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

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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’Étoile, 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 ticarcillin 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 carbapenemases genes revealed that the isolate coharbors blaOXA-48 and blaNDM-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/PPB/mbl/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 blaNDM-1. In all transconjugants, we detected blaNDM-1 and blaOXA-48, and we obtained two plasmids, the first of IncJ/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 blaNDM-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.

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