New Delhi Metallo-β-Lactamase, Ontario, Canada

To the Editor: The New Delhi metallo-β-lactamase (NDM-1) was first characterized in 2009 from *Klebsiella pneumoniae* and *Escherichia coli* isolated from a patient in Sweden who had received medical care in New Delhi, India (*1*). Further studies have shown broad dissemination of this β-lactamase gene (bla_{NDM-1}) in India, Pakistan, Bangladesh, and the United Kingdom (*2*). Additional isolates have been detected in other countries, and many of the patients with NDM-1–producing *Enterobacteriaceae* reported receiving medical care in the Indian subcontinent (*1*–*7*). We describe detection and characterization of an NDM-1–producing *K. pneumoniae* isolated in Ontario, Canada.

In August 2010, a urinary tract infection was diagnosed in a 36-year-old woman in a hospital in Brampton, Ontario. An *E. coli* strain sensitive to multiple antibacterial drugs (including carbapenems) was isolated from a midstream urine sample; the patient was successfully treated with ciprofloxacin. One week after treatment, when the patient did not have a fever or other clinical signs, a urine culture was repeated, and a carbapenem-resistant *K. pneumoniae* isolate (GN529) was recovered. Travel history indicated that the patient had recently returned from India, where in mid-July she had had a miscarriage and had been hospitalized in Mumbai for 2 days. At that time, no antimicrobial drug treatment was prescribed.

Susceptibility profiles of *K. pneumoniae* GN529 and its *E. coli* transconjugant were obtained by using Etest (bioMérieux, Marcy l'Etoile, France) and the agar dilution method based on the Clinical and Laboratory Standards Institute guidelines (*8*). Multilocus sequence typing (MLST) of isolate GN529 was performed as described (*9*). The Pasteur Institute online database (www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) was used to assign the allelic numbers and sequence type (ST).

To screen for the most commonly known β-lactamase genes in enterobacteria, we performed multiplex PCRs (*10*). Primers were designed (NDM-F, 5′-AATGGAATTGCCCAATATTATGC-3′; NDM-R, 5′-CGAAAGTCAGGCTGTGTTG C-3′) for the specific detection of bla_{NDM-1} and included in 1 of the multiplex PCRs (multiplex V). Primers NDM-F and NDM-R2 (5′-TCAGCGCAGCTTGTCGGC-3′) were used to amplify and sequence the entire bla_{NDM-1} gene. The samples were screened for the presence of six 16S methylase genes (*armA*, *rmtA–D*, and *npmA*) by PCR. *E. coli* J53 transconjugants were selected on Luria-Bertani plates containing sodium azide and meropenem (100 μg/mL and 1 μg/mL, respectively). The plasmid harboring bla_{NDM-1} was identified by Southern blot analysis by using a specific digoxigenin-labeled bla_{NDM-1} probe (Roche Diagnostics, Indianapolis, IN, USA).

K. pneumoniae GN529 was highly resistant to all β-lactams, aminoglycosides, quinolones, tetracycline, nitrofurantoin, and co-trimoxazole. MICs of 0.5 μg/mL for colistin (European Committee on Antimicrobial Susceptibility Testing colistin breakpoint for *Enterobacteriaceae*: susceptibility ≤2 μg/mL) and 1 μg/mL for tigecycline (European Committee on Antimicrobial Susceptibility Testing and US Food and Drug Administration tigecycline breakpoint for *Enterobacteriaceae*: susceptibility ≤1 and ≤2 μg/mL, respectively) were also obtained (Table).

Considering the travel history of the patient and the high level re-

Table. Antibacterial drug susceptibility profiles and resistance genes of *Klebsiella pneumoniae* GN529 clinical isolate and its *Escherichia coli* transconjugant, Ontario, Canada, 2010*

Antibacterial drug or gene	MIC, μg/mL		
	Kpn GN529	Eco J529	Eco J53
Ampicillin	≥256	≥256	6
Cefoxitin	≥256	≥256	8
Ceftazidime	≥256	≥256	0.19
Cefotaxime	≥256	≥256	0.094
Cefepime	48	48	0.064
Ertapenem	32	12	0.008
Meropenem	≥32	4	0.023
Imipenem	≥32	32	0.38
Amikacin	≥256	≥256	1.5
Gentamicin	≥256	≥256	1.5
Tobramycin	≥256	≥256	1
Ciprofloxacin	≥32	0.012	0.012
Tetracycline	≥16	0.78	1
Tigecycline	1	ND	ND
Nitrofurantoin	≥512	ND	ND
Colistin	0.5	ND	ND
Co-trimoxazole	4/76	ND	ND
PCR† and sequencing			
bla_{NDM-1}	+	+	ND
$bla_{CTX-M-15}$	+	–	ND
bla_{SHV-12}	+	+	ND
bla_{SHV-11}	+	–	ND
bla_{OXA-1}	+	–	ND
bla_{TEM-1}	+	–	ND
armA	+	+	ND

*Kpn, *Klebsiella pneumoniae*; Eco J529, *Escherichia coli* transconjugant strain; Eco J53, recipient *E. coli* J53; ND, not determined.
†PCR screening included bla_{TEM}, bla_{SHV}, bla_{OXA-1}-like, bla_{CTX-M} groups 1, 2, and 9, bla_{VEB}, bla_{PER}, bla_{GES}, bla_{OXA-48}-like, bla_{IMP}, bla_{VIM}, bla_{KPC}, bla_{NDM-1}, and 6 groups of bla_{AmpC} genes.

