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NDM-1-Producing *Klebsiella pneumoniae* Resistant to Colistin in a French Community Patient without History of Foreign Travel

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A carbapenem-resistant *Klebsiella pneumoniae* strain, Kp5196, was responsible for an uncomplicated cystitis in a patient living at home and without history of foreign travel. This isolate produced the metallocarbapenemase NDM-1 and was resistant to all antibiotics except tetracyclines and colistin. The *K. pneumoniae* strain belonged to sequence type ST15, and bla_{NDM-1} was carried by a nontypeable conjugative plasmid. Two months later, a similar ST15 isolate, Kp5241, was present in the patient but was additionally colistin resistant.

The New Delhi metallo-β-lactamase 1 (NDM-1) is a carbapenemase that hydrolyzes all β-lactams except monobactams and is susceptible to EDTA but not to clavulanic acid (13). The bla_{NDM-1} gene is usually borne by conjugative plasmids that carry multiple additional determinants, leading to multidrug resistance (14, 16). NDM-1 was first identified in 2008 in a *Klebsiella pneumoniae* strain isolated from a Swedish patient transferred from India (20). Since then, this carbapenemase has been described in various Gram-negative bacilli. NDM-1-producing strains have been shown to be endemic in the Indian subcontinent and, probably, in the Balkans (8). Sporadic cases have been observed in many other countries worldwide, in patients either repatriated after hospitalization or who recently traveled to areas of endemicity. Multiple community-acquired cases have been reported in India related to the heavy environmental contamination (13), but only two autochthonous and/or community-acquired cases have been described outside India (12, 13). However, all these isolates remained susceptible to colistin.

On 1 September 2011, a 91-year-old woman living in her own apartment within a residence for the elderly with common services (meals, help for washing and dressing, and housekeeping) in the Aquitaine region (France) developed an acute uncomplicated cystitis due to *K. pneumoniae* Kp5196. This strain was resistant to all antibiotics except tetracyclines and colistin. Since EDTA restored imipenem susceptibility, a metallocarbapenemase-encoding gene was sought by PCR amplification (15), leading to the discovery of bla_{NDM-1}, which was further confirmed by sequencing. Subsequently, the patient received minocycline (200 mg/day from 09/06/2011 [month/day/year] to 09/12/2011). A further urinary sample was taken (09/21/2011) and sent to another laboratory at the end of the treatment, in order to check for bacterial eradication. A *K. pneumoniae* isolate exhibiting a similar antibiogram to the one exhibited by Kp5196, except for nitrofuran susceptibility, was found. Unfortunately, this strain was not preserved. Based on the latter result, the patient was treated with nitrofurantoin for 41 days (150 mg/day from 09/26/2011 to 11/05/2011). Subsequently, a new urinary sample (11/05/2011) was taken, which after analysis indicated the presence of a nitrofurantoin-susceptible, colistin-resistant, and NDM-1-producing *K. pneumoniae* strain, Kp5241.

Kp5196 and Kp5241 were resistant to all β-lactams. Using the Etest strips (AB bioMérieux), Kp5196 was slightly more resistant to carbapenems than Kp5241 (MICs of 4 versus 2 μg/ml for imipenem, 3 versus 2 μg/ml for meropenem, and 12 versus 8 μg/ml for ertapenem, respectively). The colistin MICs, determined by a dilution method in Mueller-Hinton broth, were 2 μg/ml for Kp5196 versus 64 μg/ml for Kp5241. Kp5196 and Kp5241 were resistant to fluoroquinolones, aminoglycosides (gentamicin, kanamycin, tobramycin, netilmicin, and amikacin), chloramphenicol, sulfonamides, trimethoprim, and fosfomycin. Additional β-lactamase-encoding and plasmid-mediated aminoglycoside and quinolone resistance genes have been screened by a multiplex PCR approach followed by sequencing (6, 15). The results revealed that both strains also expressed the extended-spectrum β-lactamase (ESBL) $bla_{CTX-M-15}$ and the narrow-spectrum β-lactamases bla_{OXA-1} and bla_{SHV-28}. Furthermore, they harbored the plasmid-mediated quinolone resistance gene *qnrB1*, the *aac(6′)-Ib*-like gene (TNA phenotype), and the 16S rRNA methylase gene *armA*, conferring high-level aminoglycoside resistance. Plasmid analysis by pulsed-field gel electrophoresis (PFGE) following S1 nuclease DNA linearization (2) showed that Kp5196 and Kp5241 possessed four plasmids, including three with identical sizes of 220, 170, and 100 kb and another one with a slightly different size in the two strains, i.e., 270 kb in Kp5196 and 300 kb in Kp5241 (data not shown). The bla_{NDM-1} gene was successfully transferred to an azide-resistant mutant of *Escherichia coli* C600 at a frequency of approximately 10^{-3} to 10^{-4}, together with $bla_{CTX-M-15}$, bla_{OXA-1}, *aac(6′)-Ib*-like, *armA*, and *qnrB1* genes. Chloramphenicol, sulfonamide, and trimethoprim resistance was also cotransferred with NDM-1. Only the largest plasmid of 270 kb or 300 kb was present in the transconjugants obtained from Kp5196 and Kp5241, respectively. Incompatibility group typing of these two plasmids by a PCR-based replicon typing method (5) re-

