Recurrent pyelonephritis due to NDM-1 metallo-beta-lactamase producing *Pseudomonas aeruginosa* in a patient returning from Serbia, France, 2012

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We describe the first isolation in France of a New-Delhi metallo-beta-lactamase-1 (NDM-1) producing *Pseudomonas aeruginosa*. In March 2012, a patient with history of prior hospitalisation in Serbia was diagnosed in France with acute pyelonephritis due to NDM-1 producing *P. aeruginosa*. Clinical and microbiological cure was obtained under appropriate antibiotic treatment. Two months later, she presented with a recurrence due to the same bacteria, with a favourable evolution. During both hospitalisations, contact isolation precautions were implemented and no cross-transmission was observed.

In March 2012, a patient with a history of prior hospitalisation in Serbia was diagnosed in France with acute pyelonephritis due to New Delhi metallo-beta-lactamase-1 (NDM-1) producing *Pseudomonas aeruginosa*.

Case report

In February 2012, a woman in her early 60s was referred to the Infectious Diseases Department of the Military Hospital Bégin (Saint-Mandé, France) for an acute pyelonephritis. She reported having undergone a surgical intervention in Serbia in November 2011. She stayed one month in hospital, with urinary catheterisation of undetermined duration but less than one month. The medical records reported a first treatment with cefuroxime and streptomycin just after surgery, and a history of fever, drowsiness and inflammatory syndrome two weeks after surgery, treated with ceftriaxone and streptomycine. Laboratory data were unavailable.

Since her return to France, in early February 2012, she complained of urinary frequency, dysuria and urinary incontinence. In late February, she presented to her general practitioner (GP) with fever (38.7 °C), vomiting, diarrhoea and diffuse abdominal pain and she was referred to our hospital. White blood cell count was 4,470/mm^3 (norm: 4,000-10,000/mm^3), C-reactive protein 52 mg/L (norm: < 5 mg/L), creatinine 36 µmol/L (norm: 62-106 µmol/L).

The urinalysis recovered 29,106 leukocytes/mL and 10^6 CFU/ml *P. aeruginosa* serotype 011 (HIABP11). The rectal swab, performed for multidrug resistant bacteria screening according to the French recommendations for patients with a history of hospitalisation abroad in the previous year [1], was also positive for *P. aeruginosa*. Blood cultures remained negative.

Kidney ultrasonography was normal, kidney CT-scan showed a left pyelonephritis without abscess or urinary obstruction. The patient underwent urinary catheterisation and three weeks antibiotic treatment with aztreonam (2g TID) and colistin (2 million units TID).

Antimicrobial sensitivity testing and molecular diagnostics

For the isolates from urine and rectal swabs, antimicrobial drug susceptibility testing was performed by the disk diffusion method on Mueller-Hinton agar (I2A Laboratories, Perols, France) and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations [2]. It demonstrated resistance to imipenem, meropenem, doripenem, to all antipseudomonal cephalosporins, aminoglycosides, fluoroquinolones, rifampicine and tigecycline. Both isolates remained susceptible to piperacillin-tazobactam and intermediate to aztreonam (Table 1). Minimal inhibitory concentrations (MICs) of selected antimicrobials determined by microdilution and Etest (Biomérieux, Marcy l'Etoile, France) confirmed the results and showed susceptibility to colistin. The metallo-betalactamase-production screening in the meropenem-dipicolinic acid combined disk test (*Klebsiella pneumoniae* carbapenemas (KPC) + metallo-beta-lactamase (MBL) Confirm ID Pack, Rosco Diagnostica, Taastrup, Denmark) and in E-test with

Table 1

Antibiotic susceptibility of *Pseudomonas aeruginosa* serotype 011 (HIABP11) strain isolated in a patient with history of previous hospitalisation in Serbia, France 2012

Antibiotic	MIC (µg/mL)	Susceptibility
Ticarcillin	› 256	R
Ticarcillin/Clavulanic acid	›256	R
Piperacillin	12	S
Piperacillin/Tazobactam	12	S
Ceftazidime	›256	R
Cefepime	›256	R
Aztreonam	3	I
Imipeneme	› 32	R
Meropeneme	› 32	R
Doripenem	› 32	R
Tobramycin	›256	R
Gentamicin	›256	R
Amikacin	192	R
Ciprofloxacin	›32	R
Colistin	2	S
Doxycycline	32	R
Tigecycline	128	R

MIC: minimal inhibitory concentration.
I: intermediate; R: resistant; S: susceptible.

imipenem alone or combined with EDTA (Biomérieux, Marcy l'Etoile, France) was positive.

PCR for carbapenemases, including MBL bla_{IMP}, blaVIM, bla_{NDM} revealed that HIABP11 harboured bla_{NDM}. Sequencing showed 100% homology with bla_{NDM-1}. PCR for detection of other beta-lactamase genes, plasmid-borne quinolone resistance genes and methylases were negative.

Monitoring of plasmatic and urinary concentrations of antibiotics

Aztreonam plasmatic peak and residual concentrations were 40 times and 4 times the MIC respectively for a dosage of 2g TID, while urine concentration was 166 times the MIC. Colistin plasmatic residual concentration (12 hours after injection) was under the MIC (0.7 mg/L, while the expected residual concentration is 2 mg/L, eight hours after injection); urine concentration was 10 times the MIC (Table 2).