sistance to all β-lactams, molecular screening of β-lactamases in strain GN529 was initiated to identify possible carbapenemases (e.g., bla_{NDM-1}) in that isolate. Five β-lactamases genes (bla_{NDM}, bla_{SHV}, bla_{TEM}, group 1 bla_{CTX-M} , and bla_{OXA}) and one 16S rRNA methylase (*armA*) were detected. By using primers for amplification of complete genes, we obtained sequences of bla_{NDM-1}, 2 extended-spectrum β-lactamases ($bla_{CTX-M-15}$ and bla_{SHV-12}), 3 broad-spectrum β-lactamases (bla_{SHV-11}, bla_{TEM-1} and bla_{OXA-1}), and methyltransferase *armA*. No AmpC β-lactamases were linked to this isolate. Southern blotting identified a plasmid of ≈150 kb harboring bla_{NDM-1} (data not shown). A transconjugant *E. coli* positive for bla_{NDM-1} (*E. coli* J529, Table) was resistant to all β-lactams and aminoglycosides tested. In addition, bla_{SHV-12} and *armA* were detected in strain J529 (Table), indicating the potential for the horizontal spread of these resistance genes.

K. pneumoniae GN529 was typed by MLST as ST147, the same type as a clinical NDM-1–producing strain isolated in Australia (*6*) but distinct from ST14 and ST16 strains described (*1*,*7*). There are insufficient MLST data to confirm polyclonal dissemination of NDM-1, but previous pulsed-field gel electrophoresis results support that hypothesis (*2*).

K. pneumoniae GN529 was isolated from a patient who had recently received emergency medical care in India, suggesting importation of this clinical strain. In the United Kingdom, where *Enterobacteriaceae* containing bla_{NDM-1} are increasingly common, carriage of these organisms has been closely linked to receipt of medical care in the Indian subcontinent (*2*). Similar association as a risk factor was observed in other regions, including bla_{NDM-1}-positive clinical strains isolated in North America, Australia, and Africa (*3*–*6*,*10*).

The NDM-1–producing enterobacteria described in this study previously had low MICs only for colistin and tigecycline (*1*,*2*,*5*,*6*). However, an NDM-1 isolate resistant to these antimicrobial drugs has also been described (*2*). Early detection and implementation of infection control interventions is essential for preventing the spread of multidrug-resistant organisms such as these. It may be prudent to consider medical exposure in the Indian subcontinent as a risk factor for possible infection, colonization, or both with multidrug-resistant, NDM-1–producing *Enterobacteriaceae*.

Acknowledgments

We thank Prasad Rawte, Stephen Lo, Heather Siebert, Jennifer Ma, and Keisha Warren for technical support.

Nathalie Tijet, David C. Alexander, David Richardson, Olga Lastovetska, Donald E. Low, Samir N. Patel, and Roberto G. Melano

Author affiliations: Ontario Agency for Health Protection and Promotion, Toronto, Ontario, Canada (N. Tijet, D.C. Alexander, O. Lastovetska, D.E. Low, S.N. Patel, R.G. Melano); University of Toronto, Toronto (D.C. Alexander, O. Lastovetska, D.E. Low, S.N. Patel); William Osler Health Centre, Brampton Civic Hospital, Brampton, Ontario, Canada (D. Richardson); and Mount Sinai Hospital, Toronto (D.E. Low, R.G. Melano)

DOI: 10.3201/eid1702.101561

References

1. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53:5046–54. DOI: 10.1128/AAC.00774-09
2. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10:597–602. DOI: 10.1016/S1473-3099(10)70143-2
3. Centers for Disease Control and Prevention. Detection of *Enterobacteriaceae* isolates carrying metallo-β-lactamase—United States, 2010. MMWR Morb Mortal Wkly Rep. 2010;59:750.
4. Poirel L, Lagrutta E, Taylor P, Pham J, Nordmann P. Emergence of metallo-β-lactamase NDM-1–producing multidrug resistant *Escherichia coli* in Australia. Antimicrob Agents Chemother. 2010;54:4914–6. DOI: 10.1128/AAC.00878-10
5. Poirel L, Revathi G, Bernabeu S, Nordmann P. Emergence of metallo-β-lactamase NDM-1 producing *Klebsiella pneumoniae* in Kenya. In: Abstracts of the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy; Boston; 2010 Sep 12–15; Abstract C1–1334. Washington: American Society of Microbiology; 2010.
6. Sidjabat HE, Nimmo GR, Binotto E, Enbom R, George N, Paterson DL. A new threat from carbapenem resistant *Klebsiella pneumoniae*—the New Delhi metallo-β-lactamase (NDM-1). In: Abstracts of the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy; Boston; 2010 Sep 12–15. Abstract C1–1332. Washington: American Society of Microbiology; 2010.
7. Mulvey MR, Grant JM, Plewes K, Roscoe D, Boyd DA. New Delhi metallo-β-lactamase in *Klebsiella pnemoniae* and *Escherichia coli,* Canada. Emerg Infect Dis. 2011 Feb; [Epub ahead of print]. 10.3201/eid1701.101358.
8. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 20th Informational supplement M100–S20. Wayne (PA): The Institute; 2010.
9. Diancourt L, Passet V, Verhoef J, Grimont PAD, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J Clin Microbiol. 2005;43:4178–82. DOI: 10.1128/JCM.43.8.4178-4182.2005
10. Dallenne C, Da Costa A, Decre D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in *Enterobacteriaceae.* J Antimicrob Chemother. 2010;65:490–5. DOI: 10.1093/jac/dkp498

Address for correspondence: Roberto G. Melano, Ontario Agency for Health Protection and Promotion, Public Health Laboratory Branch, 81 Resources Rd, Toronto, Ontario M9P 3T1, Canada; email: roberto.melano@oahpp.ca

All material published in Emerging Infectious Diseases is in the public domain and may be used and reprinted without special permission; proper citation, however, is required.