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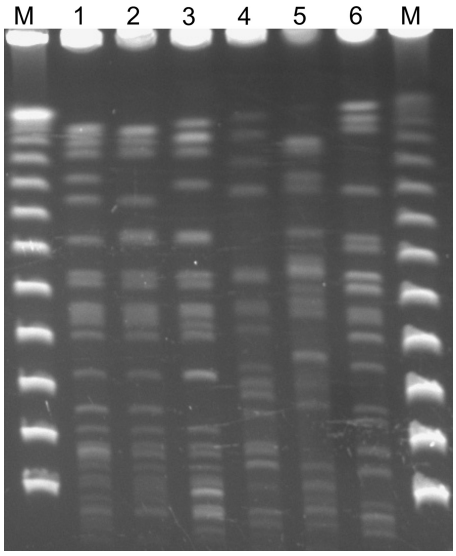

FIG 1 XbaI patterns of *K. pneumoniae* strains after pulsed-field gel electrophoresis analysis. Lanes 1 and 2, Kp5196 and Kp5241, respectively. Lane 3, Kp10197 (Belgian strain of ST15, NDM-1-producing *K. pneumoniae*) (3). Lane 4, *K. pneumoniae* ATCC 13883. Lanes 5 and 6, Kp5233 (OXA-48-producing *K. pneumoniae*, ST unknown), and Kp5069 (ST14 *K. pneumoniae*), respectively; two unrelated strains used as controls. Lanes M, lambda DNA ladder (Bio-Rad).

mained unsuccessful. Multilocus sequence typing (MLST) (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) indicated that both strains belonged to sequence type ST15. Their epidemiological relationship was determined after XbaI-digested DNA and PFGE analysis using the CHEF DRIII apparatus (Bio-Rad) (Fig. 1). They showed a similarity index of 91.3%, obtained with the FQQuest software version 4.5 (Bio-Rad) with 0.32% positional tolerance and using the unweighted-pair group method using average linkages (UPGMA) algorithm and Dice similarity coefficient.

No travel or hospital source could be found for the patient carrying these NDM-1-producing strains. Indeed, this elderly widow has never been abroad. Her family members and care givers had no recent history of foreign travel. In the preceding year, the patient was hospitalized in a clinic for a fractured vertebra for 59 days (05/03/2011 to 06/20/2011). On discharge, she entered the residence, which had just opened. During her stay in the clinic, the patient underwent two episodes of urinary tract infections (UTIs) (05/03/2011 and 05/23/2011) caused by wild-type *Proteus mirabilis* strains which were treated with pipemidic acid (400 mg/day for 4 days) and cefpodoxime (800 mg/day for 7 days), respectively. The only invasive procedure was the subsequent insertion of a urinary catheter (05/28/2011), which was removed upon the NDM-1 discovery (09/12/2011). Within at least the last 2 years, no other antibiotic treatment has been given for this patient. Fecal samples were collected (09/16/2011) from two other patients who shared the same room with the patient in the clinic in May and June 2011 and were discharged to a convalescence center. However, screening for carbapenemase producers in their feces using the selective Brilliance CRE medium (Oxoid) remained negative. Thus, this case of NDM-1-producing *K. pneumoniae* is autochthonous since the patient had no history of foreign travel. In addition, her episodes of UTIs were community acquired. No other NDM-1-positive *Enterobacteriaceae* have been found in the Aquitaine region, in particular in the University hospital or in local hospitals, in the clinic where the patient was hospitalized, and in the residence where she lived.

