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Detection of NDM-1-Producing *Klebsiella pneumoniae* in Kenya▽

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Seven carbapenem-resistant NDM-1-positive *Klebsiella pneumoniae* isolates were recovered from patients hospitalized between 2007 and 2009 in different wards at a referral and tertiary care center in Nairobi. Most of the isolates were obtained from urine. All isolates carried the bla_{NDM-1} carbapenemase gene previously reported from India, Pakistan, and the United Kingdom. These isolates were clonally related and expressed many other resistance determinants, including β-lactamases CTX-M-15, OXA-1, OXA-9, CMY-6, and aminoglycoside resistance methylase RmtC. This work corresponds to the first report of NDM-1 producers in Africa.

Carbapenem-hydrolyzing β-lactamases belonging to Ambler classes A, B, and D have been reported worldwide among *Enterobacteriaceae* (10, 14, 18). The most clinically significant are KPC-type (Ambler class A), IMP and VIM types (class B), and OXA-48 (class D), mostly identified in *Klebsiella pneumoniae* as a source of nosocomial outbreaks (14). Most of those isolates are multidrug resistant.

Recently, a novel metallo-β-lactamase (MBL) named NDM-1 (*N*ew *D*elhi *m*etallo-β-lactamase) has been identified from *K. pneumoniae* (strain 05-506) and *Escherichia coli* isolates recovered in Sweden from a patient previously hospitalized in India (19). An extensive survey performed in the United Kingdom, India, and Pakistan identified NDM-1-producing *K. pneumoniae*, *E. coli*, *Citrobacter freundii*, *Morganella morganii*, *Providencia* spp., and *Enterobacter cloacae* isolates (9). The presence of NDM-1 producers in hospitalized patients in the United Kingdom was related in many cases to previous hospitalization in the Indian subcontinent (9), and similar observations have been made in France (12, 15) and the Sultanate of Oman (11). Spread of this novel carbapenemase gene is considered a serious threat since the reservoir of NDM-1 producers is at least in part related to the Indian subcontinent, which is inhabited by the second-largest population in the world and where NDM-1 producers are reported also in community-acquired infections (9, 12).

Our study was initiated using an archival collection of bacterial isolates from the Aga Khan University Hospital, Nairobi, Kenya, for the purpose of surveillance of antibacterial resistance mechanisms and infection control audits. Seven multidrug-resistant *K. pneumoniae* strains isolated over a 3-year period (2007 to 2009) were selected, all showing an identical pattern of resistance. Those isolates had been recovered mostly from urine, from patients who were all receiving antibiotic treatments when the samples had been recovered (Table 1). However, those treatments were not targeted toward the carbapenem-resistant *K. pneumoniae* isolates that were systematically considered non-strictly invasive (Table 1).

The MICs were determined by Etest (AB bioMérieux, Solna, Sweden) on Mueller-Hinton agar plates and by liquid microdilution assays at 37°C, and results of susceptibility testing were interpreted according to the CLSI guidelines (6). The isolates were resistant to all β-lactams, including carbapenems with MIC values of >32 μg/ml for imipenem, meropenem, ertapenem, and doripenem. In addition, they were resistant to aminoglycosides, fluoroquinolones, chloramphenicol, sulfonamides, fosfomycin, and nitrofurantoin, the MIC of rifampin was >32 μg/ml, and the MICs of tigecycline and colistin measured by Etest were 0.5 and 0.5 μg/ml, respectively.

Results from MBL detection performed by using Etest MBL strips (AB bioMérieux) were positive. Thus, PCR assays were carried out with a series of primers designed for the detection of several class B β-lactamase genes, bla_{IMP}, bla_{VIM} (16), and bla_{NDM-1} (primer NDM-Fm, 5′-GGTTTGGCGATCTGGTTT TC-3′; and primer NDM-Rm, 5′-CGGAATGGCTCATCAC GATC-3′). The isolates were positive for bla_{NDM-1}, and sequencing of the PCR products revealed 100% identity with the published sequence of the bla_{NDM-1} gene (19). Noteworthy, all the patients from whom the NDM-1-producing isolates had been recovered were Kenyans living in Kenya, but the history of their travel or contact with Indian or British populations could not be recovered. All these isolates were identified from hospitalized patients, and pulsed-field gel electrophoresis (PFGE) was performed as described previously (3) to evaluate any clonal relationship. PFGE analysis showed an identical pattern for all isolates (Fig. 1). The hospitalization wards from which the infected patients were originating were located in distinct locations inside the hospital, and no source or index cases could be identified. Very interestingly, the PFGE pattern of those Kenyan isolates was very similar to that of the first reported NDM-1-producing *K. pneumoniae* strain 05-506 identified in Sweden from a patient hospitalized in India in 2008 (Fig. 1) (9). It was also very similar to that of *K. pneumoniae* strain 601, recently identified in the Sultanate of Oman (Fig. 1), for which a link with India was evidenced (11). This result indicates a clonal link between those isolates identified in Nairobi and this Indian strain. That clonal link was further

