Outbreak of Carbapenem-Resistant Enterobacteriaceae Containing $bla_{\text{NDM-1}}$, Ontario, Canada

Sergio Borgia,1,3,8 Olga Lastovetska,4,5 David Richardson,1,2,9 Alireza Eshaghi,4 Jianhui Xiong,4 Catherine Chung,4 Mahin Baqi,1,3 Allison McGeer,5,6,7 Gloria Ricci,2 Rachael Sawicki,3 Rajni Pantelidis,3 Donald E. Low,4,5,6 Samir N. Patel,4,5,a and Roberto G. Melano4,5,6,a

1Division of Infectious Diseases, 2Department of Laboratory Medicine, and 3Infection Prevention and Control, William Osler Health System, Brampton, 4Public Health Ontario, Public Health Laboratories, 5Department of Laboratory Medicine and Pathobiology, University of Toronto, 6Department of Microbiology and 7Infection Prevention and Control, Mount Sinai Hospital, Toronto, and Departments of 8Medicine and 9Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada

**Background.** New Delhi metallo-ß-lactamase (NDM) has emerged worldwide in clinically relevant gram-negative bacteria. We report an outbreak of NDM-producing *Klebsiella pneumoniae* in patients with no prior travel history to endemic regions.

**Methods.** Five NDM-1–producing *K. pneumoniae* colonizing and/or clinically infecting patients in a community tertiary hospital were detected between October and November 2011. NDM-1–producing Enterobacteriaceae (*K. pneumoniae* and *Escherichia coli*) were clinically and epidemiologically characterized, including susceptibility profiles, molecular typing, and molecular characterization of plasmids and resistant determinants.

**Results.** Five patients were identified carrying NDM-1–producing *K. pneumoniae*, all of them epidemiologically linked with each other. *K. pneumoniae* were confirmed to belong to the same clone, exhibiting multidrug-resistant phenotypes. One patient was positive for NDM-1–producing *E. coli* in blood and *E. coli* and *K. pneumoniae* in rectal specimens, both containing the same $bla_{\text{NDM}}$ plasmid, suggesting horizontal transfer between species in the patient. No environmental sources of these strains were found. Detection of positive isolates directly from rectal specimens allowed the rapid identification and isolation of colonized patients.

**Conclusions.** We report a NDM-1–producing *K. pneumoniae* outbreak in Ontario, Canada. Implementation of standard infection control practices, including active screening was able to contain the spread of this organism in the hospital setting. Of concern is the potential loss of a travel history to identify patients that are at high risk of being colonized or infected with this organism and the lack of an accurate, cost-effective test that can be implemented in the hospital setting to identify these multidrug-resistant organisms.

Carbapenem resistance is primarily mediated by the production of carbapenemases, which are ß-lactamases capable of hydrolyzing all ß-lactam antibiotics, including the most potent class, carbapenems [1]. The New Delhi metallo-ß-lactamase (NDM) was first characterized in 2008 in Sweden from *Klebsiella pneumoniae* and *Escherichia coli* isolated from a patient who had received medical care in India [2]. Subsequently, it was shown that Enterobacteriaceae harboring $bla_{\text{NDM-1}}$ were widely disseminated in India, Pakistan, Bangladesh, and the United Kingdom [3, 4]. Since then, Enterobacteriaceae containing $bla_{\text{NDM-1}}$ have been detected and reported worldwide [5, 6]. The first cases of NDM-1–producing Enterobacteriaceae in North America were detected in the United States and Canada in 2010 from patients who had received medical care in India before arriving to the continent [7–10]. Here we report an outbreak of NDM-1–producing Enterobacteriaceae in hospitalized
patients in Canada with no recent travel history to endemic areas.

MATERIALS AND METHODS

Hospital Setting and Nosocomial Measures
The Brampton Civic Hospital campus (BCH) of the William Osler Health System is a 500-bed tertiary care community health facility that serves the acute care health needs of the Brampton community, a city of >500,000 residents where approximately 36% of the population is of South Asian descent (http://www.brampton.ca/en/Info-Centre/Pages/Welcome.aspx). BCH routinely screens for carbapenem-producing Enterobacteriaceae (CPE) rectal colonization among close contacts of known CPE-colonized patients according to the Ontario Provincial Infectious Diseases Advisory Committee on Infection Prevention and Control guidelines [11].

