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SHORT REPORT
Dissemination of clonally related multidrug-resistant *Klebsiella pneumoniae* in Ireland

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SUMMARY

In October 2012, an outbreak of gentamicin-resistant, ciprofloxacin non-susceptible extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* occurred in a neonatal intensive care unit in Ireland. In order to determine whether the outbreak strain was more widely dispersed in the country, 137 isolates of *K. pneumoniae* with this resistance phenotype collected from 17 hospitals throughout Ireland between January 2011 and July 2013 were examined. ESBL production was confirmed phenotypically and all isolates were screened for susceptibility to 19 antimicrobial agents and for the presence of genes encoding bla_{TEM}, bla_{SHV}, bla_{OXA}, and $bla_{\text{CTX-M}}$; 22 isolates were also screened for bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and $bla_{\text{OXA-48}}$ genes. All isolates harboured bla_{SHV} and $bla_{\text{CTX-M}}$ and were resistant to ciprofloxacin, gentamicin, nalidixic acid, amoxicillin-clavulanate, and cefpodoxime; 15 were resistant to ertapenem, seven to meropenem and five isolates were confirmed as carbapenemase producers. Pulsed-field gel electrophoresis of all isolates identified 16 major clusters, with two clusters comprising 61% of the entire collection. Multilocus sequence typing of a subset of these isolates identified a novel type, ST1236, a single locus variant of ST48. Data suggest that two major clonal groups, ST1236/ST48 (CG43) and ST15/ST14 (CG15) have been circulating in Ireland since at least January 2011.

Key words: Antibiotic resistance, clonality, *Klebsiella*.

Infections with extended spectrum β-lactamase (ESBL)-producing bacteria are a major public health threat worldwide and are associated with significant morbidity, mortality and increased healthcare costs [1]. β-lactamases of the CTX-M group first emerged in the late 1980s and early 1990s and are now the most prevalent ESBL type reported worldwide [1]. Their widespread dissemination can be attributed to their association with specific epidemic plasmids, e.g. IncFII; and specific clones, notably *Escherichia coli* O25b:H4-ST131, *Klebsiella pneumoniae* ST258, and *K. pneumoniae* ST11 [2]. Community-acquired CTX-M-producing *E. coli* urinary tract infection frequently dominate reports; however, increasingly outbreaks associated with multidrug-resistant ESBL

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K. pneumoniae are reported [2, 3]. Most recent available data from the European Antimicrobial Surveillance Network indicate that across Europe the percentages of invasive *K. pneumoniae* resistant to third-generation cephalosporins range from 0% to 70·1%, with ESBL producers accounting for between 85% and 100% of all strains reported [4]. Further, increasing combined resistance to fluoroquinolones, aminoglycosides and third-generation cephalosporins was also widespread with a reported mean percentage of 20·9% in 2013 [5]. The most recent data for Ireland for 2014 indicate that 11·3% of invasive *K. pneumoniae* were ESBL producers, 17·3% were resistant to ciprofloxacin, 13·2% were resistant to aminoglycosides, and 13·7% were resistant to all three [5].

In late 2012 an outbreak of CTX-M ESBL-producing *K. pneumoniae* was identified at a neonatal intensive care unit (NICU) in Ireland [6], and two infants were diagnosed with bloodstream infection with this organism. All infants in the NICU during the outbreak period were screened by rectal swab for carriage of ESBL *K. pneumoniae* and 22 of 78 infants proved positive. No new cases of infection or carriage were detected after 31 October 2012 and no new rectal swabs were positive after 2 January 2013 indicating that transmission had been interrupted and no environmental reservoir was identified. All isolates were ciprofloxacin non-susceptible and gentamicin resistant in addition to ESBL (CipGeESBL). Prompted by the recognition that *K. pneumoniae* with this phenotype had been observed in other hospitals an investigation was undertaken to determine the extent of dissemination of such strains throughout Ireland.

Retrospective analysis of data stored by the Antimicrobial Resistance and Microbial Ecology (ARME) group identified 75 isolates of *K. pneumoniae* from eight hospitals throughout Ireland with the CipGeESBL phenotype since January 2011. In December 2012, hospital laboratories throughout the country were alerted to this concern and invited to submit *K. pneumoniae* isolates from all specimen types with this phenotype. Forty-four isolates were received from 11 hospital laboratories between December 2012 and July 2013 and the total collection comprised 137 isolates from 17 hospitals (including 18 isolates from the original NICU outbreak and four isolates from nursing home residents) between January 2011 and July 2013.

