Emergence of *Klebsiella pneumoniae* producing NDM-1 carbapenemase in Saint Petersburg, Russia

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Sir,

Gram-negative bacilli producing NDM-type metallo-carbapenemases present a recognized threat to the healthcare system. Most NDM-positive isolates are multidrug resistant and remain susceptible only to colistin and tigecycline.1 Although first and most often detected in *Klebsiella pneumoniae* and *Escherichia coli*, the NDM-1 gene can spread rapidly and has been found in diverse bacterial strains and species, in healthcare settings and in the environment.1,2 NDM producers have been reported from many countries and often, though not always, from patients with travel or healthcare links in other countries. We report here an NDM-1-producing strain of *K. pneumoniae* that was recovered in a Russian patient with no history of overseas travel.

In April 2012, a patient with heart failure (class III, New York Heart Association functional classification) was admitted to Almazov Centre, where the patient received standard treatment for this condition. The patient did not have a urinary catheter during this hospitalization and no antibiotics were administered; the patient was discharged 31 days after admission when the symptoms of heart failure were stabilized. The patient has resided solely in Saint Petersburg throughout their life.

At the end of May 2012, the patient complained of frequent urination. An outpatient urine sample yielded 1.5×10⁸ leucocytes/L and a multidrug-resistant *K. pneumoniae* (10⁶ cfu/mL) was cultured (isolate 1), which was susceptible in *vitro* to aztreonam, colistin, chloramphenicol, tetracyclines and tigecycline. The MIC of fosfomycin, determined by the agar dilution method, was 16 mg/L and the patient received this preparation (daily dose 3 g) for 2 days with a positive clinical effect. Microbiological reinvestigation was not performed. Multidrug-resistant *K. pneumoniae* (10⁶ cfu/mL) was again isolated from a follow-up outpatient urine sample taken 5 weeks later (isolate 2). CT revealed urolithiasis and chronic pyelonephritis. By this time, both isolates 1 and 2 were known to carry the *bla*<sub>NDM-1</sub> gene (see below) and aztreonam was initiated at a daily dose of 3.0 g after readmission of the patient to Almazov Centre. The treatment was terminated after 4 days due to adverse effects (diarrhoea and hypotension) and urine cultures yielded no bacteria up to the fifth day after cessation of aztreonam treatment. However, 2 weeks later, multidrug-resistant *K. pneumoniae* (10⁶ cfu/mL) was again recovered from a urine sample (isolate 3). No antibiotics were administered at this time. The patient’s stools did not grow Gram-negative bacilli on selective media for the detection of carbapenem-resistant Enterobacteriaceae: Brilliance CRE agar, MacConkey agar with meropenem and ertapenem discs as well as agar plates supplemented with 0.5 mg/L meropenem. *E. coli* and *Acinetobacter johnsonii* isolated on non-selective agar plates were susceptible to carbapenems and did not carry the *bla*<sub>NDM-1</sub> gene. The renal stones were recognized as uric acid deposits. A dietary change as well as medication was prescribed to diminish the size of the stones. The screening of contact patients and careful microbiological investigation of the hospital environment did not reveal carbapenem-resistant Enterobacteriaceae. The patient was discharged home, to the community. The patient was scheduled for hospitalization to the specialized urological department.

The three urinary isolates were identified as *K. pneumoniae* subsp. *pneumoniae* by sequencing of the first 500 bp of the 16S RNA gene (MicroSeq ID 16S rDNA 500 Library V. 2.1). Multilocus sequence typing was performed as described on the web site of the Pasteur Institute, France (http://www.pasteur.fr/recherche/genopole/PF8/mist/Kpneumoniae.html); all three isolates belonged to sequence type ST340, which has been associated with NDM-1-positive isolates from Oman and South Korea.2,3 The isolates were tested by PFGE of XbaI-digested DNA (PulseNet standard operating procedure for *E. coli*). The PFGE patterns of the NDM-1-producing *K. pneumoniae* isolates are shown in Figure 1. All isolates from the patient’s urine belonged to the same PFGE type. The PFGE profile of a reference strain of *K. pneumoniae* producing NDM-1 carbapenemase from Finland (*K. pneumoniae*, NDM-1, ESBL no. 2050, National Institute for Health and Welfare, Turku, Finland) was completely different (Figure 1).

Antibiotic susceptibilities were determined on Mueller–Hinton agar by disc diffusion (Oxoid, UK) and by a microdilution method (Sensititre, Trek Diagnostic Systems, USA). The results were interpreted, where possible, using CLSI guidelines5 or, in the absence of a CLSI breakpoint (e.g. colistin), using EUCAST guidelines.7 The isolates were resistant to all β-lactams except aztreonam (Table S1, available as Supplementary data at JAC Online);
carbapenemase activity was indicated by a modified Hodge test and the presence of a metallo-β-lactamase by a double-disc synergy test with EDTA as the chelating agent. 6,8 Susceptibility to chloramphenicol, colistin, fosfomycin and tetracyclines and tigecycline was confirmed. PCR (primers are presented in Table S2, available as Supplementary data at JAC Online) followed by sequencing identified the blaNDM-1 gene, together with genes encoding SHV-11 β-lactamase and the ArmA 16S methyltransferase, which confers resistance to aminoglycosides.