On 7 October 2011, the first case of CPE was identified at BCH from a wound culture. This prompted initiation of active surveillance of all patients in the ward. The surveillance identified 2 additional patients in the ward, and outbreak was declared on 27 October 2011. Weekly screening of all patients was conducted until no further transmission was detected in the affected units. Patients with confirmed CPE were placed in isolation under contact precautions. A restricted visitor policy and daily as well as terminal cleaning of all rooms were implemented [11]. Patients who were transferred from any affected unit to other locations in the hospital were placed in contact isolation until 2 negative weekly surveillance results were obtained from them. A point prevalence investigation was also conducted in the intensive care unit (ICU) to screen all patients whose stay had overlapped with a known case. Patients discharged home or to another facility were screened for CPE on discharge. These patients’ medical records were flagged as being on active surveillance in the event that they returned to the hospital before the latest microbiological results were known.

For the purposes of this study, 2 rectal swab specimens per patient were collected as part of the surveillance strategy: the first for direct detection of carbapenemase genes by multiplex real-time polymerase chain reaction (mRT-PCR; see below) and the second for culture, to identify and confirm isolates positive for blaNDM genes. In addition, 2 environmental samples from various sources including sinks, toilet seats, bed rails, and tables, were also collected from the affected units (respirology, oncology, and ICU) for culture and mRT-PCR assays.

Bacterial Isolation, Identification, and Antimicrobial Susceptibility Profiles
Carbapenem-resistant microorganisms were screened by plating specimens onto either extended-spectrum β-lactamase isolation media (Oxoid) or CHROMagar C3GR media (Alere). All suspect colonies were subcultured onto purity plates. Growth from the purity plates was used for identification and tested at BCH for antibiotic susceptibility, using the Vitek 2 system (BioMérieux). Isolates that were nonsusceptible to carbapenems (ertapenem [minimum inhibitory concentration ≥ 1 mg/L] and meropenem [minimum inhibitory concentration ≥ 2 mg/L]) were sent for confirmation to the Public Health Ontario Laboratories (PHOL), which serves as a reference laboratory for the province of Ontario. At the PHOL, isolates were screened for carbapenemase activity using the phenotypic inhibitor testing method, and if positive were confirmed for the presence of carbapenemase gene using multiplex PCR [10, 12]. In addition, susceptibility testing was performed using Etest (bioMérieux) and agar dilution method, with results interpreted according to the Clinical and Laboratory Standards Institute guidelines [13], except colistin and tigecycline, for which the 2011 European Committee on Antimicrobial Susceptibility Testing break points were used (available at http://www.eucast.org/clinical_breakpoints/).

Bacterial Typing
Clinical K. pneumoniae and E. coli isolates were genotyped by both pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), as described elsewhere [14–16]. The allelic numbers and sequence types (STs) were assigned using online databases (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html for K. pneumoniae, and http://mlst.ucc.ie/mlst/dbs/Ecoli for E. coli).

mRT-PCR for Detection of blaNDM
Rectal and environmental swab specimens were tested directly for the presence of blaNDM using an mRT-PCR assay that can simultaneously detect both blaNDM and blaKPC genes. Briefly, each rectal swab specimen was vortexed in 500 µL of saline. DNA was extracted from 250 µL of the sample using an automated NucliSENS easyMAG extractor (bioMérieux), according to the manufacturer’s recommendations. The protocol of the Centers for Disease Control and Prevention was used for the amplification assays (available at http://www.cdc.gov/HAI/settings/lab/kpc-ndm1-lab-protocol.html).

Molecular Screening of Resistance Determinants
Enterobacterial isolates exhibiting carbapenemase activity were further screened for the presence of β-lactamase and 16S ribosomal RNA methylase genes [10, 17], and alleles were identified by sequencing the complete genes. The nucleotide and deduced amino acid sequences were analyzed with the Vector NTI analysis software package (version 10.3.0; Invitrogen). Searches of sequences were performed with the BLAST program, available at the National Center for Biotechnology Information Web site (http://www.ncbi.nlm.nih.gov/). Multiple-sequence alignments were performed with the ClustalX
program, available at the European Bioinformatics Institute Web site (http://www.ebi.ac.uk/Tools/msa/clustalw2).

