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Laurent Poirel
_Hôpital de Bicêtre_

Gunturu Revathi
_Aga Khan University_

Sandrine Bernabeu
_Hôpital de Bicêtre_

Patrice Nordmann
_Hôpital de Bicêtre_

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

Laurent Poirel,1 Gunturu Revathi,2 Sandrine Bernabeu,1 and Patrice Nordmann1*

Service de Bactériologie-Virologie, INSERM U914 Emerging Resistance to Antibiotics, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris Sud, K.-Bicêtre, France,1 and Department of Pathology, The Aga Khan University Hospital, Nairobi, Kenya2

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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*<sub>NDM-1</sub> 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 (New Delhi metallo-*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 MICs 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*<sub>IMP</sub>, *bla*<sub>VIM</sub> (16), and *bla*<sub>NDM-1</sub> (primer NDM-Fm, 5′-GGTTTGGCGATCTGGTTT-CGAACAG-3′; and primer NDM-Rm, 5′-CGGAATGGCTCATCAC-GATC-3′). The isolates were positive for *bla*<sub>NDM-1</sub> and sequencing of the PCR products revealed 100% identity with the published sequence of the *bla*<sub>NDM-1</sub> 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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* Corresponding author. Mailing address: Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 Rue du Général Leclerc, 94275 Le Kremlin-Bicêtre Cedex, France. 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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confirmed by performing multilocus sequence typing as described previously (7) which showed that the \textit{K. pneumoniae} isolates of Kenya belonged to the ST14 sequence type, as reported for \textit{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 \(\beta\)-lactamase (ESBL) and AmpC productions was carried out by PCR analysis using specific primers to detect broad-spectrum \(\beta\)-lactamase genes (4), followed by sequencing. All isolates harbored the \textit{bla} \textit{CMY-6} AmpC gene and the \textit{bla} \textit{SHV-28} and \textit{bla} \textit{TEM-15} ESBL genes, together with the \textit{bla} \textit{TEM-15} ESBL genes, had a 75-kb plasmid, belonged to the \textit{IncA/C} 2 \(\beta\)-lactamase genes. Yong et al. (19) reported that the NDM-1-positive \textit{K. pneumoniae} strain 05-506 was \textit{bla} \textit{CMY-4} positive. Screening of 16S rRNA genes encoding methylase, performed by using a multiplex PCR approach as described previously (1), identified the \textit{rmtC} methylase gene conferring high-level resistance to all aminoglycosides in the Kenyan isolates.

Transferability of the \textit{bla} \textit{NDM-1} gene was studied by conjugation experiments as described previously (17), with a selection based on ceftazidime (30 \(\mu\)g/ml) and azide (100 \(\mu\)g/ml), using \textit{K. pneumoniae} \textit{Kp} 7 as the donor strain and \textit{E. coli} J53 (resistant to azide) as the recipient strain. One of the obtained transconjugants, \textit{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 \(\mu\)g/ml, respectively [MICs for these same molecules were 0.12 \(\mu\)g/ml for the \textit{E. coli} J53 recipient strain]). It was resistant to all aminoglycosides and to sulfonamides, and PCR analysis confirmed that plasmid pKp7 coharbored the \textit{rmtC} gene. This plasmid, analyzed by using the Kieser technique (8), was 120 kb in size and belonged to the \textit{IncA/C2} incompatibility group as demonstrated by the PCR-based replicon typing method (2), whereas the \textit{bla} \textit{NDM-1}-positive plasmid in \textit{K. pneumoniae} 05-506 was untypeable (9), and that of \textit{K. pneumoniae} 601 from the Sultanate of Oman was reported to belong to the InL/M group (11). Kumarasamy et al. (9) reported \textit{bla} \textit{NDM-1}-positive plasmids from the Indian isolates that were either untypeable or belonged to the \textit{IncA/C} or \textit{IncFII} plasmid incompatibility group. Another type of transconjugant was obtained, \textit{E. coli}(pKp7-B), expressing an ESBL phenotype. It harbored the \textit{bla} \textit{CTX-M-15} and \textit{bla} \textit{TEM-1} genes, had a 75-kb plasmid, belonged to the \textit{IncF} group, and did not carry any other non-\(\beta\)-lactam resistance markers.

PCR mapping was performed to identify the genetic sequences surrounding the \textit{bla} \textit{NDM-1} gene in \textit{K. pneumoniae} \textit{Kp} 7 for comparison with the sequences surrounding the \textit{bla} \textit{NDM-1} gene in \textit{K. pneumoniae} 05-506 (19). PCR experiments based on primers located in those surrounding sequences produced negative results, indicating that the structures identified in \textit{K. pneumoniae} 05-506 were not present on plasmid pKp7.

Our study further underlines the spread of the \textit{bla} \textit{NDM-1} gene worldwide, as exemplified by the newly reported NDM-1-producing \textit{E. coli} isolate identified in Australia (13), together with the report of NDM-1-producing \textit{E. coli}, \textit{K. pneumoniae}, and \textit{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 in Sweden (19). Histories of contacts or the origin of the 936 POIREL ET AL. ANTIMICROB. AGENTS CHEMOTHER. 5046–5054.

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