ESBL production was determined in all isolates by the combination disk method using cefpodoxime (30 g), and cefpodoxime plus clavulanic acid (10 μg/1 μg). All isolates were screened for susceptibility to the following antimicrobial agents by disk diffusion in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria where available: ampicillin (10 μg); cefpodoxime (10 μg), cefotaxime (5 μg), ceftazidime (10 μg), cefoxitin (30 μg), amoxicillin-clavulanate (20 μg/10 μg), piperacillin-tazobactam (30 μg/6 μg), ertapenem (10 μg), meropenem (10 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]. Clinical Laboratory Standard Institute (CLSI) interpretive criteria was applied to antimicrobial agents for which EUCAST interpretive criteria were not available.

Confirmed ESBL-producing strains were screened for bla_{TEM}, bla_{SHV}, bla_{OXA}, and $bla_{\mathrm{CTX\text{-}M}}$ by multiplex polymerase chain reaction (PCR) assays using primers and protocols as described previously [8, 9], and carbapenem-resistant isolates [$n = 22$] were screened for bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} and $bla_{\mathrm{OXA\text{-}48}}$ by real time PCR [10]. Pulsed-field gel electrophoresis (PFGE) was performed on all isolates according to the PulseNet protocol with *Xba*I and DNA banding patterns were analysed using the Dice coefficient with clustering by the unweighted pair-group method with arithmetic averaging (UPGMA) [11]. One or more isolates representative of major PFGE clusters were subjected to multilocus sequence typing (MLST) as described previously [12, 13]. Allele and profile were determined by comparison to data from the *K. pneumoniae* MLST database (www.pasteur.fr/mlst).

All isolates were confirmed as ESBL producers and harboured bla_{SHV} and a $bla_{\mathrm{CTX\text{-}M}}$ gene ($bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}1}}$, $n = 126$; $bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}25}}$, $n = 5$), $bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}2}}$ ($n = 5$), and $bla_{\mathrm{CTX\text{-}M\text{-}group\text{-}9}}$ ($n = 1$). Four isolates received from hospital 11 (see Table 1 for hospital numbers) ($n = 2$), hospital 9 ($n = 1$) and hospital 10 ($n = 1$) were positive for $bla_{\mathrm{KPC\text{-}2}}$ and one isolate from hospital 9 harboured bla_{NDM} and $bla_{\mathrm{OXA\text{-}48}}$ (Table 1). All isolates were resistant to ampicillin, amoxicillin-clavulanate, cefpodoxime, ciprofloxacin, gentamicin and nalidixic acid, and at least $\geqslant 2$ other antimicrobial agents giving 66 individual antibiograms in the 137 isolates. One isolate was resistant to all antimicrobial agents tested; 15 isolates were resistant to ertapenem, seven of which were also resistant to meropenem.

Table 1. *Correlation of pulsed-field profile with location, date of isolation, β-lactamase, and sequence types of multidrug-resistant* Klebsiella pneumoniae