**Plasmid Characterization**

For conjugative assays, cells of both the donor (*K. pneumoniae* Kp-1 and *E. coli* Ec-2) and the recipient strains (sodium azide-resistant *E. coli* J53) were mixed on Luria-Bertani agar at a ratio of 1:1, and the mixtures were incubated for 18 hours at 36°C. Transconjugants were selected on LB agar supplemented with sodium azide (100 mg/L) and meropenem (0.125 mg/L). To estimate sizes of *bla<sub>NDM-1</sub>* plasmids, genomic DNA agarose plugs of each clinical and transconjugant strains were partially digested with the endonuclease S1 [18]. DNA bands were separated by PFGE under the following conditions: 0.5 Tris-Borate-EDTA, 1% agarose, 14°C, and 6 V/cm voltage gradient for 6 hours with switch time ramping from 5 to 25 seconds and 18 hours with switch time ramping from 30 to 45 seconds. Plasmids were transferred and immobilized on a nylon membrane and identified by Southern blot analysis, using specific *bla<sub>NDM</sub>* digoxigenin-labeled probes (Roche Diagnostics). Plasmid replicons were determined using the PCR-based replicon typing procedure [19]. Probes of the positive replicons were also prepared and used to identify the *bla<sub>NDM-1</sub>* plasmids.

**RESULTS**

**Epidemiological Investigation**

Patient A, a 73-year-old man with no recent travel history, was admitted on 9 September 2011 to the BCH ICU with presumptive community-acquired pneumonia. Broad-spectrum antibiotic treatment was started. On 23 September, the patient was transferred to the respirology ward where a multidrug-resistant *K. pneumoniae* (strain Kp-1) was isolated from a wound culture on 7 October. Patient A remained in long-term rehabilitation persistently colonized with carbapenem-resistant *K. pneumoniae* from rectal surveillance swab specimens as well as from urine specimens. He was asymptomatic from the urine and remained off any antibiotics. A Kp-1 isolate was submitted to the PHOL, where it was confirmed as a metallo-ß-lactamase–producing strain with the inhibitor test and was subsequently positive for the *bla<sub>NDM-1</sub>* gene by PCR and sequencing. This finding prompted rectal surveillance cultures of all patients in the unit (23 rooms with a 37-patient capacity; Figures 1 and 2). Rectal swab specimens collected on 19 October identified 2 additional patients colonized with a *K. pneumoniae* isolate carrying the *bla<sub>NDM-1</sub>* gene (strains Kp-2 and Kp-3).

![Figure 1. Timeline of events in the epidemiologically linked cases of New Delhi metallo-ß-lactamase 1–producing *Klebsiella pneumoniae* and *Escherichia coli* in 2011. Dates are given as date/month. Abbreviations: ER, emergency room; ICU, intensive care unit; swab, swab specimen.](https://academic.oup.com/cid/article-abstract/55/11/e109/370217)
Patient B, a 76-year-old woman, was admitted to the BCH Emergency Department on 26 August and transferred to the respirology unit on 27 August with *Salmonella enterica* bacteremia. On 25 October, for unrelated comorbid conditions, she was discharged to home for palliative care. The patient had last traveled to Vietnam in March 2011, returning to Canada with no hospitalization abroad.

Patient C was a 77-year-old woman admitted to the BCH emergency department with hepatic encephalopathy on 30 September, transferred to the respirology unit on 3 October into a room shared with 3 other patients, and discharged back to a long-term care facility on 19 October. Patients B and C were contemporaneously in close geographic proximity to the index case patient on the respirology unit (Figures 1 and 2). As a result of this investigation, an outbreak was declared on 28 October, with closure of the respirology unit and implementation of contact precautions. At this point, rectal and environmental swab specimens were obtained (2 swab specimens each, as described in Materials and Methods) to check for the presence of *bla*NDM-1 with mRT-PCR.

