Early (2008–2010) hospital outbreak of Klebsiella pneumoniae producing OXA-48 carbapenemase in the UK


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Abstract

OXA-48 β-lactamase is one of the several emerging carbapenemases. Pre-2007 reports were almost exclusively from Turkey, but subsequently its distribution has expanded. We report an early and prolonged outbreak in the UK of Klebsiella pneumoniae producing OXA-48 carbapenemase affecting a predominantly renal cohort in a West London hospital. Carbapenemase production was detected by the modified Hodge test, with confirmation by PCR for blaOXA-48. Isolates were typed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Risk factors for acquisition were determined. Between January 2008 and April 2010, 20 K. pneumoniae isolates with reduced susceptibility to carbapenems were identified from 13 patients, comprising 12 renal cases and 1 oncology patient; 8 were outpatients and 5 were inpatients; 7 were deemed to be colonised and 6 infected, including 2 with bacteraemia, 1 of whom died. Hodge tests were positive for all isolates and all had blaOXA-48. PFGE showed strain similarity in isolates from nine patients, whereas four patients’ isolates were distinct, representing three further PFGE profiles and suggesting horizontal spread of blaOXA-48. Most patients had received antibiotics in the preceding 3 months and all had healthcare contact, but none had recent travel to areas with endemic OXA-48 Enterobacteriaceae. The renal cohort was screened and a prevalence rate of 0.17% was found. Interventions that collectively brought the outbreak under control included strict infection control precautions, screening, improved laboratory detection protocols and antibiotic stewardship rounds.

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1. Introduction

The perception of antibiotics as a limitless resource has been challenged over the last decade by the accumulation of Enterobacteriaceae with quinolone, aminoglycoside and cephalosporin resistance. Carbapenems have been increasingly used in hospitals [1], either as regimen escalation or as first-line empirical agents in settings where multidrug resistance is endemic. Carbapenems remain active against most extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, although their activity is compromised if these enzymes, or AmpC type β-lactamases, are combined with porin loss [2]. More worryingly, Enterobacteriaceae with carbapenem-hydrolysing β-lactamases are an emerging problem [3]. These ‘carbapenemases’ principally include metallo-enzymes of the NDM, IMP and VIM families as well as the KPC and OXA-48-like non-metallo-enzymes [4].

OXA-48 carbapenemase (Ambler class D) was first reported in Enterobacteriaceae in Turkey in 2001 and originated as a genetic escape from Shewanella spp. where it is chromosomal and inherent [5,6]. Subsequently, spread has been reported not only across Turkey [7] but also in the Middle East, North Africa and Europe [8]. In 2013, OXA-48 also began to be reported in North America [9,10], whilst the closely related OXA-163 and OXA-181 carbapenemases have been reported in South America and India, respectively [8]. Enterobacteriaceae with OXA-48 enzyme have additionally been
found in the final effluent of a wastewater treatment plant [11] and recently from pet dogs in Germany [12].

Here we report the first UK outbreak of Klebsiella pneumoniae with OXA-48 carbenpenemase, which was prolonged, affected 13 patients in a West London hospital, and pre-dated other non-Turkish outbreaks. Preliminary outbreak findings were presented in part at the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) in 2009 [13].

2. Materials and methods

2.1. Patients and bacterial isolates

Twenty carbenpenem-intermediate or -resistant K. pneumoniae isolates were identified from 13 patients attending a West London teaching hospital between January 2008 and April 2010 (Table 1). Isolates from 11 patients were from clinical samples; those from the other 2 patients were obtained during an extensive screening programme undertaken in the renal cohort (see below).

Risk factors potentially relevant to the acquisition of carbenpenem-non-susceptible K. pneumoniae were sought with respect to all 13 patients. Based upon literature reports, factors considered were: recent travel history outside the UK (within the last 6 months); exposure to antibiotics (last 3 months); and healthcare facility contact (last 3 months).

2.2. Screening for infected or colonised patients

Subsequent to identification of the first case, ertapenem was included in the primary antibiotic panel for all Gram-negative organisms cultured from patients attending all units within the hospital. As part of the outbreak management, an extensive prospective screening programme for carbenpenemase-producing Enterobacteriaceae was undertaken over 14 weeks from March 2009 to June 2009 in the renal cohort, reviewing two haemodialysis units (23 beds in the West London teaching hospital and 26 beds in a satellite unit), all patients at two renal transplant and chronic kidney disease clinics, and the 28-bed inpatient ward from which the inpatient cases were identified. Patients from areas were identified, with rectal/perianal swabs being the screening sample of choice, and samples were plated on to MacConkey agar as above. Environmental screening was also undertaken from all sinks and toilets in areas from which cases were identified, including the renal transplant and chronic kidney disease clinics, the West London hospital dialysis unit and the inpatient ward. Screening for carbenpenemase-producing Enterobacteriaceae used methods that match those now advocated by the UK Advisory Committee on Antimicrobial Resistance and Healthcare-Associated Infection (ARHAI) [14].