Fever and abdominal pain resolved within 48 hours after administration of antibiotics while urinary incontinence persisted, requiring prolonged urinary catheterisation. Urinalysis 72 hours after the beginning of antibiotic treatment was normal and did not show any bacteria growth. The patient was discharged from hospital 21 days after admission.

During the stay at our hospital, a squamous-cell carcinoma of the oropharynx had been discovered and the patient underwent a first chemotherapy course at the beginning of May and was discharged from our hospital. One week later, she experienced dysuria and abdominal pain, without fever or flank pain. She was treated with ofloxacin (10 days) and prednisolone (seven days) by her GP. The urinalysis showed $42x10^4$ leukocytes/mL (significant leukocytes count $›10^4$/mL) and *P. aeruginosa* 10^3 CFU/mL. *P. aeruginosa* with the same antibiotic susceptibility pattern as previously was isolated and PCR for bla_{NDM-1} was again positive. Kidney ultrasound was normal.

The patient received piperacillin-tazobactam (4g TID) for three weeks, and her second chemotherapy course, without complication. The urinalysis after 72 hours of treatment was negative and the patient was discharged for at-home hospitalisation.

During both hospitalisations, contact isolation precautions with dedicated healthcare personnel were implemented. All patients hospitalised in the same ward were screened weekly with a total of 111 rectal swabs performed in 52 patients and no transmission of NDM-1 producing *P. aeruginosa* occurred.

Discussion and conclusion

Since the first description in 2008, of an NDM-1 carbapenemase in single isolates of *Klebsiella pneumoniae* and *Escherichia coli* [3], NDM-1-producing *Enterobacteriaceae* have been reported worldwide, mostly in patients with an epidemiological link to India or Pakistan [4,5]. However, among 77 patients infected or colonised by NDM-1 producing *Enterobacteriaceae* reported in Europe from 2008 to 2010, five had been hospitalised previously in the Balkan region [6]. Clinical isolates of NDM-1-producing *A. baumanii* are also increasingly reported in Europe [7] and importation of NDM-1 -producing *A. baumannii* from Serbia has been reported in 2011 [8].

We report here the first case of infection due to NDM-1-producing *P. aeruginosa* in France. To date,

Table 2

Monitoring of plasmatic and urinary concentrations of antibiotics in patient with pyelonephritis caused by *Pseudomonas aeruginosa* serotype 011 (HIABP11) and history of previous hospitalisation in Serbia, France 2012

	Aztreonam	Colistin
Plasmatic peak concentration (mg/L)	127.6	NA
Plasmatic residual concentration (mg/L)	14.3	0.7
Urinary concentration (mg/L)	› 500	22.3

NA: not available.

only two other cases of colonisation or infection by NDM-1-producing *P. aeruginosa* have been reported worldwide, occurring in two patients hospitalised in Belgrade,Serbia. Both had undergone invasive surgical interventions and none of them had travelled outside Serbia. No epidemiological connection was evidenced between them [9, 10].

NDM-1 producing bacteria are undoubtly challenging: firstly, they are usually multiresistant to antibiotics because bla_{NDM-1} encoding plasmids co-harbor multiple resistance determinants. *P. aeruginosa* shows a high level of intrinsic resistance to antimicrobial agents. Its ability to acquire and combine different resistance determinants represents a major threat, compromising therapeutic options. The acquisition of MBL-carbapenemase (Verona integron-encoded metallo-beta-lactamase (VIM), imipenemase (IMP), Sao Paulo Metallo-beta-lactamase (SPM), Australia imipenemase (AIM), German imipenemase (GIM), Dutch IMipenemase (DIM), NDM, led to emergence of multidrug-resistant (MDR) or extensively drug-resistant (XDR) *P. aeruginosa*. The case presented highlights the difficulties of therapeutic management, with only three antibiotics categorised as susceptible or intermediate (colistin, aztreonam, piperacillin-tazobactam). Due to the low MIC recommended for the inferior breakpoint for aztreonam by EUCAST, wild type *P. aeruginosa* are reported as 'intermediate'. However MBL-carbapenemases do not hydrolyse the monobactam aztreonam and high dose therapy can be useful for patients infected with MBL-producing *P. aeruginosa* [11,12].

Secondly, NDM-1 producers have a potential for spread through the transfer of the plasmid-borne bla_{NDM1} gene [5]. In *P. aeruginosa*, there is no complete documentation for plasmid-borne or chromosomal localisation for bla_{NDM1} gene yet. However, many outbreaks including carbapenemase-producing *P. aeruginosa* and spread of MDR *P. aeruginosa* clones have been recently reported, underlining that cross-transmission plays a major role in the spread of MDR *P. aeruginosa* in hospital settings [13, 14]. These considerations combined with the emerging character of our isolate in France are reason why all members of the medical and paramedical staff agreed to set up a dedicated team to care for the patient and a weekly screening of all contemporary patients on the same ward.

This strategy is recommended in France for carbapenemase-producing *Enterobacteriae* [1] without any mention of carbapenemase-producing Acinetobacter or Pseudomonas. However, in this particular case of a first isolation of NDM-1 producing *P. aeruginosa* in France, this strategy allowed us to assess the absence of cross-transmission for this isolate.

This observation highlights the emergence of NDM-1 not only in Enterobacteriaceae, but also in *P. aeruginosa* in Balkan area and France. In our view, NDM screening should be performed when a carbapenemase-producing *Pseudomonadaceae* clinical isolate is identified.

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