NDM-1-producing *Klebsiella pneumoniae*, now in Turkey

The emergence of NDM producers among Gram-negative rods is being reported worldwide, with *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* being the main hosts for this resistance trait (7). Whereas UK, India, and Pakistan have been identified as reservoirs of NDM producers, it appears also now that Balkan countries might constitute a secondary reservoir (5-7).

Here we report a 16-years-old male patient who was admitted in the Haematology Unit of a hospital situated near Istanbul, Turkey, in October 2011. That leukemic patient had been transferred from a hospital in Baghdad, Iraq, and received allogenic haematopoeitic stem cell transplantation immediately the day after his admission. Two weeks later, he developed severe respiratory failures and fever and was transferred to the intensive care unit. Blood cultures grew two types of multidrug-resistant enterobacterial isolates, being *K. pneumoniae* and *E. coli*. Despite receiving a treatment based on meropenem, teicoplanin, and colistin, the patient died of septic shock. Susceptibility testing performed and interpreted according to the updated CLSI guidelines (3) showed that *K. pneumoniae* SAL1 was resistant to all β-lactams including carbapenems (MICs of imipenem, meropenem, and ertapenem being at 32 µg/ml),
to all aminoglycosides, to fluoroquinolones, nitrofurantoin, chloramphenicol, and trimethoprim-sulfamethoxazole. It was susceptible only to tigecycline and colistin (MICs at 0.5 µg/ml for both). *E. coli* SAL2 was resistant to all β-lactams, with MICs of carbapenems being all ≥32 µg/ml, and also to fluoroquinolones, tetracycline, gentamicin, tobramycin, and sulfonamides, being only susceptible to tigecycline and colistin (same MICs as above).

According to phenotypic test results (synergy between aztreonam and clavulanate), both isolates produced extended-spectrum β-lactamases (ESBLs). Metallo-β-lactamase (MBL) detection performed by using the Etest combining imipenem and EDTA (AB bioMérieux, Solna, Sweden) gave a positive result for *K. pneumoniae* SAL1.

PCR, sequencing and plasmid analysis revealed that *K. pneumoniae* SAL1 harbored the *bla*<sub>NDM-1</sub> carbapenemase gene (10), in addition to the ESBL gene *bla*<sub>CTX-M-15</sub>, both being located on different plasmids (80 and 160 kb in size, respectively). On the other hand, *E. coli* SAL2 harbored the *bla*<sub>OXA-48</sub> carbapenemase gene, in addition to *bla*<sub>CTX-M-15</sub>, both being also located on different plasmids (62 and 100 kb in size, respectively). Screening for additional β-lactamase genes (10) and for 16S RNA methylase genes as reported (1) showed that *K. pneumoniae* SAL1 was co-harboring the *bla*<sub>OXA-1</sub>, *bla*<sub>SHV-28</sub> and *bla*<sub>TEM-1</sub> gene (a total of five β-lactamase genes), in addition to the *rmtB* gene encoding high level resistance to all
aminoglycosides. On the other hand, *E. coli* SAL2 co-harbored the \( \text{bla}_{\text{CTX-M-15}} \), \( \text{bla}_{\text{OXA-1}} \), and \( \text{bla}_{\text{TEM-1}} \) genes.

Mating-out experiments performed as described (8) using the two clinical isolates as donors gave *E. coli* transconjugants expressing NDM-1 and OXA-48, respectively. The \( \text{bla}_{\text{NDM-1}} \)-positive transconjugant harbored a single IncFIIb-type and 80-kb plasmid (2), carrying the \( \text{rmtB} \) gene. The \( \text{bla}_{\text{OXA-48}} \)-positive transconjugant harbored a single IncL/M-type 62-kb plasmid corresponding to the recently identified and worldwide disseminated scaffold (9).

Since occurrence of NDM-1-producing *K. pneumoniae* is now increasingly reported, tracing the strain backgrounds is interesting and therefore multilocus sequence typing was performed as described (4) and results analyzed by eBURST (http://pubmlst.org). It showed that isolate SAL1 belonged to the ST38 sequence type that corresponds to an ST distantly related to those of other NDM-1-positive *K. pneumoniae* isolate identified so far, including that one identified from Iraq (11).

This study constitutes the very first report of an NDM-1-positive isolate in Turkey, further underlining the wide spread of that resistance trait. Since NDM-1 had been already identified in Iraq, it is likely here that the patient was already colonized when admitted at the
Turkish hospital. This would further indicate that the Middle East and Iraq in particular may be reservoirs of NDM-producing isolates, in addition to the Indian subcontinent and the Balkans (5, 6). On the other hand, the co-infection with an OXA-48-producing *E. coli* might be nosocomial, since Turkey is known to be an endemic area for OXA-48-producing *Enterobacteriaceae*. However, since no other OXA-48-producing *E. coli* have been identified in the same hospital during the same period of time, the hypothesis of an importation together with the NDM-1-producing isolate remains plausible.

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Laurent Poirel

*INSERM U914 “Emerging Resistance to Antibiotics”, Hôpital de Bicêtre, K.-Bicêtre, France*

Melda Özdamar

*Department of Microbiology, Anadolu Medical Center, Kocaeli, Turkey*

Alain A. Ocampo-Sosa

*INSERM U914 “Emerging Resistance to Antibiotics”, Hôpital de Bicêtre, K.-Bicêtre, France*

Salih Türkoglu

*Department of Microbiology, Anadolu Medical Center, Kocaeli, Turkey*
Ufuk Guney Ozer
Department of Haematology, Anadolu Medical Center, Kocaeli, Turkey

Patrice Nordmann*
Service de Bactériologie-Virologie, INSERM U914 “Emerging Resistance to Antibiotics”, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, K.-Bicêtre, France

*Corresponding author. Phone: 33-1-45-21-36-32. Fax: 33-1-45-21-63-40.
E-mail: nordmann.patrice@bct.aphp.fr
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