2.3. Phenotypic and genotypic investigation of resistance

Isolates were identified by API 20E (bioMérieux, Marcy l’Étoile, France) and were initially recognised on the basis of reduced susceptibility to one or more of ertapenem, imipenem and meropenem according to the British Society for Antimicrobial Chemotherapy (BSAC) disc methods of the time [15]. Minimum inhibitory concentrations (MICs) of selected antimicrobials were determined by BSAC agar incorporation or by Etest (bioMérieux). Phenotypic evidence of carbenpenemase production was sought by modified Hodge tests, and metallo-carbenpenemases were sought with imipenem–ethylene diamine tetra-acetic acid (EDTA) synergy tests.
[14]. Genes encoding KPC and OXA-48 enzymes were detected by PCR, with sequencing of representative amplicons [5]. Selected isolates were also screened for insertion sequence IS1999 as well as the genes blaTEM-1, blaSHV, blaOXA-1, blaOXA-9 and blaCTX-M [5,16].

2.4. Strain typing and plasmid analysis

Isolates were compared by pulsed-field gel electrophoresis (PFGE) of XbaI-digested genomic DNA as previously described [17]. Multilocus sequence typing (MLST) was performed on representatives of two outbreak strains using standard primers and protocols [18]. Alleles were defined with reference to the Pasteur scheme [19], and a novel allelic profile was assigned a sequence type (ST) designation by the curators of this scheme.

Plasmid DNA was extracted from a representative isolate by alkaline lysis and was electrotransformed into Escherichia coli strain DH5α, with selection for ampicillin resistance. Transformants were screened by PCR to confirm acquisition of blaOXA-48, and MIC determinations were performed to identify co-transferred resistances [20].

3. Results

3.1. Outbreak and screening programme

Identification of the first case in January 2008 followed ertapenem susceptibility testing performed as a prelude to outpatient intravenous antimicrobial therapy. This detected a K. pneumoniae isolate with MICs as follows: ertapenem, 4 mg/L; meropenem, 1 mg/L; and imipenem, 4 mg/L. Including this first case, a total of 13 patients were identified over the next 27 months, comprising 12 renal patients and 1 oncology outpatient. Among the renal patients, seven were outpatients (two haemodialysis and five chronic kidney disease or renal transplant clinics) and five were inpatients. Two patients were identified as carriers through screening; the other eleven were identified from clinical samples (although only six of these were clinically deemed to have an infection with the OXA-48-producing K. pneumoniae: three with urinary tract infections, one with a peripancreatic infection and two with bacteraemia).

The prospective screening programme targeted the renal cohort linked to the majority of clinical cases. In total, samples were received from 1146 patients (958 renal transplant and chronic kidney disease patients, 102 patients from the West London dialysis unit and 86 patients from the satellite dialysis unit); as noted already, only 2 were positive, equating to a prevalence of 0.17%. The acceptance of urine as a screening sample was pragmatic rather than optimal, since gut carriage is more likely; consequently, this prevalence rate is likely to be an underestimate. One of the patients identified through screening (patient K; Table 1) was a household contact of one of the clinical cases (patient G; Table 1); both were patients of the renal unit. Screening also identified one further isolate with MICs as follows: ertapenem, 4 mg/L; meropenem (MICs of 0.5–32 mg/L), imipenem (MICs of 2–64 mg/L) and ceftazidime (MICs of 0.5–32 mg/L). Both strain 1, strain 2 and the unique isolates were related to outbreak strains A and B from Istanbul as described by Carrère et al. [7].

In addition to blaOXA-48, outbreak strain 1 was positive by PCR for blaTEM, blaSHV and IS1999, which is commonly associated with blaOXA-48 [20], but was negative for blaCTX-M and blaOXA-9 genes (blaOXA-9 was sought because it was present in some early Turkish isolates with OXA-48 enzyme). Strain 2 had blaOXA-48, IS1999, blaOXA-1 and a group 1 blaCTX-M gene. Strain ‘Unique 1’ was positive for blaOXA-48, IS1999, blaTEM, blaSHV, blaOXA-1, and a group 1 blaCTX-M gene, but was negative for blaOXA-9. Strain ‘Unique 2’ was not examined.

