AAC Journals.ASM.org

A Case of New Delhi Metallo-β-Lactamase 1 (NDM-1)-Producing *Klebsiella pneumoniae* with Putative Secondary Transmission from the Balkan Region in the Netherlands

New Delhi metallo-beta-lactamase 1 (NDM-1) was first identified in 2008 in a *Klebsiella pneumoniae* strain from a Swedish patient of Indian origin with a history of medical treatment in a New Delhi hospital for a urinary tract infection (12). Since then, more such cases in different parts of the world have been reported (6). The majority of these patients were found to have traveled to or been admitted to hospitals in the Indian subcontinent. A few patients had a travel history in the Balkan area (11). Here we describe a Dutch patient proven to be carrying an NDM-1-producing *K. pneumoniae* strain that was imported from the Balkan area. Furthermore, a second patient apparently acquired this strain during her stay in the same hospital as the index case.

Patient A, a 66-year-old female with a cerebrovascular accident, was transferred from a hospital in Belgrade, Serbia, to the neurology department of a hospital in the east of the Netherlands on 27 August 2008. Since the patient was known to carry methicillin-resistant *Staphylococcus aureus* (MRSA), she was directly placed in a separate room in isolation. During admission, extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae*, as determined according to the 2008 CLSI guidelines (2), was isolated from different sites, including throat, rectum, and urinary tract. The patient was treated for the urinary tract infection with nitrofurantoin, to which the isolate was susceptible, and with removal of the urinary catheter. She was discharged from the hospital on 15 October to a nursing home, where contact isolation measures were maintained. Follow-up screening cultures remained positive for ESBL-producing *K. pneumoniae* up to March 2009, but subsequent cultures obtained on several occasions between April and September 2009 were negative.

Patient B, a 73-year-old female with no travel history outside the Netherlands, was admitted with exacerbation of pulmonary symptoms to the department of pulmonary disease (DPD) in the same hospital as the first patient between 10 October and 7 November 2008. At the end of October, ESBL-producing *K. pneumoniae* was cultured from a urine specimen. The patient was treated with oral amoxicillin-clavulanate in addition to removal of the urinary catheter and was discharged from the hospital in good condition. A urinary culture obtained during readmission to the DPD on 25 June 2010 with pulmonary symptoms and urinary tract infection yielded only *Escherichia coli*. For the urinary tract infection, the patient was treated with ciprofloxacin, to which the *E. coli* strain was susceptible. The patient died on 18 July 2010.

Two ESBL-producing *K. pneumoniae* isolates, one from each of these two patients, were selected for further analysis aimed at the presence of carbapenemase because of their elevated MICs to meropenem according to the 2008 CLSI guidelines (2). Antimicrobial susceptibility testing using the EUCAST breakpoints (www.eucast.org) was performed with the Vitek2 automated system (bioMérieux). Ertapenem, imipenem, meropenem, tigecy-

Received 16 January 2012 **Returned for modification** 29 January 2012
Accepted 4 February 2012
Published ahead of print 13 February 2012
Address correspondence to Teysir Halaby, t.halaby@labmicta.nl.
* Present address: Laboratory for Medical Microbiology and Public Health, Enschede, The Netherlands.

doi:10.1128/AAC.00111-12

TABLE 1 Results of antimicrobial susceptibility testing and phenotypic confirmations for two ESBL-producing *K. pneumoniae* isolates, one from each of the two cases

Patient	Specimen	MIC (μg/ml)										Phenotypic confirmation[c]				
													MHT for[e]:			
		Ceftazidime	Cefepime	Gentamicin	Tobramycin	Ciprofloxacin	Imipenem	Meropenem	Ertapenem	Colistin	Tigecycline	ESBL[d]	Imipenem	Meropenem	CD[f] imipenem-EDTA	Ertapenem-boronic acid[g]
1[a]	Urine	>64	32	>16	>16	>4	8	3	6	0.25	1.5	Pos	Pos	Pos	Pos	Neg
2[b]	Urine	>64	32	>16	>16	>4	6	3	6	0.19	1.5	Pos	Pos	Pos	Pos	Neg

[a] Index patient.
[b] Secondary case.
[c] Pos, positive; neg, negative.
[d] ESBL, extended-spectrum beta-lactamase (laboratory detection of highly resistant microorganisms [HRMO]; http://www.nvmm.nl).
[e] MHT, modified Hodge test (3).
[f] CD, combined disc (7).
[g] See reference 5.

