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Polyclonal Outbreak of KPC-3-Producing *Enterobacter cloacae* at a Single Hospital in Montréal, Québec, Canada

Louis-Patrick Haraoui,[a] Simon Lévesque,[b] Brigitte Lefebvre,[b] Ruth Blanchette,[a] Melissa Tomkinson,[a] Laura Mataseje,[c] Michael R. Mulvey,[c] Mark A. Miller[a]

Jewish General Hospital, Montréal, Québec, Canada[a]; Laboratoire de Santé Publique du Québec/Institut National de Santé Publique du Québec, Ste. Anne-de-Bellevue, Québec, Canada[b]; National Microbiology Laboratory, Winnipeg, Manitoba, Canada[c]

From September 2010 to December 2011, 26 KPC-3-producing *Enterobacter cloacae* isolates were identified from 16 patients at a single hospital. Analyses revealed the bla_{KPC} gene to be localized on multiple plasmids in a diverse nonclonal *E. cloacae* genetic background. These findings highlight the potential complexity of a KPC outbreak at a single hospital.

Enterobacter cloacae ranks as the tenth most common organism isolated in Canadian hospitals (1). As with other *Enterobacteriaceae*, carbapenem-hydrolyzing activity has predominantly been associated with the *Klebsiella pneumoniae* carbapenemases (KPCs) encoded within the Tn*4401* transposon carrying one of many variants of the bla_{KPC} gene (2, 3). Starting in September 2010, the Jewish General Hospital (JGH), in Montréal, Canada, a tertiary-care hospital with 637 acute-care beds, began screening for the presence of carbapenem-resistant *Enterobacteriaceae* (CRE) from both clinical and screening specimens. This report discusses the subset of KPC-producing *E. cloacae* isolated at the JGH.

Screening was performed using rectal swabs transported in Amies media (Becton Dickinson, Franklin Lakes, NJ) that were then directly plated onto a CRE screening plate (4). Resistance to ertapenem in isolates from the screen plates were confirmed by Etest (AB Biodisk, Solna, Sweden), with interpretations based on iterations of CLSI document M100 (5). Isolates with an ertapenem MIC of ≥1 μg/ml were sent for confirmation of carbapenem resistance to the Laboratoire de Santé Publique du Québec (LSPQ). The LSPQ confirmed species identification using API 20E strips (bioMérieux, Lyon, France). Extended biochemical panel testing (6) with or without *rpoB* sequencing was used to resolve discrepancies in species identification. The presence of bla_{KPC} was confirmed by PCR technique at the LSPQ (7, 8). The bla_{KPC} subtypes were obtained by sequencing the amplicon using two additional internal primers (2). Further characterization of the genetic environment of bla_{KPC} at five distinct intergenic regions within the Tn*4401* transposon was performed by PCR and sequencing, as previously described (9). Pulsed-field gel electrophoresis (PFGE) using the XbaI and BioNumerics software (version 6.5; Applied Maths, Belgium) generated clustering of strains. A cutoff of ≥85% similarity was used for cluster determination. Plasmids harboring bla_{KPC} were transferred using electroporation to *Escherichia coli* DH10B and fingerprinted using EcoRI as previously described (10). Plasmids were subsequently typed by a PCR-based replicon typing method as previously described (11). Antimicrobial susceptibility was evaluated among clinical isolates by various established methods (Etest, broth microdilution, and disk diffusion).

Between September 2010 and December 2011, 26 isolates of KPC-3-producing *E. cloacae* were identified from 16 patients: 12 men and 4 women (Fig. 1). Of the 26 isolates, 7 were recovered from clinical specimens (six patients): 2 from the blood, 3 from urine, and 2 from pus. In addition, 19 isolates from 11 patients were recovered from rectal screens. All of the patients except one were hospitalized at the time of their positive culture determination. The exception (strain G) was detected in the emergency room from a patient who had been recently hospitalized at the JGH. The other patients were located on six different wards at the time of the positive culture (Fig. 1). Macrorestriction analysis using PFGE revealed four clusters of three or more isolates (Fig. 1). The seven clinical isolates identified were found in four distinct PFGE types.

Transfer of the bla_{KPC} plasmids to *E. coli* DH10B was successful for all isolates. Plasmid size ranged from 28 to 103 kb, with a median size of 58 kb. Subsequent molecular analysis revealed numerous incompatibility types, including IncN ($n = 9$), IncP,L/M ($n = 5$), and IncN,A/C ($n = 1$), and 11 with an unknown incompatibility type, labeled NEG (Fig. 1). Plasmids harboring IncN were identified in three different *E. cloacae* fingerprint groups. The five IncP,L/M plasmids were identified in two different *E. cloacae* fingerprint clusters, suggesting that this plasmid had transferred between strains during the course of the outbreak.