3.3. Susceptibility in relation to strain types and resistance genes

Ertapenem MICs for members of strain 1 ranged from 2 mg/L to >16 mg/L, indicating consistent resistance, whereas those of meropenem (MICs of 0.5–32 mg/L), imipenem (MICs of 2–64 mg/L) and cefotaxime (MICs of 0.5–32 mg/L) were resistant to BSAC/European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) breakpoints, with most isolates intermediate or resistant (Table 1). Of the nine strain 1 isolates, two were non-susceptible to tigecycline with MICs of 24 mg/L and all were resistant to piperacillin/tazobactam (MICs of >64 mg/L), but all were susceptible to amikacin (MICs of 1–2 mg/L) and, with a single exception, to gentamicin (MICs of 0.25–1 mg/L; exception, 8 mg/L) and ceftazidime (MICs of 0.25–1 mg/L, except 2 mg/L, which then counted as susceptible but now intermediate). Resistance to cefotaxime was variable but mostly low level, with MICs of 0.5–8 mg/L except for one isolate (32 mg/L). Both strain 2 isolates were highly resistant to oxyimino-cephalosporins, with MICs of 32 mg/L to >256 mg/L, consistent with production of a group 1 CTX-M ESBL, as also found by PCR; one was ‘susceptible’ to all three carbapenems (MICs of 0.25–1 mg/L), whereas the other was resistant (MICs all 8 mg/L). The two unique isolates were resistant to all three carbapenems (MICs of 0.25–1 mg/L), whereas the other was resistant (MICs all 8 mg/L). All 13 patients had contact with the renal service. Two other patients had isolates with unique PFGE profiles (Fig. 1). A representative of strain 1 was shown by MLST to belong to a novel sequence type, ST353 (allelic profile, gapA 3, infB 9, mdh 47, pgi 1, phoE 13, rpoB 1, tonB 16). Representatives of strain 1 were recovered over a period of 27 months and included the initial case. Strain 2 was found by MLST to correspond to ST15. None of the strain 1, strain 2 or the unique isolates were related to outbreak strains A and B from Istanbul as described by Carrère et al. [7].

3.4. Risk factors for acquisition of carbapenem-resistant K. pneumoniae

Epidemiological risk factors for acquisition of an organism with OXA-48 carbapenemase were investigated in all confirmed cases. Patients were interviewed and healthcare records were interrogated to identify prior healthcare contact, overseas travel (especially to Turkey) and antibiotic use. None of the 13 patients reported overseas travel in the preceding 6 months. Of the 13 patients, 10 had received antibiotics in the 3 months prior to isolation of OXA-48-positive K. pneumoniae, but only 5 (3 with strain 1) had been given a carbapenem. All 13 patients had contact with healthcare facilities in the 3 months before identification of their OXA-48-positive strains. Although predominant strain 1 was also isolated from one non-renal (oncology) patient, no epidemiological link could be found between this individual and the renal service.
inherent in detection of OXA-48-producing Enterobacteriaceae.

true index case escaped detection, in part due to the difficulties be identified, actually represented secondary spread and that the ground, with strong familial links to the Middle East as well as South accounted for 12 of the identified patients, is of diverse ethnic back-should be noted that the West London renal cohort, which UK-born donor, about whom we have no further information avail-

tive index case had not travelled in the year prior to identification, any of the present infected or colonised individuals. The presump-
machte, in 2007, was a of the two outbreak strains.

The particular challenge to detection of isolates with OXA-48 enzyme stems from Enterobacteriaceae producing this enzyme exhibiting variable susceptibility or resistance to carbapenems at EUCAST and CLSI breakpoints [4]. This almost certainly leads to under-reporting and may challenge detection by some automated systems [27]. The behaviour is likely to relate to differential porin expression and may also reflect enzyme quantity. Moreover, those isolates, like members of our strain 1, that do not co-express ESBLs retain in vitro susceptibility to some oxymino-cephalosporins, partic-

The first ‘UK’ isolate confirmed to produce OXA-48 carbapenemase, in 2007, was a K. pneumoniae from a patient repatriated from a Turkish healthcare provider (D.M. Livermore and N. Woodford, unpublished data); whereas no overseas link was established for any of the present infected or colonised individuals. The presumpt-

Microbiology records for renal patients during the 6 months prior to the ‘index’ case were filtered, seeking organisms with the ertapenem-resistant, piperacillin/tazobactam-resistant, ceftazidime-susceptible K. pneumoniae phenotype of strain 1. No examples were identified, but caveats are (i) ertapenem was not routinely tested and (ii) not all OXA-48-positive isolates were ceftazidime-susceptible.

Useful pointers to production of OXA-48 enzyme, irrespective of ESBL co-production, include reduced carbapenem susceptibility combined with high-level resistance to both piperacillin/tazobactam and temocillin [20,28]. Carbapenem resistance is not reduced by divalent cation chelators (e.g. EDTA) or boronic acid (which inhibit KPC enzymes). Confirmation of the presence of blaOXA-48 needs genotypic methods, based on PCR, sequencing or the use of arrays [29]. As with all resis-

4. Discussion

The emergence of carbapenemases in Enterobacteriaceae is of great concern as these provide a far more efficient and stable mechanism of resistance to carbapenems than combinations of an ESBL and impermeability. Moreover, acquired carbapenemases have the potential for horizontal spread, including into E. coli, which is the commonest cause of community-acquired sepsis.