cline, and colistin MIC values were determined by the Etest. The results of retrospective antimicrobial susceptibility testing and phenotypic confirmations are shown in Table 1. PCR and sequencing (9) confirmed the presence of NDM-1, CTX-M-15, SHV-12, and OXA-1. Molecular plasmid analysis (9) revealed the presence of a 70-kb IncII plasmid containing $bla_{\text{NDM-1}}$ and a 140-kb plasmid. By amplified fragment length polymorphism (AFLP) typing (10), the strains were shown to be identical (data not shown), a result which strongly suggests that the strain was transmitted from the index patient to the secondary case, although the transmission pathway remains unknown.

By multilocus sequence typing (MLST) (4), the strain of the index patient was found to belong to sequence type 15 (ST15). This MLST type was recently described in Belgium for an NDM-1-producing *K. pneumoniae* isolate from a patient who had been previously hospitalized in Podgorica, Montenegro (1). The strain from the secondary case was ST431, an MLST type with only one base pair change from ST15. In conclusion, our study describes one of the first cases of NDM-1-producing *K. pneumoniae* with apparent secondary transmission, as evidenced by the findings from AFLP typing. Furthermore, the strain belongs to ST15, which has recently been isolated from a patient returning from the Balkan area, supporting the hypothesis of the Balkan region as a reservoir of NDM-1-producing Gram-negative bacteria (8).

REFERENCES

1. **Bogaerts P, et al.** 2011. Emergence of NDM-1-producing *Enterobacteriaceae* in Belgium. Antimicrob. Agents Chemother. **55**:3036–3038.
2. **Clinical and Laboratory Standards Institute.** 2008. Performance standards for antimicrobial susceptibility testing; 18th informational supplement. CLSI M100-S18. Clinical and Laboratory Standards Institute, Wayne, PA.
3. **Clinical and Laboratory Standards Institute.** 2010. Performance standards for antimicrobial susceptibility testing: 20th informational supplement. CLSI document M100-S20. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S.** 2005. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J. Clin. Microbiol. **43**:4178–4182.
5. **Doi Y, et al.** 2008. Simple disk-based method for detection of *Klebsiella pneumoniae* carbapenemase-type β-lactamase by use of a boronic acid compound. J. Clin. Microbiol. **46**:4083–4086.
6. **Grundmann H, et al.** 2010. Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Euro Surveill. **15**:pii=19711.
7. **Lee K, Lim YS, Yong D, Yum JH, Chong Y.** 2003. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J. Clin. Microbiol. **41**:4623–4629.
8. **Livermore DM, Walsh TR, Toleman M, Woodford N.** 2011. Escape or transplant? Lancet Infect. Dis. **11**:164.
9. **Poirel L, et al.** 2011. Cross-border transmission of OXA-48-producing Enterobacter cloacae from Morocco to France. J. Antimicrob. Chemother. **66**:1181–1182.
10. **Savelkoul PH, et al.** 1999. Amplified-fragment length polymorphism analysis: the state of an art. J. Clin. Microbiol. **37**:3083–3091.
11. **Struelens MJ, et al.** 2010. New Delhi metallo-beta-lactamase 1-producing Enterobacteriaceae: emergence and response in Europe. Euro Surveill. **15**:pii=19716.
12. **Yong D, et al.** 2009. Characterization of a new metallo-beta-lactamase gene, $bla_{\text{NDM-1}}$, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob. Agents Chemother. **53**:5046–5054.

Teysir Halaby
Laboratory for Medical Microbiology and Public Health
Enschede, The Netherlands

Ascelijn E. Reuland
Nashwan al Naiemi*
Department of Medical Microbiology & Infection Control
VU University Medical Center
Amsterdam, The Netherlands

Anais Potron
Service de Bactériologie-Virologie
Hôpital de Bicêtre et Unité INSERM U914
Le Kremlin Bicêtre, France

Paul H. M. Savelkoul
Christina M. J. E. Vandenbroucke-Grauls
Department of Medical Microbiology & Infection Control
VU University Medical Center
Amsterdam, The Netherlands

Patrice Nordmann
Service de Bactériologie-Virologie
Hôpital de Bicêtre et Unité INSERM U914
Le Kremlin Bicêtre, France