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Inter-hospital outbreak of *Klebsiella pneumoniae* producing KPC-2 carbapenemase in Ireland

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Objectives: To describe an outbreak of KPC-2-producing *Klebsiella pneumoniae* with inter-hospital spread and measures taken to control transmission.

Methods: Between January and March 2011, 13 *K. pneumoniae* isolates were collected from nine patients at hospital A and two patients at hospital B. Meropenem, imipenem and ertapenem MICs were determined by Etest, carbapenemase production was confirmed by the modified Hodge method and by a disc synergy test, and confirmed carbapenemase producers were tested for the presence of carbapenemase-encoding genes by PCR. PFGE, plasmid analysis, multilocus variable-number tandem-repeat analysis (MLVA) and multilocus sequence typing (MLST) analysis were performed on all or a subset of isolates.

Results: Meropenem, imipenem and ertapenem MICs were 4 to >32, 8–32 and >16 mg/L, respectively. PCR and sequencing confirmed the presence of bla_{KPC-2}. PFGE identified four distinguishable (≥88%) pulsed-field profiles (PFPs). Isolates distinguishable by PFGE had identical MLVA profiles, and MLST analysis indicated all isolates belonged to the ST258 clone. Stringent infection prevention and control measures were implemented. Over a period of almost 8 months no further carbapenemase-producing Enterobacteriaceae (CPE) were isolated. However, KPC-2-producing *K. pneumoniae* was detected in two further patients in hospital A in August (PFP indistinguishable from previous isolates) and October 2011 (PFP similar to but distinguishable from previous isolates).

Conclusions: Stringent infection prevention and control measures help contain CPE in the healthcare setting; however, in the case of hospital A, where CPE appears to be established in the population served, it may be virtually impossible to achieve eradication or avoid reintroduction into the hospital.

Keywords: antimicrobial resistance, *K. pneumoniae*, dissemination

Introduction

Clinically, the most prevalent of the carbapenemases are the *Klebsiella pneumoniae* carbapenemase (KPC) enzymes. The bla_{KPC} genes are predominantly plasmid encoded and are frequently associated with the Tn3-type transposon Tn*4401* and, in some cases, with particularly successful sequence types (STs) (e.g. *K. pneumoniae* ST258 and ST11), which has facilitated their rapid dissemination.[1,2] KPC-producing isolates are common in the USA, Colombia, Israel, the Zhejiang province of China and in Greece, where they have been associated with large nosocomial outbreaks.[3] Reports from other European countries are increasing. In 2009, Roche *et al.*[4] reported the first *K. pneumoniae* isolate with a KPC enzyme (KPC-2) in Ireland. In 2011, we reported KPC-2 in an *Escherichia coli* isolate belonging to the pandemic clone ST131.[5] In this paper we describe an outbreak of KPC-2-producing *K. pneumoniae* with inter-hospital spread and measures taken to control transmission.

Outbreak description

Hospital A is a 412 bed tertiary care centre serving a population of ~361 000. Affected patients were cared for in four areas of the hospital, the intensive care unit (ICU), the high-dependency unit (HDU) and two surgical wards (1 and 2). Hospital B is a 700 bed tertiary care centre serving ~300 000 patients per year in another city. Affected patients were cared for in the ICU of hospital B.

The index case of this outbreak was identified on 6 January 2011. Following identification of a second case in the same unit from a central venous catheter (CVC) tip on 24 January, an outbreak was declared (Table 1). Prior to this outbreak six cases of colonization or infection with carbapenemase-producing Enterobacteriaceae (CPE) were identified at hospital A between 2009 and 2010 (Table 2).[4,5]

Stringent infection and prevention control (IPC) measures were implemented, including contact precautions, access restrictions to affected areas, instruction in hand hygiene, audit of hand hygiene compliance and increased frequency of environmental cleaning, including hydrogen peroxide treatment of all affected wards of hospital A. Weekly screening of patients on affected units for carriage of carbapenem-resistant *K. pneumoniae* and enhanced antimicrobial stewardship were introduced. Screening was also performed on all discharges and transfers from affected wards in hospital A to other hospitals and long-term care facilities during the outbreak.

In January 2011 carbapenem-resistant *K. pneumoniae* was identified in a patient in the ICU of hospital B. This patient had recently self-discharged from the HDU of hospital A, but was subsequently admitted to the ICU of hospital B on 24 January 2011. IPC measures similar to those implemented in hospital A were invoked. One further case was identified on screening (Table 1).

In August 2011 a further isolate of carbapenem-resistant *K. pneumoniae* was detected in a patient admitted from the community with a record of admission to hospital A during December 2010, when both this patient and the index case were admitted to the same surgical ward. In October 2011 an additional isolate of carbapenem-resistant *K. pneumoniae* was detected in a patient admitted to a ward implicated in the outbreak.