A single case of an autochthonous and community-acquired NDM-1-producing *K. pneumoniae* strain has been previously reported (12). This first case also occurred in France but in a distant region. Although the clinical context was similar, the latter strain was different since it belonged to the ST1 type and harbored two self-transferable plasmids, including an IncA/C plasmid that carried $bla_{\text{NDM-1}}$ and $bla_{\text{CMY-2}}$ and another one with $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, and *qnrB* (12). In the literature, NDM-1-producing *K. pneumoniae* strains have been shown to belong to a wide variety of ST types (16), and $bla_{\text{NDM-1}}$ has been found on a number of plasmid scaffolds (16). The *K. pneumoniae* strain isolated in our study belonged to sequence type ST15, which is a single-allele variant of ST14 (16). Only two ST15 NDM-1-producing *K. pneumoniae* strains have been reported. The first strain was identified in a Belgian patient transferred from Montenegro (3). The second report referred to three clonally related strains isolated from two patients and one outpatient of the same hospital in Morocco (15). In both strains, the $bla_{\text{NDM-1}}$ gene was located on nontypeable plasmids of 130 and ca. 250 kb, respectively. Similar ($bla_{\text{CTX-M15}}$ and $bla_{\text{OXA-1}}$) but also different ($bla_{\text{OXA-9}}$, $bla_{\text{TEM-1}}$, and SHV-type ESBL) resistance determinants coexisted with $bla_{\text{NDM-1}}$. Only the report on the Belgian strain mentioned the presence of a *qnrB1*-like gene, and no *armA* gene was found in any of these strains (3, 15). The XbaI-digested PFGE pattern comparison showed that Kp5196 and Kp5241 were probably related to the Belgian strain, Kp10197, since their Dice similarity indices reached 59.1% and 68.2%, respectively (Fig. 1) (18). These results suggest either the dissemination of an evolving NDM-1 ST15 *K. pneumoniae* clone or the spread of $bla_{\text{NDM-1}}$-carrying plasmids in this common type of strain.

Kp5196 and Kp5241, isolated with a 2-month interval, showed similarities which clearly indicate their epidemiological relatedness. Common features included identical ST types, similar pulsotypes and plasmid contents, and identical NDM-1 coresistance profiles. However, differences were observed between these two strains. The natural strain evolution could account for such variations. In particular, according to the criteria of Tenover et al. (18), two or three distinct bands visualized after PFGE analysis may be due to a single genetic event. Furthermore, the different sizes of NDM-1-carrying plasmids could result from the insertion or deletion of a mobile genetic element (1). Additional chromosomal mutations are certainly involved in the differences in carbapenem, nitrofuran, and colistin sensitivities (9, 11, 17). However, it is difficult to believe that so many genetic events occurred in such a short period. Moreover, although the correlation between antibiotic selective pressure and the emergence of colistin resistance is not always evident (4, 10), it could not be demonstrated in this case. Thus, either the patient was colonized for a long time by one of these strains which has evolved or she had been recently and simultaneously contaminated by both strains. However, transfer of the NDM-1-carrying plasmid into two variants of the same strain, already resident in the patient's gut flora, with sequential transfer to her urinary tract, cannot be totally excluded. NDM-1 producers are generally resistant to most antibiotics, except tigecycline and colistin (15, 16). Recent increases in the use of colistin for the treatment of infections with multidrug-resistant

Gram-negative bacilli, such as *K. pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* strains, have led to increased frequencies of resistance to this antibiotic (4). In contrast, very few colistin-resistant NDM-1-producing *K. pneumoniae* (7) and cases of acquired resistance to colistin (19) in the community setting have been reported. In recent years, colistin has become the last resort for the treatment of infections due to multidrug-resistant Gram-negative bacteria. The currently growing rate of colistin resistance shows that this antibiotic should no longer be considered a panacea.

Conclusion. The isolation of a novel autochthonous and community-acquired case of infection due to an NDM-1-producing strain in a country where such strains are still scarce is alarming. The acquisition of colistin resistance by an NDM-1-producing *K. pneumoniae* strain highlights the risk of the emergence of panresistant strains. What is worrisome is the occurrence of such an event at a time when we are seeing very few new drugs under development. Considering the dramatic impact on the treatment options, a high degree of awareness and monitoring is warranted.

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