsuperscript{7} revealed that E. cloacae isolates carried two plasmids (60 and 165 kb), whereas E. coli, both K. pneumoniae isolates and all \(bla\textsubscript{OXA-48}\)-positive transconjugants carried a single plasmid that co-migrated with the 60 kb plasmid of E. cloacae isolates. The \(bla\textsubscript{OXA-48}\) gene was part of the plasmid-borne Tn19992 transposon, since an insertion sequence IS1999 disrupted by an IS1IR was detected by PCR mapping upstream of the \(bla\textsubscript{OXA-48}\) gene.\textsuperscript{8}

This is the first report of a patient colonized with three enterobacterial isolates (E. cloacae, E. coli and K. pneumoniae) harbouring the \(bla\textsubscript{OXA-48}\) gene. The emergence of this gene has been linked to the spread of a peculiar Tn19999-type transposon, but also to the dissemination of specific clones. Poirel et al.,\textsuperscript{9} 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.\textsuperscript{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
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**Patient A**
- E. coli
- K. pneumoniae
- E. cloacae
- Transfer in another healthcare setting

**Patient B**
- K. pneumoniae
- E. cloacae
- Transfer in another healthcare setting

**Patient C**
- K. pneumoniae
- E. cloacae
- Transfer in another healthcare setting

**Patient D**
- K. pneumoniae
- E. cloacae
- Transfer in another healthcare setting

Boxes, hospitalization at Nantes Hospital:
- Internal medicine unit
- Palliative care unit
- Intensive care unit
- Infectious diseases unit

Rectal swab culture for OXA-48-producing enterobacteria screening:
- First positive
- Other positive
- Negative

Length of antimicrobial therapy

**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, ceftiraxone; ETP, ertapenem; FEP, ceftepime; IPM, imipenem; MEM, meropenem; TZP, piperacillin/tazobactam; VAN, vancomycin.
carbapenem-resistant K. pneumoniae. 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 blaOXA-48 in K. pneumoniae would be explained by the horizontal transmission of an OXA-48-encoding plasmid within strains belonging to different STs.

Our experience raises concern about a possible rapid rise in carbapenem resistance in enteric bacteria through the spread of blaOXA-48-positive plasmids and/or strains. Outbreaks involving different OXA-48-producing species have already been described. 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.

References