Methods

In both hospitals A and B, rectal swabs were screened by the CDC protocol for detection of carbapenem-resistant *Klebsiella* spp. and *E. coli*.[6] Suspect CPE were forwarded to the Antimicrobial Resistance and Microbial Ecology (ARME) Group at the National University of Ireland Galway for further analyses.

Confirmation of carbapenemase production was performed by the modified Hodge method[7] and by a disc synergy test (Rosco Diagnostica, Taastrup, Denmark). The MICs of meropenem, ertapenem and imipenem were determined by Etest. Susceptibility to the following 15 antimicrobial agents was determined by disc diffusion methods: ampicillin (10 μg); cefpodoxime (10 μg); cefotaxime (30 μg); ceftazidime (30 μg); cefoxitin (30 μg); nalidixic acid (30 μg); ciprofloxacin (5 μg); gentamicin (10 μg); kanamycin (30 μg); streptomycin (10 μg); chloramphenicol (30 μg); sulphonamides (250 μg); tetracycline (30 μg); trimethoprim (5 μg); and minocycline (30 μg).[7]

Confirmed CPE were tested for the presence of bla_{VIM}, bla_{IMP}, bla_{GIM}, bla_{SIM}, bla_{SPM}, bla_{KPC}, $bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}40\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$, $bla_{OXA\text{-}48}$, bla_{GES} and bla_{NDM} by PCR.[8–11] Analysis of Tn*4401* insertion sites was performed by PCR.[12] PFGE was performed on KPC-producing *K. pneumoniae* using XbaI. Plasmid analysis was performed by S1 nuclease PFGE.

The Antimicrobial Reference Laboratory at HPA UK carried out multilocus variable-number tandem-repeat analysis (MLVA) at loci A, E, H, J, K, D, N1, N2 and N4.[13] Multilocus sequence typing (MLST) was performed on isolates representative of each PFGE type at the Institut Pasteur.[14] Alleles and profiles were determined by comparison with the *K. pneumoniae* MLST database at www.pasteur.fr/mlst.

Results

The 13 isolates had MICs of meropenem, imipenem and ertapenem of 4 to >32, 8–32 and >16 mg/L, respectively, were positive by the modified Hodge method and the disc synergy test indicated KPC activity (Table 2). Phenotypic results were similar for the additional isolates of *K. pneumoniae* isolated at hospital A (Table 2). Isolates showed resistance to multiple non-β-lactam agents including ciprofloxacin, but were generally susceptible to streptomycin, gentamicin, colistin and tigecycline.

PCR and sequencing confirmed the presence of $bla_{KPC\text{-}2}$ in all isolates and that this was carried in an isoform 'a' Tn*4401* element. Isolates harboured one to four plasmids of 5–191 kb. PFGE analysis of outbreak isolates revealed significant similarities (≥88%) with four distinguishable pulsed-field profiles (PFPs) identified. PFGE analysis of previous isolates indicated that four of these isolates (including the isolate from 2009—isolate number 2028) were very closely related to the outbreak strains (≥88%). The PFP of the isolate detected in August 2011 was indistinguishable from that of a number of the outbreak strains, whereas the isolate identified in October 2011 was similar but distinguishable from the outbreak strains and from isolates detected in 2009 and 2010 (Table 2). MLVA revealed that representatives of individual PFPs shared the same MLVA profile (3, 2, 2, 14, -, 1, 3, 3, 1). MLST indicated that all isolates belonged to the pandemic sequence type ST258.

Discussion

The capacity of clinical laboratories to detect CPE is fundamental to mapping trends and the detection of CPE outbreaks. Routine testing of isolates of Enterobacteriaceae for susceptibility to a carbapenem should be performed whenever possible to ensure early detection of such strains. Current EUCAST and CLSI criteria correctly identified all of the isolates reported here as resistant to ertapenem. All isolates were also categorized as non-susceptible to both meropenem and imipenem by both sets of criteria. All isolates were resistant by current CLSI criteria, whereas nine and six isolates were classified as intermediate to meropenem and imipenem, respectively, by EUCAST criteria. The synergy disc test used in this study correctly identified KPC activity in all isolates; however, some carbapenemases, such as OXA-48, are associated with only a modest increase in carbapenem MIC and inhibitors of these enzymes have not been identified. It is thus not yet possible to identify production of all carbapenemases based solely on phenotypic methods. Therefore, evaluation for the presence of carbapenemase-encoding genes using molecular methods is optimal to confirm the presence of a carbapenemase.