All isolates had a similar Tn*4401* transposon structure surrounding bla_{KPC}, suggesting that the same transposon was present among all isolates and plasmids. No deletion was observed in the polymorphic region between the *istB* of the ISK*pn7* and the bla_{KPC} gene (3).

Using MICs of ≤2 and ≥4 mg/liter to express, respectively, sensitivity and resistance to colistin, 6 of the 26 strains were found to be resistant to this antibiotic. For tigecycline, using cutoff MICs of ≤2 and ≥8 mg/liter to establish sensitivity and resistance, only one strain was found to be resistant, with six in the intermediate range with an MIC of 4 mg/liter. Only half of the strains underwent susceptibility testing to chloramphenicol, and all of these were sensitive to this antibiotic except for two in the intermediate

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Address correspondence to Louis-Patrick Haraoui, louis-patrick.haraoui@rrsss16.gouv.qc.ca.

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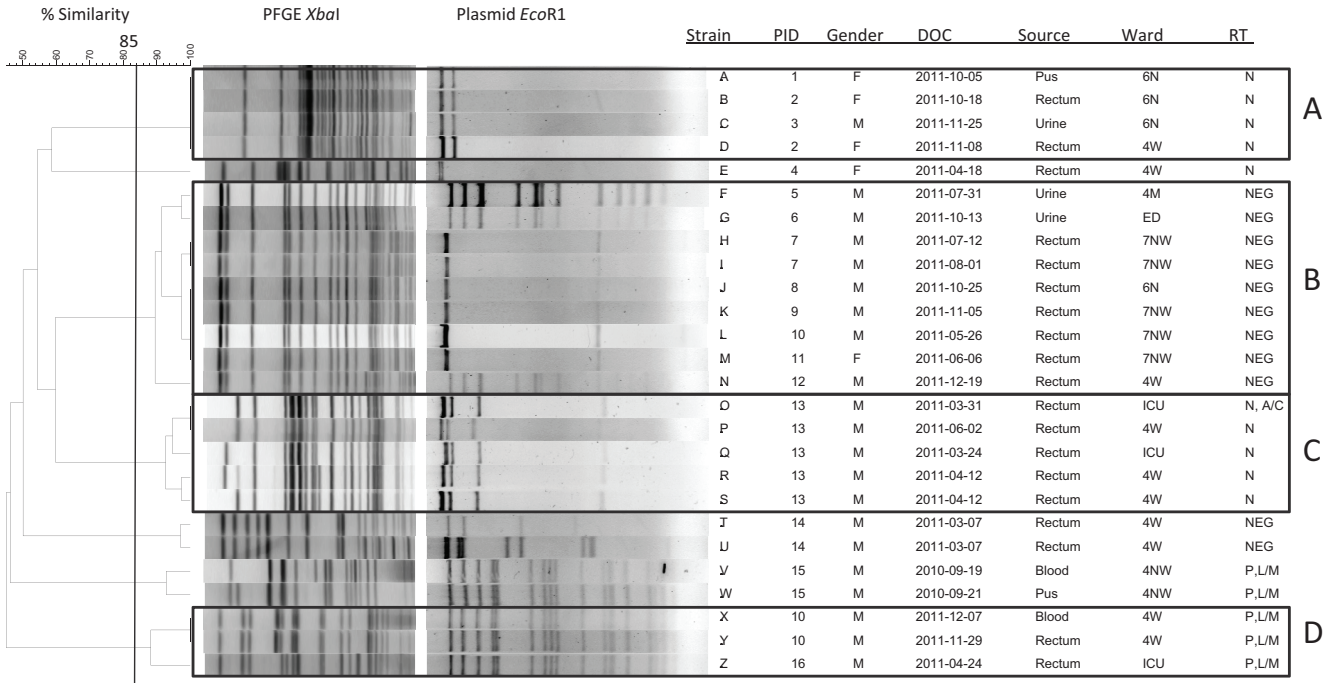

FIG 1 Dendrogram of all 26 strains of *E. cloacae* isolated at the JGH between September 2010 and December 2011. PID, patient identifier; DOC, date of collection; N, north; NW, northwest; W, west; M, main; ED, emergency department; ICU, intensive care unit; RT, replicon type; NEG, negative.

range (5). These and other antimicrobial susceptibility profiles to non-beta-lactam antibiotics are detailed in Table 1.