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TABLE 1. Clinical features of the studied *K. pneumoniae* isolates and patient characteristics[a]

Isolate no.	Sex of patient	Age of patient (yr)	Hospital unit	Treatment of patient	Total duration of patient hospitalization (days)	Outcome of patient	Source of isolation	Date of first isolation (mo/day/yr)
1	F	58	Medical ward	Amoxicillin-clavulanate	4	Discharged		
2	M	72	ICU	Imipenem + amikacin	2	Died[b]	Urine	05/23/2007
3	M	42	Medical ward	Imipenem + ciprofloxacin	8	Discharged	Urine	12/24/2007
4	F	76	Medical ward	Ciprofloxacin	6	Discharged	Urine	11/01/2008
5	F	65	Medical ward	Meropenem + amikacin	11	Discharged	Urine	12/16/2008
6	M	56	ICU	Meropenem + amikacin + ciprofloxacin	11	Discharged	Urethral pus	01/02/2009
7	F	26	Maternity	High-dose ofloxacin	7	Discharged	Urine	03/11/2009

[a] F, female; M, male; ICU, intensive care unit.
[b] Death not attributed to infection (patient had terminal-stage cancer).

confirmed by performing multilocus sequence typing as described previously (7) which showed that the *K. pneumoniae* isolates of Kenya belonged to the ST14 sequence type, as reported for *K. pneumoniae* 05-506 (19).

Since high-level resistance to cephalosporins and monobactam aztreonam (a substrate spared by NDM-1) was observed for all isolates, detection of extended-spectrum β-lactamase (ESBL) and AmpC productions was carried out by PCR analysis using specific primers to detect broad-spectrum β-lactamase genes (4), followed by sequencing. All isolates harbored the $bla_{\text{SHV-28}}$ and $bla_{\text{CTX-M-15}}$ ESBL genes, together with the $bla_{\text{CMY-6}}$ AmpC gene and the $bla_{\text{OXA-1}}$ and $bla_{\text{OXA-9}}$ class D β-lactamase genes. Yong et al. (19) reported that the NDM-1-positive *K. pneumoniae* strain 05-506 was $bla_{\text{CMY-4}}$ positive. Screening of 16S rRNA genes encoding methylase, performed by using a multiplex PCR approach as described previously (1), identified the *rmtC* methylase gene conferring high-level resistance to all aminoglycosides in the Kenyan isolates.

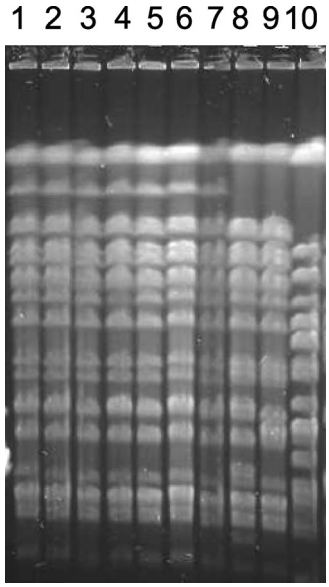

FIG. 1. Pulsed-field gel electrophoresis patterns of the seven NDM-1-producing *K. pneumoniae* isolates from Nairobi and the single NDM-1-producing *K. pneumoniae* isolate from India. Lane 1, Kp1; lane 2, Kp2; lane 3, Kp3; lane 4, Kp4; lane 5, Kp5; lane 6, Kp6; lane 7, Kp7; lane 8, *K. pneumoniae* 05-506 (19); lane 9, *K. pneumoniae* 601 (11); lane 10, unrelated *K. pneumoniae* strain (included as a comparative strain).