Table 1. Case descriptions involved in multi-hospital outbreak, January to March 2011

Case no.	Hospital	Unit	Type of specimen	Date of isolation	Infection/ colonization	Antimicrobial used prior to isolation of KPC-2 producer	Antimicrobial used as treatment for infection	Relationship to other cases	Outcome
1	A	ward 1 and HDU	blood sample	06/01/2011	infection	1 course of TZP and 1 course of GEN	2 courses of TGC and 2 courses of CST	index case hospital A	death
2	B	ICU (prior admission to ward 1 and HDU of hospital A)	femoral CVC tip	30/01/2011	colonization and infection[a]	1 course of TZP	1 course of TGC, 1 course of CST and 1 course of GEN	index case hospital B and contact of case 1	discharged
3	B	ICU	rectal swab	03/02/2011	colonization	1 course of MEM, 1 course of GEN, 1 course of TZP and 1 course of SXT	1 course of TGC, 1 course of CST and 1 course GEN	contact of case 2	discharged
4	A	HDU	rectal swab	27/02/2011	colonization	1 course of TZP, 1 course of CRO, 1 course of FCX and 1 course of PEN		contact of case 1	discharged
5	A	ICU	rectal swab	28/02/2011	colonization	1 course of MEM		no known contact with other cases	discharged
6	A	HDU	urine	02/03/2011	infection	1 course of AMC, 1 course of MEM and 1 course of GEN	1 course of TGC and 1 course of CST	contact of case 4	death
7	A	HDU	rectal swab	08/03/2011	infection	1 course of AMC and 1 course of GEN	1 course of TGC	contact of case 6	death
8	A	ward 2	rectal swab	11/03/2011	colonization	1 course of MEM and 1 course of CIP		contact of case 1	transfer to another hospital
9	A	ward 2	rectal swab	11/03/2011	infection	1 course of CIP	1 course of TGC	contact of case 7	discharged
10	A	ward 2	rectal swab	14/03/2011	colonization	1 course of AMC		contact of case 7	discharged
11	A	ward 2	rectal swab	29/03/2011	colonization	1 course of AMC		contact of cases 1, 8 and 9	discharged

TZP, piperacillin/tazobactam; GEN, gentamicin; CRO, ceftriaxone; FCX, flucloxacillin; PEN, benzyl penicillin; MEM, meropenem; CIP, ciprofloxacin; AMC, amoxicillin/clavulanic acid; TGC, tigecycline; CST, colistin; SXT, co-trimoxazole.

[a]The KPC was first isolated from a CVC tip, but at that time represented colonization only. Subsequently, the isolate was recovered from a broncho-alveloar lavage and the patient was treated for ventilator-associated pneumonia with tigecycline, colistin and gentamicin.

Table 2. Microbiological and molecular analyses of all KPC-2-producing Enterobacteriaceae