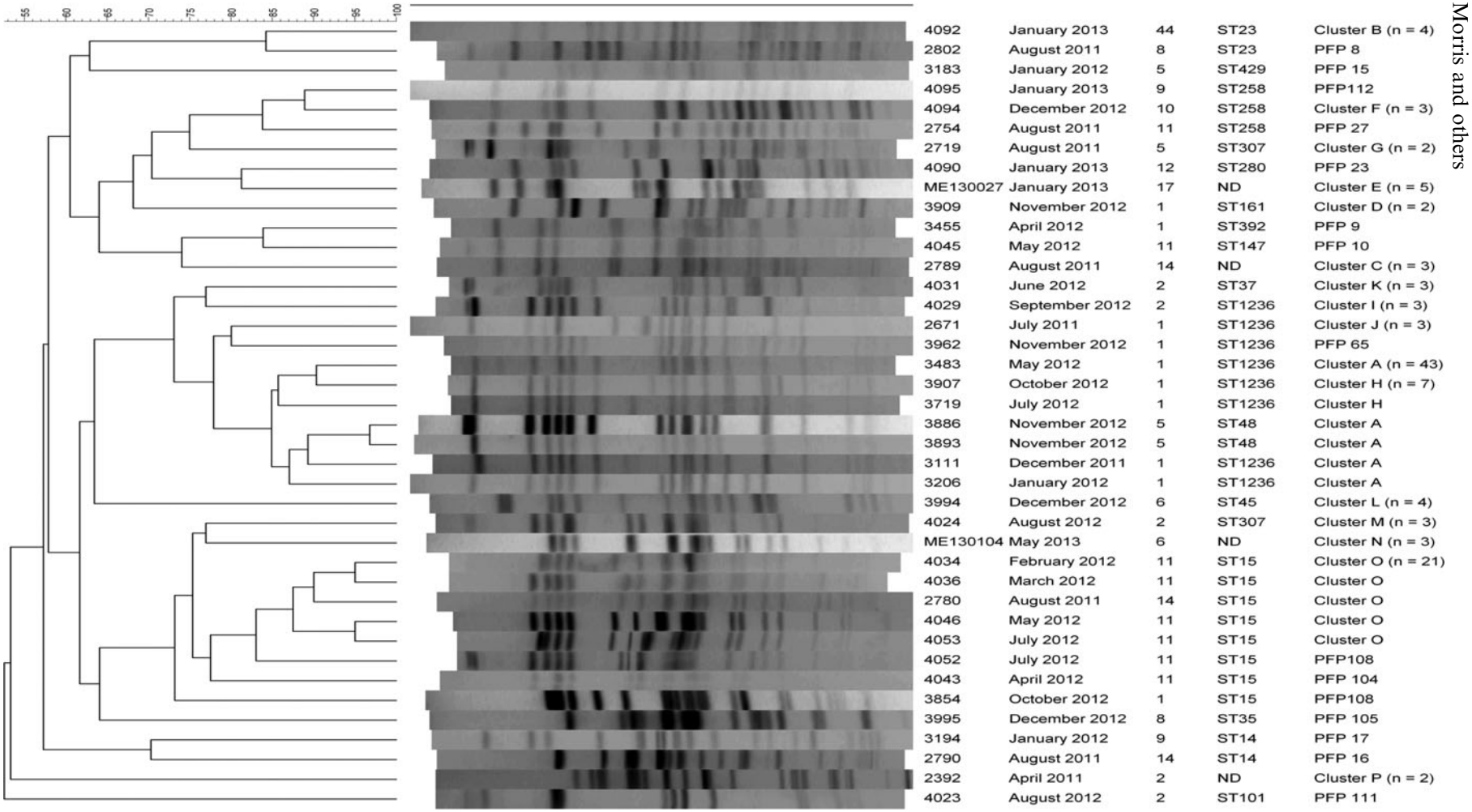
PFGE cluster	No.	Hospital(s)*	Date of isolation	*bla* type identified							MLST type†
				$bla_{\text{CTX-M}}$ group	bla_{TEM}	bla_{SHV}	$bla_{\text{OXA-1}}$	$bla_{\text{KPC-2}}$	$bla_{\text{OXA-48}}$	bla_{NDM}	
A	43	1, 5, 11	Feb. 2011-Oct. 2013	1 (42), 9 (1)	0	43	43	0	0	0	ST1236 ($n = 3$), ST48 ($n = 2$)
B	4	2, 13	Nov. 2012-May 2013	1 (4)	0	4	4	0	0	0	ST23 ($n = 1$)
C	3	5, 14	Aug. 2011	1 (3)	3	3	0	0	0	0	n.d.
D	2	1	Oct.–Nov. 2012	1 (2)	0	2	0	0	0	0	ST161 ($n = 1$)
E	5	17	Jan.–Apr. 2013	2 (5)	0	5	0	0	0	0	n.d.
F	3	1, 10, 11	Feb. 2011–Dec. 2012	25 (3)	3	3	0	2	0	0	ST258 ($n = 1$)
G	2	2, 5	Aug. 2011–June 2012	1 (2)	0	2	2	0	0	0	ST307 ($n = 1$)
H	7	1	Jul. 2012–June 2013	1 (7)	0	7	7	0	0	0	ST1236 ($n = 2$)
I	3	1, 2	Sept. 2012–July 2013	1 (3)	0	3	3	0	0	0	ST1236 ($n = 1$)
J	3	1, 3	July 2011–Nov. 2012	1 (3)	0	3	3	0	0	0	ST1236 ($n = 1$)
K	3	2	June–Jul. 2012	1 (3)	0	3	3	0	0	0	ST37 ($n = 1$)
L	4	4, 6, 7	2011–Dec. 2012	1 (4)	0	4	4	0	0	0	ST45 ($n = 1$)
M	3	2	July–Oct. 2012	1 (3)	0	3	0	0	0	0	ST307 ($n = 1$)
N	3	6	Apr.–May 2013	1 (3)	0	3	0	0	0	0	n.d.
O	21	11, 14	Jan.–Aug. 2012	1 (21)	21	21	0	0	0	0	ST15 ($n = 5$)
P	2	1, 2	Apr. 2011–July 2012	1 (2)	0	2	2	0	0	0	n.d.
Ind. PFPs	26	12 hospitals	2011–2013	1 (24), 25 (2)	4	26	20	2	1	1	Various‡
Total	137	17	2011–2013	137	31	137	90	4	1	1	36