On 12 November, a 79-year-old man (patient D) admitted to the BCH ICU on 18 October with pyelonephritis and gastrointestinal bleeding was identified with *E. coli* (strain Ec-1) containing *bla*NDM-1 from blood and urine specimens. This patient had stayed on the respirology unit 21–24 October before his surgery and subsequently transferred to the oncology unit (Figures 1 and 2). Using mRT-PCR, it was possible to detect the *bla*NDM-1 gene from patient D’s rectal swab specimens within 24 hours of specimen collection. The second rectal swab specimen collected for culture on the same day yielded *K. pneumoniae* and *E. coli* isolates 48 hours later, both of them positive for *bla*NDM-1 gene (strains Kp-4 and Ec-2, respectively). Patient D was treated with tigecycline (50 mg/d intravenous) for 14 days, and his condition clinically improved. He was discharged on 29 November in good condition with outpatient follow-up. This patient had returned from a 3-week trip to Guyana before his admission to BCH and had not been hospitalized outside of Canada.

Patient E, the roommate of patient D, was a 60-year-old man admitted to BCH with diabetic foot infection and osteomyelitis on 7 November and transferred to the oncology unit on 8 November; a swab specimen was subsequently obtained for CPE colonization on 16 November. This patient was identified as having the *bla*NDM-1 gene by mRT-PCR, but his culture was negative for CPE. Two additional rectal swab specimens collected 48 hours later yielded a *K. pneumoniae* isolate containing the *bla*NDM-1 gene (strain Kp-5). The identification of patient E on the oncology unit led to the initiation of weekly surveillance to identify any additional CPE-colonized patients on the oncology and respirology units, as described
above; no new CPE-colonized patients were identified. No further transmission was detected in any affected unit, and the outbreak was declared over on 24 November.

**Bacterial Typing, Susceptibility Profiles, ß-Lactamase Content, and Plasmid Characterization**

Both PFGE and MLST data showed that *K. pneumoniae* isolates from 5 patients were clonally related and belonged to the clonal group ST231. *E. coli* isolates from patient D were identical by PFGE and were typed as ST1193 (Figure 3). All *K. pneumoniae* and *E. coli* isolates were highly resistant to most of the antibiotics tested (Table 1). Molecular characterization of *K. pneumoniae* showed that all of the isolates harbored blaNDM-1, blaCTX-M-15, blashv, blaTEM-1, blacmy-6 and rmtC genes. Plasmids from the same incompatibility groups were identified (Table 1). On the other hand, clinical isolates Ec-1 and Ec-2 carried only blaNDM-1, blacmy-6 and rmtC genes, with both isolates harboring the same plasmid types but only one in common with the *K. pneumoniae* isolates (ie, A/C incompatibility plasmid group, IncA/C). This IncA/C plasmid was transferred by conjugation from clinical isolates Kp-1 and Ec-2 to *E. coli* J53, co-harboring blaNDM-1, blacmy-6 and rmtC (Table 1). By S1 nuclease-PFGE method and Southern blot analysis, a plasmid of ~130 kb was positive for the blaNDM probe in all the strains except Kp-1, which showed a slightly smaller plasmid (Figure 4). All the NDM-1 plasmids were also positive for the IncA/C probe (data not shown).

**DISCUSSION**

This report describes the detection and containment of an outbreak of NDM-1–producing Enterobacteriaceae in Ontario, Canada. The 5 NDM-1–producing *K. pneumoniae* isolates were genetically related by PFGE and belonged to the same clonal group, ST231. They also displayed the same plasmid profile, genetic determinants of antimicrobial resistance, and plasmid replicon types. There are few reports of outbreaks due to NDM-1–producing Enterobacteriaceae in South Asia, Europe, and very recently in the United States [20–23]. However, to the best of our knowledge, there are no previous descriptions of outbreaks with organisms that produce NDM-1 in Canada. Previous studies have been limited to single case reports of NDM-1–producing Enterobacteriaceae [8–10]. The blaNDM-1 gene has been identified mostly in nonclonally related isolates of *E. coli* and *K. pneumoniae* [3, 24]. In a very recent study, the most frequently detected STs found in clinical isolates of NDM-1–producing *K. pneumoniae* from India, the United Kingdom and Sweden were ST14, ST11, ST149, ST231, and ST147 [25]. ST14 and ST147 were also found in other countries [26]. ST231 isolates from the United Kingdom have also been recently identified in India and Mauritius [27, 28], indicating its role in the NDM-1 dissemination. Both *E. coli* strains isolated from patient D belonged to the ST1193, not previously linked to the production of NDM. Nevertheless, the susceptibility profile displayed by this ST expressing NDM-1, susceptible only to tigecycline, is of concern but we were unable to assess its potential for clonal dissemination.