Transferability of the $bla_{\text{NDM-1}}$ gene was studied by conjugation experiments as described previously (17), with a selection based on ceftazidime (30 μg/ml) and azide (100 μg/ml), using *K. pneumoniae* Kp7 as the donor strain and *E. coli* J53 (resistant to azide) as the recipient strain. One of the obtained transconjugants, *E. coli*(pKp7-A) expressing NDM-1, had decreased susceptibility to carbapenems (MIC values of imipenem, meropenem, ertapenem, and doripenem were 4, 1.5, 1.5, and 1.5 μg/ml, respectively [MICs for these same molecules were 0.12 μg/ml for the *E. coli* J53 recipient strain]). It was resistant to all aminoglycosides and to sulfonamides, and PCR analysis confirmed that plasmid pKp7 coharbored the *rmtC* gene. This plasmid, analyzed by using the Kieser technique (8), was 120 kb in size and belonged to the IncA/C_2 incompatibility group as demonstrated by the PCR-based replicon typing method (2), whereas the $bla_{\text{NDM-1}}$-positive plasmid in *K. pneumoniae* 05-506 was untypeable (9), and that of *K. pneumoniae* 601 from the Sultanate of Oman was reported to belong to the IncL/M group (11). Kumarasamy et al. (9) reported $bla_{\text{NDM-1}}$-positive plasmids from the Indian isolates that were either untypeable or belonged to the IncA/C or IncFI/FII plasmid incompatibility group. Another type of transconjugant was obtained, *E. coli*(pKp7-B), expressing an ESBL phenotype. It harbored the $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1}}$ genes, had a 75-kb plasmid, belonged to the InF group, and did not carry any other non-β-lactam resistance markers.

PCR mapping was performed to identify the genetic sequences surrounding the $bla_{\text{NDM-1}}$ gene in *K. pneumoniae* Kp7 for comparison with the sequences surrounding the $bla_{\text{NDM-1}}$ gene in *K. pneumoniae* 05-506 (19). PCR experiments based on primers located in those surrounding sequences produced negative results, indicating that the structures identified in *K. pneumoniae* 05-506 were not present on plasmid pKp7.

Our study further underlines the spread of the $bla_{\text{NDM-1}}$ gene worldwide, as exemplified by the newly reported NDM-1-producing *E. coli* isolate identified in Australia (13), together with the report of NDM-1-producing *E. coli*, *K. pneumoniae*, and *Enterobacter cloacae* in the United States (5). This work is the first to report the dissemination of that gene in Africa which actually corresponds to the very first known identification of an NDM-1 producer. It has been traced to 2007, prior to the first known identification of an NDM-1 producer in 2008

in Sweden (19). Histories of contacts or the origin of the Kenyan patients to the Indian subcontinent could not be evidenced. However, taking into account the size of the Indian diaspora in Kenya (100,000 people), it remains possible that introduction of NDM-1-producing *K. pneumoniae* in the Kenyan population could be linked to the Indian subcontinent. At the same time it may be speculated that the large international community traveling in and out of Kenya and the air traffic between Europe, the United Kingdom, and East Africa could have easily facilitated such an event. The clonal relationship between the NDM-1-positive *K. pneumoniae* isolate 05-506 from Sweden, the *K. pneumoniae* isolate 601 from the Sultanate of Oman, and the Kenyan isolates presents a very strong argument that this link might exist. Interestingly, *K. pneumoniae* isolates belonging to the same clonal lineage were found to harbor different $bla_{\text{NDM-1}}$-positive plasmids. This is in accordance with the observations made by Kumarasamy et al. (9), who showed an important diversity of plasmid size and scaffolds among NDM-1-positive isolates recovered from the United Kingdom, India, and Pakistan.

Finally, this report described the first occurrence of NDM-1 producers in Africa. However, this may not be considered a nosocomial outbreak since these isolates were recovered over a long period of time and from patients who did not have any obvious epidemiological connection. The multidrug resistance pattern of the *K. pneumoniae* isolates identified here mirrors that of NDM-1 producers previously identified in India, Pakistan, and the United Kingdom and is a source of deep concern. As extensively reported for outbreaks of ESBL producers, *K. pneumoniae* is a bacterial species that is prone to being an excellent vector for hospital dissemination of resistance genes. We fear that gene exchanges similar to those observed extensively for ESBL genes may occur for the $bla_{\text{NDM-1}}$ gene, i.e., gene circulation in community-acquired *E. coli* which is then transferred to hospital-based *K. pneumoniae*.

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