Cross-transmission of *Escherichia coli* producing OXA-181 in hospitalized patients and failure of carbapenemase detection by commercial and in-house PCR assays

The dissemination of carbapenemase-producing *Enterobacteriaceae* (CPE) is a global public health challenge. Early and accurate detection of CPE, as well as prompt infection prevention and control measures, are required to minimize the risk of dissemination in healthcare settings (CDC, 2009).

In October 2014, *Escherichia coli* isolated from a rectal swab was submitted by Hospital 1 to our CPE reference service in Galway for confirmation of carbapenemase production. The isolate was identified as a possible CPE through screening using in-house ertapenem–cloxacillin–zinc-containing MacConkey agar, which is a modification of the SUPERCARBA medium (Girlich et al., 2013). The isolate also tested positive in the referring hospital laboratory for an OXA-48-like carbapenemase using a commercial assay (Check-Direct CPE Real-Time PCR; Check-Points). Tests in our reference service confirmed that the isolate was resistant to ertapenem (MIC 4 mg l\(^{-1}\)) and demonstrated reduced susceptibility to meropenem (MIC 0.5 mg l\(^{-1}\)). Phenotypic synergy tests (Rosco Diagnostica) also suggested the presence of an OXA-48-like carbapenemase. However, both the in-house and commercial (GeneXpert CarbaR Rev.2; Cepheid) PCR assays that were used to confirm these findings yielded negative results for genes encoding OXA-48-like, VIM, IMP-1-like, NDM and KPC carbapenemases. In-house assays for OXA-23-like, OXA-24-like, OXA-51-like, OXA-58-like, IMI and SME carbapenemases were also negative.

The discrepancy in the two laboratories’ findings prompted an evaluation of test methods and a review of our database for previous isolates with similar patterns of antibiogram, synergy test and PCR results (i.e. isolates with reduced susceptibility to carbapenems as well as with OXA-48-like profile on synergy testing, but with negative results for carbapenemase genes based on our original PCR assays). Two additional *E. coli* isolates were identified, both from adults in Hospital 2. The first was isolated from a rectal swab of a haematology patient (A) in June 2014, and the second came from a rectal swab of an oncology patient (B) in October 2014. In view of the multidrug-resistant phenotypes in both cases, infection control measures were recommended initially that included the use of isolation facilities and implementation of contact precautions. Patient A also had several admissions from June to November 2014, and had been hospitalized in Ghana in April 2014.

Re-evaluation of our in-house carbapenemase assays indicated that our existing OXA-48-like PCR primers were not optimized to detect newer variants, particularly OXA-181 and OXA-232. Following the design and validation of new primers (forward primer, 5’-TTCCGGTACCAGCAAAATCGG-3’; reverse primer, 5’-GATGGTCATATCCCATATTCAATGGCGA-3’; probe, 5’-FAM-CTGACTACGGCTCAGAAACGTGCAGCTTGTTG-BHQ1-3’) targeting these newer variants, all three isolates yielded positive results with this new PCR assay for an OXA-48-like carbapenemase, which was subsequently confirmed as OXA-181 via PCR and sequencing of amplicons by the Antimicrobial Resistance and Healthcare-Associated Infections Reference Unit, Public Health England. The isolates from patients A and B also tested positive for an OXA-48–like carbapenemase using the Check-Direct CPE PCR assay. Both isolates had indistinguishable banding patterns after PFGE, and epidemiological investigations revealed that the two cases were present in the same unit of Hospital 2 in June and October 2014. The conclusion of likely cross-transmission of OXA-181-producing *E. coli* between two patients in the unit was made, with patient A being the likely index case. There was no epidemiological link between the index case in Hospital 1 and the two cases in Hospital 2; the isolate from Hospital 1 also had a PFGE pattern that was clearly distinguishable from that of the two isolates from Hospital 2.

Enhanced training and education of the unit’s healthcare staff on the importance of adherence to recommended infection control measures for multidrug-resistant organisms including CPE were instituted. Rectal screening using selective agar (Brillance CRE agar; Oxoid) of other patients epidemiologically linked to the two cases was performed. Brilliance CRE agar was found to be of comparable sensitivity to SUPERCARBA medium, and possibly more sensitive than CHROMagar KPC medium, in the detection of isolates producing OXA-181, although the number of such isolates investigated in the study was small (Girlich et al., 2013). The screening did not reveal further CPE acquisition in the unit. Following the incident, the reference service also introduced, as a quality improvement measure, quarterly surveillance of published literature and gene databases to identify new carbapenemases of potential clinical significance or new variants of established carbapenemase families, thereby monitoring regularly the validity of the detection assays in use.

OXA-181 and OXA-232 are newer variants of OXA-48-like carbapenemases reported in 2011 and 2013, respectively (Potron et al., 2011, 2013). The OXA-181 enzyme is reported mainly in *Klebsiella pneumoniae* and predominantly in Asian and Middle Eastern countries (Balm et al., 2013; Potron et al., 2011; Ruppe et al., 2014; Williamson et al., 2011), although OXA-181-producing *E. coli* originating from Nigeria and the

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\[\text{OXA-232} \]

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Comoro Islands have also been reported (Decousser et al., 2015; Walkty et al., 2015). Amongst isolates of E. coli and K. pneumoniae confirmed to have an OXA-48-like carbapenemase by AMRHAI in 2014, 40 % produced OXA-181 or one of its close variants (OXA-232 and OXA-484; K. L. Hopkins, D. Meunier & N. Woodford, unpublished data). Of note, patient A from our hospital had recently been admitted to a hospital in Ghana prior to isolation of his CPE. It is likely that our index case represented a case of intercontinental transfer of OXA-181-producing E. coli from Ghana to Ireland. Elsewhere, the international transfer of OXA-181-producing CPE has been documented from the Indian subcontinent to France and New Zealand (Ruppé et al., 2014; Williamson et al., 2011).

This report highlights the inherent potential problem with in-house and commercial molecular assays in the failure to detect newer variants of existing carbapenemase families, unless they share sufficient sequence similarity with known variants. Recent reports have highlighted the potential for false-negative results for OXA-181 in Enterobacteriaceae with early versions of the GeneXpert Carba-R and eazyplex SuperBug complete kits, although both assays have since been updated to improve detection of this and related variants (Decousser et al., 2015; Findlay et al., 2015). This report reiterates the consequences of non-detection. It should be noted that failure to detect OXA-181 E. coli carriage in the index case was not the sole cause of subsequent cross-transmission, as infection prevention and control measures may have been suboptimal owing to a delay in obtaining appropriate isolation facilities. Nonetheless, detection of CPE would likely have heightened the awareness of healthcare staff to the urgency for and stringency of recommended control measures. The incident further reiterates the need for in-house assay users and commercial manufacturers to conduct regular relevant audits on the coverage of tests to ensure and maintain maximum theoretical sensitivity for CPE detection in a rapidly evolving area of antimicrobial resistance.

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Abbreviation: CPE, carbapenemase-producing Enterobacteriaceae.

References


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