Enterobacteriaceae with OXA-48 carbapenemases have been reported in Turkey since around 2001, sometimes causing sizeable outbreaks [8]. Since around 2007–2008 there has been extensive spread across the Middle East and North Africa, although surveillance is weak in these regions [8] and the enzyme may have been circulating earlier. Moreover, there have been several outbreaks in European hospitals [21–25]. The outbreak described here pre-dates all of these European outbreaks and represents the first sustained transmission within the UK of an organism with OXA-48 enzyme. Whilst the general mortality rate in infection due to organ-

 whilst retained susceptibility to ceftazidime and other cephalosporins may mean that there may be more treat-

ment options left against strains with OXA-48 enzyme than against, for example, those with NDM enzymes, this is by no means reliable and the non-strain 1 isolates found here were very broadly resistant. Useful pointers to production of OXA-48 enzyme, irrespective of ESBL co-production, include reduced carbapenem susceptibility combined with high-level resistance to both piperacillin/tazobactam and temocillin [20,28]. Carbapenem resistance is not reduced by divalent cation chelators (e.g. EDTA) or boronic acid (which inhibit KPC enzymes). Confirmation of the presence of blaOXA-48 needs genotypic methods, based on PCR, sequencing or the use of arrays [29]. As with all resis-

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ally given that (i) 8 patients in the renal cohort had the same strain and (ii) that strain had an unusual sequence type (ST353). However, temporospatial plotting of identified patients by Gantt chart failed to delineate any clear points of transmission (not shown).
The outbreak was considered to have ended in April 2010; nevertheless, a few sporadic isolates with OXA-48 enzyme have subsequently been isolated. In July and August 2011 three further OXA-48-positive *K. pneumoniae* were detected in the renal cohort, two identified as belonging to strain 1 and one to strain 2. In 2012, OXA-48 carbapenemases were found in an *E. coli* and in a *Citrobacter freundii*, again both from patients under the care of renal medicine. In 2013 we have seen one further OXA-48-positive *K. pneumoniae* from a patient in the renal cohort, found to be unique amongst isolates from this hospital but confirming by MLST to ST11; and an OXA-48-positive *Enterobacter* spp. in a non-renal patient repatriated from Romania.

The combined impact of prospective cohort screening, contact precautions and barrier nursing where appropriate, and antimicrobial stewardship, are all likely to have contributed to the evidenced decrease in incidence of OXA-48-positive organisms from 2010 onwards in this patient cohort. Hand hygiene, adoption of contact precautions, and indications for barrier isolation were reinforced through a strong rolling educational programme enacted through infection prevention and control nurses and the microbiology and infectious diseases medical staff. This focused on healthcare professionals in the renal unit, but also engaged the wider hospital staff and raised awareness in other London hospitals through broader educational events. Electronic tags were added to patient records as an alert mechanism, prompting appropriate transmission precautions when identified patients were re-admitted. Antimicrobial stewardship in the renal unit was reinforced through review of the antimicrobial guidelines for this patient cohort as well as initiation of regular multidisciplinary antimicrobial review rounds by microbiology and pharmacy staff, particularly focusing on addressing broad-spectrum antimicrobial use. Despite the negative environmental screening samples, a pragmatic approach was adopted to contain potential environmental persistence, and deep cleaning was undertaken of bays and side rooms utilised by inpatients. Infection control guidelines for carbapenem-resistant Enterobacteriaceae have now been produced by the ARHAI Advisory Committee in the UK [14] and by the US Centers for Disease Control and Prevention (CDC) [30].

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**Competing interests:** CPT has received travel sponsorship from Novartis. DML is partly self-employed and consults for numerous pharmaceutical and diagnostic companies, including Achaogen, Adeniun, Allegra, Astellas, AstraZeneca, Bayer, Basilea, bioMérieux, Cubist, Curetis, Discuva, Fedora, GSK, Merck, Meiji Seika, Pfizer, Roche, Shionogi, Tetraphase, VenatoRx and Wockhardt; he holds grants or contracts from AstraZeneca, Cubist, Meiji Seika, Merck and Wockhardt; has received lecture honoraria or travel reimbursements from AstraZeneca, Bruker, Curetis, GSK, J&J, Merck, Novartis, Pfizer and Tetraphase; and holds shares in Dechra, Eco Animal Health, GSK, Merck and Pfizer, collectively amounting to <10% of portfolio value. AHH has consulted for bioMérieux. All other authors declare no competing interests.

**Ethical approval:** Not required.

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