To the best of our knowledge, no NDM-1-positive bacilli have been reported from Russia until now. NDM-1-positive bacteria have been reported in many countries,1,2,4,5 but are often linked with travel to or healthcare contact in the Indian subcontinent or, less frequently, in the Balkan region.7 Our patient had never travelled abroad and had never been hospitalized outside Saint Petersburg, and so adds to a growing number of cases of ‘autochthonous’ emergence of NDM-1;5,10,11 these cases suggest that NDM-1 producers are possibly infiltrating community settings.

The NDM-1 gene confers resistance to all β-lactams, except aztreonam, although aztreonam resistance is observed in most NDM-positive isolates and is mediated by CMY-type and CTX-M-15 β-lactamases.1 No ESBL or AmpC β-lactamases were found in our isolates, which remained susceptible to aztreonam. Although the patient received this agent, treatment was curtailed due to adverse effects and only a short-term microbiological effect was achieved.

The early recognition of NDM-1-producing bacteria in low-incidence areas, such as Russia, is important to prevent such isolates becoming endemic in healthcare facilities. Prompt treatment of NDM-1-carrying patients is needed to minimize the opportunity for new regional reservoir formation.

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Supplementary data
Tables S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References
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**Tenofovir plasma concentrations in post-menopausal versus pre-menopausal HIV-infected women**

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Sir,

Menopause is associated with age-related physiological changes—such as a decline in renal and hepatic function, altered genital secretions and/or impaired production of sexual hormones—that can potentially affect the disposition of several drugs.1 The role of menopause on the pharmacokinetics of antiretrovirals, however, has not been investigated so far. Given the potential impact of tenofovir on long-term kidney and bone complications,2,3 which are known co-morbidities affecting post-menopausal women, we decided to perform a retrospective analysis of data from post-menopausal and pre-menopausal HIV-infected women receiving 300 mg of tenofovir/day as part of their maintenance antiretroviral therapy regimen for at least 3 months who underwent therapeutic drug monitoring of tenofovir plasma trough concentrations as routine outpatient follow-up. Menopause was established by menstrual and medical history (12 months of lack of menstrual cycle for women aged 50 years or more, with hormonal confirmation for women younger than 50 years). Creatinine clearance was calculated by using the formula of Cockcroft–Gault as follows: glomerular filtration rate = (140 − age)xbody weight/serum creatinine x 72. As the patients were female, the formula was multiplied by a constant of 0.85.

As per our clinical practice, tenofovir plasma trough concentrations were collected immediately before the following morning's tenofovir dose (a time window of ±5 min was considered as acceptable). For patients given tenofovir once daily in the evening, plasma trough (24 h) concentrations were estimated from the concentrations collected at 12 h after drug intake and taking into account the terminal portion of the pharmacokinetic drug profile.4,5 Tenofovir concentrations were assessed by a validated liquid chromatography–tandem mass spectrometry ('LC-MS/MS') method.6

A total of 50 HIV-infected women were identified from our database: 22 were post-menopausal women, while the remaining 28 were pre-menopausal women.

A wide distribution of plasma tenofovir trough concentrations was observed when considering the data from the overall study population (n=50), tenofovir concentrations ranged from 10 to 452 ng/mL. We first compared tenofovir plasma trough concentrations, together with demographic and haemato-chemical characteristics, in the post-menopausal women (n=22) versus the pre-menopausal women (n=28). As shown in Table 1, no major differences were observed between the two groups of patients in terms of concomitant antiretroviral therapy, kidney and liver function, as well as for other demographic characteristics with the (expected) exceptions of age and time on tenofovir therapy. It is worth mentioning that tenofovir plasma concentrations measured in post-menopausal women exactly matched those assessed in pre-menopausal women (119±64 versus 121±98 ng/mL, P=0.924).

In order to further investigate the potential effect of menopause on tenofovir exposure, we also performed multiple regression analysis in the overall population (n=50) using tenofovir plasma trough concentrations as the dependent variable and menopause, serum transaminase, serum creatinine, age, body weight, concomitant antiretroviral therapy and days on tenofovir therapy as independent covariates. Using this approach we found that the only factors independently associated with tenofovir plasma concentrations were serum creatinine (r=0.411, P=0.043) and body weight (r=−0.432, P=0.021), whereas no effect of menopause was documented (r=−0.049, P=0.852). This trend was also confirmed when using creatinine clearance (r=−0.384, P=0.022) and body mass index (r=−0.305, P=0.037) instead of serum creatinine and body weight, respectively.

Taken together, our results argue against a significant effect of menopause on tenofovir exposure. These findings are at odds with those presented by Patterson et al.,7 who reported significantly higher (up to 160%) tenofovir plasma and genital tract concentrations in 6 post-menopausal versus 12 pre-menopausal African American HIV-infected women. The discrepant results of