PFGE, Pulsed-field gel electrophoresis; MLST, multilocus sequence typing; n.d., not determined; Ind. PFPs, individual pulsed-field profiles.

* Hospital locations: Ulster (hospitals 6, 7, 14); Munster (hospitals 4, 5, 11, 13, 15); Leinster (hospitals 2, 8, 9, 10, 12, 16, 17); Connaught (hospitals 1, 3).

† MLST was performed on 37 representatives of individual pulsed-field clusters and profiles

‡ ST258 ($n = 2$), ST429 ($n = 1$), ST23 ($n = 1$), ST392 ($n = 1$), ST147 ($n = 1$), ST280 ($n = 1$), ST15 ($n = 3$), ST35 ($n = 1$), ST1236 ($n = 1$), ST14 ($n = 2$), ST101 ($n = 1$), ST16 ($n = 1$).



Isolate no.	Date of isolation	Hospital code	MLST	PFGE cluster
4092	January 2013	44	ST23	Cluster B (n = 4)
2802	August 2011	8	ST23	PFP 8
3183	January 2012	5	ST429	PFP 15
4095	January 2013	9	ST258	PFP112
4094	December 2012	10	ST258	Cluster F (n = 3)
2754	August 2011	11	ST258	PFP 27
2719	August 2011	5	ST307	Cluster G (n = 2)
4090	January 2013	12	ST280	PFP 23
ME130027	January 2013	17	ND	Cluster E (n = 5)
3909	November 2012	1	ST161	Cluster D (n = 2)
3455	April 2012	1	ST392	PFP 9
4045	May 2012	11	ST147	PFP 10
2789	August 2011	14	ND	Cluster C (n = 3)
4031	June 2012	2	ST37	Cluster K (n = 3)
4029	September 2012	2	ST1236	Cluster I (n = 3)
2671	July 2011	1	ST1236	Cluster J (n = 3)
3962	November 2012	1	ST1236	PFP 65
3483	May 2012	1	ST1236	Cluster A (n = 43)
3907	October 2012	1	ST1236	Cluster H (n = 7)
3719	July 2012	1	ST1236	Cluster H
3886	November 2012	5	ST48	Cluster A
3893	November 2012	5	ST48	Cluster A
3111	December 2011	1	ST1236	Cluster A
3206	January 2012	1	ST1236	Cluster A
3994	December 2012	6	ST45	Cluster L (n = 4)
4024	August 2012	2	ST307	Cluster M (n = 3)
ME130104	May 2013	6	ND	Cluster N (n = 3)
4034	February 2012	11	ST15	Cluster O (n = 21)
4036	March 2012	11	ST15	Cluster O
2780	August 2011	14	ST15	Cluster O
4046	May 2012	11	ST15	Cluster O
4053	July 2012	11	ST15	Cluster O
4052	July 2012	11	ST15	PFP108
4043	April 2012	11	ST15	PFP 104
3854	October 2012	1	ST15	PFP108
3995	December 2012	8	ST35	PFP 105
3194	January 2012	9	ST14	PFP 17
2790	August 2011	14	ST14	PFP 16
2392	April 2011	2	ND	Cluster P (n = 2)
4023	August 2012	2	ST101	PFP 111

