AAC Journals.ASM.org

Emergence of NDM-1 in Association with OXA-48 in *Klebsiella pneumoniae* from Tunisia

Adam Ben Nasr,[a,b] Dominique Decré,[c,d] Fabrice Compain,[c] Nathalie Genel,[c] Farouk Barguellil,[b,e] Guillaume Arlet[c,d]

Laboratoire de Biologie Médicale, Hôpital Habib Thameur, Tunis, Tunisia[a]; Département de Microbiologie, Faculté de Pharmacie de Monastir, Université de Monastir, Monastir, Tunisia[b]; Université Pierre et Marie Curie, Faculté de Médecine, Département de Bactériologie, Paris, France[c]; Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Est Parisiens, Département de Bactériologie, Paris, France[d]; Service de Microbiologie, Hôpital Militaire de Tunis, Tunis, Tunisia[e]

The carbapenemase New Delhi metallo-β-lactamase-1 (NDM-1), initially identified in *Escherichia coli* and *Klebsiella pneumoniae* in 2008 in a Swedish patient who was repatriated to Sweden from India (1), is spreading rapidly worldwide except in Central and South America. Most of the reported cases indicated a link with the Indian subcontinent or Balkan countries.

In May 2012, a 73-year-old female Libyan patient was admitted to the intensive care unit in a hospital in Tunis, Tunisia. Culture from a sternal pus sample revealed carbapenem-resistant *Klebsiella pneumoniae*. Species identification was performed with the API20E (bioMérieux, Marcy l'Etoile, France).

Antimicrobial drug susceptibility testing was performed by using the Vitek 2 compact automated system (bioMérieux) and disk diffusion assay, and the results were interpreted according to the latest documents from the European Committee on Antimicrobial Susceptibility testing (EUCAST) (2). The isolate was susceptible to gentamicin, amikacin, netilmicin, fosfomycin, colistin, and tigecycline and resistant to all of the β-lactams except aztreonam. The imipenem MIC was 8 μg/ml, which is considered intermediate resistance according to EUCAST guidelines. The isolate was confirmed as a carbapenemase producer by the modified Hodge test (3), and metallo-β-lactamase activity was indicated by a combined disk assay (4). PCR and sequencing for carbapenemase genes revealed that the isolate coharbors bla_{OXA-48} and bla_{NDM-1} genes. This carbapenemases association was already described in Lebanon (5). We also checked for additional acquired β-lactamase genes by PCR. A product was obtained only with OXA-1 primers. Notably, no extended-spectrum β-lactamase (ESBL) was found in our isolate, in contrast with most OXA-48 and NDM-1 producers (6, 7). Strain genotyping was performed by multilocus sequence typing according to the Institut Pasteur scheme (www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). Our strain belongs to sequence type 11 (ST11). This sequence type has a worldwide distribution and was found in NDM-1 producers in India, Sweden (8), Norway (9), and New Zealand (10), always with an epidemiological link to the Indian subcontinent. The patient in this report had no apparent link to the Indian subcontinent or Balkan countries. Conjugation experiments with azide-resistant *E. coli* J53 used as a recipient and selection on Drigalski agar plates containing sodium azide (100 μg/ml) and cefotaxime (2 μg/ml) were attempted in order to characterize the plasmid carrying bla_{NDM-1}. In all transconjugants, we detected bla_{NDM-1} and bla_{OXA-48}, and we obtained two plasmids, the first of IncL/M type (of about 65 kb in size) and the second of IncN type (of about 50 kb in size), by PCR-based replicon typing (11). In a second conjugation experiment between the first, tetracycline-resistant *E. coli* strain, J53, and the streptomycin-resistant *E. coli* HB101, selecting with cefotaxime (2 μg/ml) and streptomycin (50 μg/ml), we obtained four transconjugants producing only bla_{NDM-1} associated with the IncN plasmid. Similar results have already been described (12).