To our knowledge, this study is unique with regard to the breadth and depth in the reporting of an outbreak of KPC-producing *E. cloacae*. The various analyses performed on this cluster of *E. cloacae* strains revealed a highly conserved Tn*4401* transposon expressing the bla_{KPC-3} gene. As opposed to traditional outbreak investigations that reveal one or a few strains spreading among individuals, our findings in this outbreak instead suggest that multiple modes of spread were responsible for the dissemination of these strains. In some cases, a

TABLE 1 Antimicrobial testing results using non-beta-lactam antibiotics for all KPC-positive *E. cloacae* strains[a]

Strain information		Etest MIC (mg/liter)		Broth microdilution MIC (mg/liter)					Disk diffusion (mm)		
Strain ID	Patient ID	AZT	TGC	CST	GEN	TOB	AMI	CIP	LEV	TMP/SMX	CHL
A	1	256	4	4	64	>64	2	16	8	6	16
B	2	256	4	4	64	>64	4	16	6	6	15
C	3	128	4	4	64	>64	4	8	8	6	20
D	2	≥256	4	2	64	>64	8	8	9	6	21
E	4	256	2	4	32	16	2	0.5	23	6	NA
F	5	64	0.5	2	1	1	2	1	23	30	25
G	6	64	0.5	2	0.5	0.5	1	1	19	26	18
H	7	≥256	1	2	0.25	0.5	1	2	21	27	22
I	7	128	0.5	2	0.5	1	2	1	24	34	25
J	8	64	0.5	2	0.5	1	2	1	23	29	25
K	9	256	0.5	2	0.5	0.5	2	0.5	21	28	24
L	10	256	1	2	4	4	2	1	22	29	NA
M	11	64	0.5	2	0.5	0.5	1	1	22	15	NA
N	12	64	0.5	2	0.5	1	2	1	23	30	26
O	13	≥256	2	2	32	32	2	64	6	6	NA
P	13	≥256	2	2	32	32	2	>64	6	6	NA
Q	13	≥256	4	2	32	32	2	4	15	6	NA
R	13	≥256	2	2	32	32	1	64	6	6	NA
S	13	≥256	2	2	32	32	2	64	6	6	NA
T	14	≥256	0.5	1	0.5	0.5	1	1	22	27	NA
U	14	≥256	0.5	2	0.5	0.5	1	1	22	27	NA
V	15	≥256	8	2	16	8	1	4	15	6	NA
W	15	≥256	4	4	8	16	0.5	2	14	6	NA
X	10	≥256	2	4	16	16	1	1	20	6	21
Y	10	≥256	2	2	16	16	1	0.5	25	6	22
Z	16	256	1	2	16	16	2	0.25	25	6	NA

[a] AZT, aztreonam; TGC, tigecycline; CST, colistin; GEN, gentamicin; TOB, tobramycin; AMI, amikacin; CIP, ciprofloxacin; LEV, levofloxacin; TMP/SMX, trimethoprim-sulfamethoxazole; CHL, chloramphenicol; NA, not available.

highly promiscuous plasmid carrying the Tn*4401* transposon disseminated among polyclonal *E. cloacae* strains (Inc type P,L/M among strains V to Z). However, the multiple plasmid Inc types observed harboring KPC-3 also indicate a high mobility of Tn*4401*. Indeed, in some cases a single patient could harbor at the same time different *E. cloacae* strains with different plasmids (strains T and U of patient 14); in another instance, a single patient was found to have distinct *E. cloacae* strains with different plasmid Inc types over a period of a few months (strains L, X, and Y of patient 10). The variability of strains and plasmids observed could also be explained by multiple-point source outbreaks occurring at the same hospital during the time frame under study. A more detailed epidemiologic investigation and the use of whole-genome sequencing may provide answers to these questions.

Outbreaks of KPC-producing organisms are being increasingly reported and represent a challenge to infection control practitioners as well as to clinicians. The findings from our study suggest that sophisticated methods are increasingly required to develop a thorough understanding of the spread of KPC-producing organisms. Whereas traditional tools such as PFGE initially pointed to a group of polyclonal strains of phenotypically similar bacteria expressing carbapenem resistance, additional molecular methods have revealed that the plasmid or the transposon was the entity spreading among patients and not the bacteria themselves. Rather than investigating suspected outbreaks of KPC-producing organisms by focusing on clonal strains, infection control practitioners, clinicians, and medical microbiologists should supplement the traditional techniques of phenotypic typing and PFGE with the necessary molecular tools to focus on the genetic structure of the KPC-bearing transposon and plasmid in order to better identify and follow the spread of these resistant organisms.

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