*K. pneumoniae* Kp-1 and *E. coli* Ec-2 transferred only the IncA/C plasmid to a susceptible *E. coli* when meropenem was used as selector, and both *E. coli* transconjugant strains (J-Kp-1 and J-Ec-2) were positive only for blaNDM-1, blacmy-6 and rmtC genes. These horizontal transfers proved the capability of this NDM-1 plasmid for in vitro dissemination. Similarly, both clinical *E. coli* strains Ec-1 and Ec-2 recovered from patient D also carried an IncA/C plasmid, similar in size to that observed in *K. pneumoniae* from the same patient (Figure 4), and harbored only blaNDM-1, blacmy-6, and rmtC genes, as in the transconjugant strains. Mulvey and collaborators described a possible in vivo horizontal transfer of an IncA/C plasmid carrying blaNDM-1, blacmy-6, and probably a
16S methylase gene like \(rmtC\) (based on the aminoglycoside susceptibility profiles of these isolates) between \(K.\ pneumoniae\) and \(E.\ coli\) [8]. Considering this study and the fact that \(bla_{NDM-1}\) genes are commonly carried on IncA/C broad-host-range plasmids [3, 4, 25], our results also suggest a possible in vivo interspecies dissemination of this plasmid in a single patient. Additional studies are ongoing to confirm this suspicion. IncA/C plasmids are large plasmids isolated from diverse groups of \(Proteobacteria\) found in the environment, food animals, food products, and human pathogens [29–32]. These plasmids have flexibility in the acquisition of resistant-encoding determinants and their presence increase the potential of reaching any gram-negative bacterium, providing an additional risk of dissemination in the community [33].

There is epidemiological evidence that travel to the Indian subcontinent is a significant risk factor for acquisition and infection with NDM-1-producing bacteria [3, 4]. The widespread presence of multidrug-resistant microorganisms carrying

### Table 1. Antibiotic Susceptibility Profiles, Resistance Genes, Multilocus Sequence Typing, and Replicon Types of \(Klebsiella\ pneumo-niae\) and \(Escherichia coli\) Clinical Isolates and \(E.\ coli\) Transconjugant Strains

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<th>Kp-3</th>
<th>Kp-4</th>
<th>Kp-5</th>
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**PCR**

| **bla**_{NDM-1} | + | + | + | + | + | + | + | + | ND |
| **bla**_{CTX-M-15} | + | + | + | + | + | + | + | + | ND |
| **bla**_{SHV} | + | + | + | + | + | + | + | + | ND |
| **bla**_{TEM-1} | + | + | + | + | + | + | + | + | ND |
| **bla**_{TEM-6} | + | + | + | + | + | + | + | + | ND |
| **rmtC** | + | + | + | + | + | + | + | + | ND |

MLST

| ST231 | ST231 | ST231 | ST231 | ST231 | ST1193 | ST1193 | ND | ND | ND |

Replicon typing

| FIA | FIA | FIA | FIA | FIA | FIA | FIA | FIA | FIA | FIA |

Abbreviations: Ec, \(E.\ coli\) strains; Eco J53, recipient \(E.\ coli\) J53; J-Kp-1 and J-Ec-2, \(E.\ coli\) J53 transconjugant strains derived from Kp-1 and Ec-2 clinical isolates; Kp, \(K.\ pneumoniae\) strains; MIC, minimum inhibitory concentration; MLST, multilocus sequence typing; ND, not determined; PCR, polymerase chain reaction. Plus signs indicate positive results; minus signs, negative results.