Fig. 1. Dendogram showing relatedness of representative isolates from clusters and individual profiles identified by pulsed-field gel electrophoresis (PFGE) and correlation with multilocus sequence typing (MLST) analysis

PFGE identified 111 different profiles which fell into 16 major clusters (A–P) in 112 isolates based on a similarity of ⩾85% (Table 1, Fig. 1) with each cluster comprising 2–43 isolates. The largest cluster (A, $n = 43$) accounted for 31% of all isolates and contained isolates from three hospitals between February 2011 and October 2013, including the 18 isolates from the initial NICU outbreak (Table 1, Fig. 1).

Seventeen sequence types (STs) were identified in 36 representatives of distinguishable pulsed-field patterns: ST1236 ($n = 8$), ST15 ($n = 8$), ST258 ($n = 3$), ST23 ($n = 2$), ST48 ($n = 2$) and ST307 ($n = 2$) (Fig. 1). Eight isolates from two hospitals representing PFGE clusters A, H, I and J and one unique isolate were designated a novel MLST type, ST1236, a single locus variant of ST48. All isolates of ST1236 were ⩾73% similar by PFGE analysis. Eight isolates received from three hospitals of PFGE cluster O and individual DNA profiles with ⩾73% similarity were assigned to ST15. Three isolates from individual hospitals were of ST258, and harboured bla_{KPC}, $bla_{CTX\text{-}M\text{-}25}$ and bla_{SHV} and the fourth *K. pneumoniae* carbapenemase (KPC)-producing isolate fell in PFGE cluster F and by extrapolation to ST258. The single bla_{NDM}, $bla_{OXA\text{-}48}$ isolate was assigned to ST14.

The dissemination of multidrug-resistant *K. pneumoniae* represents a very significant threat to patients' safety and public health with several reports of outbreaks in hospitals particularly in NICUs [14, 15]. Data generated in this study suggests that two major clonal groups (CG) of multidrug-resistant *K. pneumoniae*, ST1236/ST48 (CG43) and ST15/ST14 (CG15) have been circulating in Ireland since at least January 2011. By extrapolation from PFGE analysis, 57 isolates from five hospitals and a nursing home fell into CG43, and 26 isolates from three hospitals into CG15, both groups together accounting for 61% of the entire collection. Since the establishment of the MLST scheme for *K. pneumoniae* in 2005 [13], the database now comprises 1874 STs, several of which have been described as successful pandemic clones. Attempts have been made by a number of workers to group individual STs into clonal groups and clonal complexes although it is recognized that this is challenging in *K. pneumoniae* due to the high rate of recombination between STs [2, 12, 16, 17]. ST1236 was first defined in this study and is a single locus variant of ST48, a member of CG43 [16] which has been disseminated worldwide [3] along with ST15 (CG15) [17]. The single ST14 isolate harbouring bla_{NDM} and $bla_{OXA\text{-}48}$, bla_{SHV}, $bla_{OXA\text{-}1}$, and $bla_{CTX\text{-}M\text{-}group1}$ is a single locus variant of ST15, and has been widely reported in association with CTX-M, and to a lesser extent carbapenemases including NDM-1, OXA-181 (a variant of OXA-48) [2]. The three KPC-producing isolates of ST258 are representative of a major pandemic clone comprising 96 STs [2].

This study therefore demonstrates that although there is considerable diversity among the CipGeESBL *K. pneumoniae* in Irish hospitals, a high proportion (61%) of the isolates were accounted for by two clonal groups, and in at least one case spread had extended into nursing home residents in the community.

Active surveillance to enhance knowledge of the distribution of these STs/clonal groups among ESBL-producing *K. pneumoniae* is required to inform the development and implementation of appropriate infection control and prevention procedures. Schwaber *et al.* recently described containment of a nationwide outbreak of KPC-producing *K. pneumoniae* in Israel by stringent application and monitoring of infection control procedures [18] but, nevertheless, they acknowledged that the success of such an approach is highly dependent on the commitment of leaders in health policy planning to act on the data produced by studies such as described here.

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DECLARATION OF INTEREST

None.

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