Isolate no.	Case/ patient no.	Meropenem MIC (mg/L)	Ertapenem MIC (mg/L)	Imipenem MIC (mg/L)	Modified Hodge test result	Synergy test result	KPC variant	PFP	MLVA	MLST analysis	Plasmid analysis (kb)
Outbreak isolates											
2198	1	32	>16	32	positive	KPC activity	bla_{KPC-2}	KpB3	ND	ST258	190, 130, 120, 16
2261	1	8	>16	8	positive	KPC activity	bla_{KPC-2}	KpB3	ND	ST258	100, 5
2262	1	8	>16	8	positive	KPC activity	bla_{KPC-2}	KpB3	ND	ST258	190, 120, 41, 14
2250	2	4	>16	6	positive	KPC activity	bla_{KPC-2}	KpB3	ND	ST258	133
2255	3	4	>16	12	positive	KPC activity	bla_{KPC-2}	KpB3	ND	ST258	133
2297	4	32	>16	32	positive	KPC activity	bla_{KPC-2}	KpB4	3, 2, 2, 14, -, 1, 3, 3, 1	ST258	144
2310	5	8	>16	16	positive	KPC activity	bla_{KPC-2}	KpB4	3, 2, 2, 14, -, 1, 3, 3, 1	ST258	144
2314	6	4	>16	16	positive	KPC activity	bla_{KPC-2}	KpB3	3, 2, 2, 14, -, 1, 3, 3, 1	ST258	120, 12
2320	7	16	>16	32	positive	KPC activity	bla_{KPC-2}	KpB4	3, 2, 2, 14, -, 1, 3, 3, 1	ST258	132
2328	8	>32	>16	64	positive	KPC activity	bla_{KPC-2}	KpB5	3, 2, 2, 14, -, 1, 3, 3, 1	ST258	144
2319	9	16	>16	32	positive	KPC activity	bla_{KPC-2}	KpB5	3, 2, 2, 14, -, 1, 3, 3, 1	ST258	144
2318	10	4	>16	8	positive	KPC activity	bla_{KPC-2}	KpB3	3, 2, 2, 14, -, 1, 3, 3, 1	ST258	120, 11
2348	11	32	>16	32	positive	KPC activity	bla_{KPC-2}	KpB6	ND	ST258	130, 120, 19
Previous isolates—2009 and 2010											
2028 (2009)	12	4	12	4	positive	KPC activity	bla_{KPC-2}	KpB1	ND	ST258	190, 120, 40, 16
1949	13	32	>16	32	positive	KPC activity	bla_{KPC-2}	KpA	3, 2, 2,14, -, 1, 3, 3, 1	ST258	133
1950	14	>32	>16	>128	positive	KPC activity	bla_{KPC-2}	KpB	3, 2, 2,14, -, 1, 3, 3, 1	ST258	190, 40, 16
1951	15	>32	>16	64	positive	KPC activity	bla_{KPC-2}	KpB1	3, 2, 2,14, -, 1, 3, 3, 1	ST258	190, 120, 40, 16
1952	15	8	>16	16	positive	KPC activity	bla_{KPC-2}	EcA	ND	ST131[5]	140, 120, 80, 7
2046	16	32	>16	32	positive	KPC activity	bla_{KPC-2}	KpB2	3, 2, 2,14, -, 1, 3, 3, 1	ST258	190, 16
2054	17	>32	>16	>128	positive	KPC activity	bla_{KPC-2}	KpC	3, 2, 2,14, -, 1, 3, 3, 1	ST258	40
2055	17	>32	>16	>128	positive	KPC activity	bla_{KPC-2}	KpD	3, 2, 2,14, -, 1, 3, 3, 1	ST258	180, 40, 16
Additional 2011 isolates											
2754	18	>32	>32	4	positive	KPC activity	bla_{KPC-2}	KpB3	ND		120
2946	19	>32	>32	>32	positive	KPC activity	bla_{KPC-2}	KpP	ND		191, 109, 14, 11

ND, not determined.

The outbreak strain corresponds to the predominant KPC variant (KPC-2) and MLST type (ST258) reported elsewhere in Europe.[2] The correlation of clustering of PFGE patterns (at ≥88% in this study) with MLST type ST258 has previously been reported.[1]

Stringent enforcement of IPC and antimicrobial stewardship measures appears to have been effective in termination of this specific outbreak. This is consistent with the report of Agodi *et al.*[15] that cleaning and disinfection of the ICU, segregation of affected patients, barrier nursing and strict compliance with hand hygiene procedures led to containment of an outbreak of KPC-3-producing *K. pneumoniae*.

However, the overall picture since 2009 suggests that KPC-2-producing *K. pneumoniae* is now endemic in this region of Ireland. As there is no established method for decolonization, there is likely to be a continuing risk of reintroduction of KPC-2-producing *K. pneumoniae* or other Enterobacteriaceae into the hospital that serves the endemic area and a continuing risk of extension to other regions by inter-hospital transfer. National guidelines advocate source isolation and screening of all inter-hospital transfers of patients with a history of admission to hospitals with an ongoing problem with CPE.[16]

The risk of dissemination of CPE is magnified by the increasing dependence on the use of carbapenems for empirical therapy of critically ill patients related to a relatively high frequency of isolation of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. In response to the emergence of this and other CPE in Ireland, infection with such isolates is now notifiable to the Department of Public Health and a national study to establish the baseline prevalence of CPE in intensive care units has been performed.[17] In addition, interim national guidance on detection of and screening for CPE and IPC response have been prepared.[16] A national programme for antimicrobial stewardship and surveillance of hospital and community antimicrobial consumption is also in place. Although such measures may have contributed to substantial recent reductions in the proportion of *Staphylococcus aureus* bloodstream infection accounted for by methicillin-resistant *S. aureus* (MRSA) in Ireland, we are not optimistic that it will be possible to effectively control or eradicate CPE. Antimicrobial resistance in Enterobacteriaceae is likely to be more difficult to control because: (i) screening based on rectal swabs is unlikely to identify all colonized patients efficiently, given the density and diversity of gastrointestinal flora; (ii) experience with ESBL-producing Enterobacteriaceae suggests that colonization, once established, may persist indefinitely; and (iii) there are no established protocols that are effective in decolonization. It is therefore likely that a national response incorporating IPC, antimicrobial stewardship and public health measures may, at best, delay dissemination of CPE. Since the onset of this outbreak a number of other CPE have emerged in a number of hospitals in other regions of Ireland, making effective control still more challenging.

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