\(a\) Susceptibility tests for tigecycline and colistin were interpreted according to the 2011 European Committee on Antimicrobial Susceptibility Testing break points. Otherwise, 2011 Clinical Laboratory Standards Institute break points were used.

\(b\) PCR screening included \(bla_{TEM},\) \(bla_{SHV},\) \(bla_{CTX-M-15},\) \(bla_{CTX-M-15a,b,c} \) groups 1, 2 and 9, \(bla_{VEB},\) \(bla_{PER},\) \(bla_{GES},\) \(bla_{OXA-48-like},\) \(bla_{IMP},\) \(bla_{VIM},\) \(bla_{KPC},\) \(bla_{NDM},\) and 6 groups of \(bla_{spp}\) \(\beta\)-lactamase genes, as well as \(armA,\) \(mtraE,\) and \(nmpA\) 16S methylase genes.
NDM-1 has also been described in the United Kingdom, Balkan states, and the Middle Eastern countries [3, 24, 34]. In this study, none of the 5 patients detected in the outbreak had any reported travel history to, or hospitalization in, an area where NDM-1 is endemic. We were unable to determine an obvious source of this organism because results of environmental screening were negative and additional patient screening did not identify a patient with a history of travel to an endemic area. Possible source of this outbreak includes transmission from another unrecognized colonized patient who may have been discharged before the identification of patient A. There is also a theoretical possibility that patient B was already colonized at the time of her admission. This patient was in Vietnam some months before her hospitalization in BCH. Very recently, a small outbreak was described in the United States involving a patient hospitalized in Vietnam [23]. NDM-1–positive K. pneumoniae isolated from environmental samples were also documented in that country [35]. However, because Vietnam is not considered endemic for that carbapenemase, patient B was not screened for CPE until the first case in the unit was identified.

Considering the global emergence of NDM-1, screening for CPE colonization only in patients with prior history of travel to endemic regions may no longer be prudent. However, it may not be cost-effective for hospitals to routinely screen for CPE colonization (even under the risk of underestimating these resistant isolates) and they should at least screen high-risk patients, particularly those returning from known endemic regions. Another possible explanation for this outbreak might be that one of the patients was exposed to a person in the community colonized with NDM-1–producing microorganisms. According to the PHOL results from a continuous surveillance of CPE in Ontario, the number of NDM-1–positive cases in the province has increased from 9 in 2010, when this metallo-ß-lactamase was first described in Ontario [10], to 22 cases in 2011 [36]. Approximately half of

Figure 4. Identification of New Delhi metallo-ß-lactamase 1 plasmids. A, S1 nuclease-pulsed – field gel electrophoresis plasmid profiles. B, Autoradiograph of gel A hybridized with blaNDM-1 probe (same results were obtained using IncA/C probe). Arrowheads indicate positive bands. S, reference standard strain H9812 restricted with XbaI (sizes are given in kilobases).
these cases were isolated from the Brampton area, which has large proportions of residents who travel to the Indian subcontinent. Locally acquired community infection with NDM-1 producers have been described in endemic areas [37] and also reported in Canada and France [38, 39]. In addition, NDM-1 producers in human flora have been shown to persist for >1 year [40]. However, community transmission would be difficult to prove without knowing the prevalence of CPE colonization in the community settings.

Our results demonstrate the value of molecular methods, such as PFGE, MLST, and mRT-PCR, as indispensable tools in outbreak investigation and management. In particular, the mRT-PCR assay allowed us to detect positive CPE isolates directly from rectal specimens, which may be of particular importance in the rapid identification and isolation of colonized patients.

Although standard infection-control methods were able to limit the spread of this organism in the hospital setting, the greater threat may be its eventual dissemination in the community and the loss of the ability to identify high-risk persons on whom to carry out surveillance testing. The needs are now for sensitive, specific, and cost-effective testing methods for routine laboratory use. In addition, newer antibiotics are needed to provide clinicians with options to treat infections caused by these multidrug-resistant organisms.

Notes

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