We report the first case, to our knowledge, of NDM-1-producing *Klebsiella pneumoniae* infection in Tunisia, a country where OXA-48 producers are already endemic. This report, in addition to recent observations in neighboring countries (13, 14), indicates the emergence of this resistance mechanism in North Africa. Microbiologists and clinicians should now be aware of this threat and implement the necessary control measures to prevent a possible wide spread in the population.

REFERENCES

1. **Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR.** 2009. 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. **53:**5046–5054.
2. **European Committee on Antimicrobial Susceptibility Testing.** 2012. Breakpoint tables for interpretation of MICs and zone diameters, version 2.0. EUCAST, Basel, Switzerland.
3. **CDC.** 2009. Modified Hodge test for carbapenemase detection in *Enterobacteriaceae*. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/HAI/pdfs/labSettings/HodgeTest_Carbapenemase_Enterobacteriaceae.pdf.
4. **Galani I, Rekatsina PD, Hatzaki D, Plachouras D, Souli M, Giamarellou H.** 2008. Evaluation of different laboratory tests for the detection of metallo-β-lactamase production in Enterobacteriaceae. J. Antimicrob. Chemother. **61:**548–553.
5. **El-Herte RI, Araj GF, Matar GM, Baroud M, Kanafani ZA, Kanj SS.** 2012. Detection of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* producing NDM-1 in Lebanon. J. Infect. Dev. Ctries. **6:**457–461.
6. **Nordmann P, Poirel L, Walsh TR, Livermore DM.** 2011. The emerging NDM carbapenemases. Trends Microbiol. **19:**588–595.
7. **Poirel L, Bonnin RA, Nordmann P.** 2012. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. Antimicrob. Agents Chemother. **56:**559–562.
8. **Giske GC, Fröding, Hasan IMC, Turlej-Rogacka A, Toleman M, Livermore D, Woodford N, Walsh TR.** 2012. Diverse sequence types of *Klebsiella pneumoniae* contribute to the dissemination of bla_{NDM-1} in India, Sweden, and the United Kingdom. Antimicrob. Agents Chemother. **56:**2735–2738.
9. **Samuelsen Ø, Thilesen MC, Heggelund L, Vada NA, Kümmel A, Sundsfjord A.** 2011. Identification of NDM-1-producing Enterobacteriaceae in Norway. J. Antimicrob. Chemother. **66:**670–683.
10. **Williamson AD, Sidjabat EH, Freeman TJ, Roberts AS, Silvey A, Woodhouse R, Mowat E, Dyet K, Paterson LD, Blackmore T, Burns A, Heffernan**

Published ahead of print 10 June 2013

Address correspondence to Guillaume Arlet, guillaume.arlet@tnn.aphp.fr.

doi:10.1128/AAC.00536-13

H. 2012. Identification and molecular characterisation of New Delhi metallo-β-lactamase-1 (NDM-1)- and NDM-6-producing Enterobacteriaceae from New Zealand hospitals. Int. J. Antimicrob. Agents **39:**529–533.
11. **Carattoli A, Bertini A, Villa L, Fablo V, Hopkins LK, Threfall E.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63:**219–228.
12. **Poirel L, Bonnin RA, Nordmann P.** 2011. Analysis of the resistome of a multidrug-resistant NDM-1-producing *Escherichia coli* strain by high-throughput genome sequencing. Antimicrob. Agents Chemother. **55:**4224–4229.
13. **Boulanger A, Naas T, Fortineau N, Figueiredo S, Nordmann P.** 2012. NDM-1-producing *Acinetobacter baumannii* from Algeria. Antimicrob. Agents Chemother. **56:**2214–2215.
14. **Hammerum AM, Larsen AR, Hansen F, Justesen US, Friis-Møller A, Lemming LE, Fuursted K, Littauer P, Schønning K, Gahrn-Hansen B, Ellermann-Eriksen S, Kristensen B.** 2012. Patients transferred from Libya to Denmark carried OXA-48-producing *Klebsiella pneumoniae*, NDM-1-producing *Acinetobacter baumannii* and meticillin-resistant *Staphylococcus aureus*. Int. J. Antimicrob. Agents 40:191–192.