Outbreak Caused by *Enterobacteriaceae* Harboring NDM-1 Metallo- β -Lactamase Carried in an IncFII Plasmid in a Tertiary Care Hospital in Mexico City

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Carbapenem-resistant *Enterobacteriaceae* carrying New Delhi metallo- β -lactamase 1 (NDM-1) have rarely been reported in Latin America. We report of an outbreak caused by a *bla*_{NDM-1}-harboring plasmid spread through different bacterial species, including *Escherichia coli* (ST617) and *Enterobacter cloacae* (ST182) isolates from the same patient and three *Klebsiella pneumoniae* isolates (ST22) derived from three epidemiologically related patients. IncFII plasmids were found in all strains. Measures to control the outbreak were applied successfully.

The spread of carbapenem-resistant *Enterobacteriaceae* (CRE) is a global concern. The most common mechanism of CRE is the acquisition of plasmid-borne β -lactamases that can hydrolyze carbapenems. The New Delhi metallo- β -lactamase (MBL) 1 (NDM-1) has been reported in several regions of the world, mostly in patients with a history of travel to areas where it is endemic but scarcely in Latin America (1–4). We report an outbreak caused by NDM-1-harboring *Enterobacteriaceae*, where horizontal gene transfer likely occurred between different species with further clonal expansion.

Hospital setting and bacterial strains. Ours is a 230-bed tertiary care hospital in Mexico City with a 14-bed intensive care unit (ICU) to which adult patients from all of Mexico are referred for medical treatment. The first case of CRE carrying an MBL was detected on 5 November 2013 in a patient transferred from a southwestern city in Mexico who was admitted to the ICU. A second case was identified on 14 December 2013 in the ICU. After this, we established active screening of CRE carriers via rectal swab cultures. Rectal swabs were cultured using a 10- μ g ertapenem disk in 5 ml Trypticase soy broth (6). Other clinical samples were collected from the patients according to the usual protocol.

Antimicrobial susceptibility tests and molecular detection of β -lactamases. Identification and antimicrobial susceptibility of the isolates were done with the Vitek 2 (bioMérieux, Durham, NC) and interpreted according to CLSI standards (7). Additionally, the EDTA double-disc synergy phenotypic test was performed to detect metallo- β -lactamases (8). Presence of β -lactamases was determined by PCR (*bla*_{IMP}, *bla*_{VIM}, *bla*_{NDM}, *bla*_{GES}, *bla*_{CTX-M-15}, *bla*_{OXA-48}, *bla*_{SHV}, *bla*_{TEM}), as described elsewhere (8, 9). PCR-generated fragments were purified by Qiaquick PCR purification spin columns (Qiagen, Venlo, Netherlands), and sequenced with a 3130xl genetic analyzer (AB Applied Biosystems, Hitachi, San Francisco, CA). The nucleotide sequences were analyzed through the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov>). Five isolates (1 *Escherichia coli*, 1 *Enterobacter cloacae*, and 3 *Klebsiella pneumoniae*) from four patients were evaluated. All isolates showing resistance to third-generation cephalosporins, quinolones, monobactams,

carbapenems, and PCR were positive for NDM-1 and CTX-M-15 (Table 1).

Genotyping. Pulsed-field gel electrophoresis (PFGE) was performed for all *K. pneumoniae* clinical ($n = 3$) and rectal swab ($n = 2$) isolates using XbaI for digestion (Bio-Rad, Hercules, CA). Electrophoresis was carried out in a CHEF DRII apparatus (Bio-Rad), as recommended. *Salmonella braenderup* H9812 standard was used as a molecular size marker. Multilocus sequence typing (MLST) was performed for *K. pneumoniae*, *E. coli*, and *E. cloacae* isolates following the protocols described at <http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>, <http://mlst.warwick.ac.uk/mlst/dbs/Ecoli>, and <http://pubmlst.org/ecloacae>, respectively. The sequences of the PCR products were aligned and edited using BioEdit software (Ibis Bioscience, Carlsbad, CA) and compared with the sequences at the corresponding MLST website for each species. The number of alleles was determined for each sequence, and the ST was assigned: *E. coli* and *E. cloacae* were typed as ST617 and ST182, respectively. All *K. pneumoniae* isolates were typed as ST22 and showed an identical pulsotype by PFGE.

Plasmid analysis and conjugation. Plasmid extraction was performed by the Eckhardt technique (10). In order to detect the plasmid harboring the *bla*_{CTX-M-15} and *bla*_{NDM-1} genes, Southern blotting and hybridization were performed. The plasmid incompatibility group was determined by PCR replicon typing (10, 11). Conjugative assays were performed by the Miller method (12), using the *E. cloacae bla*_{NDM-1}-positive isolate (Table 1, patient A)

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TABLE 1 Clinical characteristics of the outbreak cases, isolate antimicrobial susceptibility profile, and molecular mechanisms of resistance

Patient information	Data for patient:				Data for transconjugant strain
	A	B	C	D	
Age (yr)/sex	30/male	41/male	50/male	35/male	
Underlying disease	Necrotizing pancreatitis	Acute lymphoblastic leukemia/urosepsis	Chronic myeloid leukemia, hospital acquired pneumonia	Chronic ulcerative colitis, abdominal sepsis	
History of travel abroad	No	No	No	No	
Antibiotic treatment for CRE	Imipenem-clastatin, colistin	Imipenem-clastatin, colistin	Meropenem, colistin	Imipenem-clastatin, colistin	
ICU stay (day/mo/yr)	29/10/13 to 26/11/13	02/12/13 to 22/12/13	24/12/13 to 28/12/13	No	
Outcome	Death	Cure	Cure	Cure	
Specimen origin	Abdominal abscess	Urine	Sputum	Abdominal abscess	
Isolation date (day/mo/yr)	05/11/13	14/12/13	03/02/14	30/12/13	
Bacterial species	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	<i>E. coli</i> J53
MIC (mg/liter)					
Ceftazidime	>64	>64	>64	>64	>64
Ceftriaxone	>64	>64	>64	>64	>64
Gentamicin	>16	>16	>16	>16	>16
Amikacin	>64	>64	>64	>64	>64
Ciprofloxacin	>4	2	>4	2	0.5
Aztreonam	>64	16	64	64	<1
Ertapenem	>8	>8	>8	>8	>8
Imipenem	>16	8	>16	>16	>16
Meropenem	16	8	>16	>16	>16
EDTA double-disc synergy test outcome	Positive	Positive	Positive	Positive	Positive
Molecular resistance genes	NDM-1, CTX-M-15	NDM-1, CTX-M-15	NDM-1, CTX-M-15	NDM-1, CTX-M-15	NDM-1
MLST	ST182	ST617	ST22	ST22	NA ^a
Plasmid incompatibility group	IncFII	IncFII	IncFII	IncFII	IncFII

^a NA, not available.

as donor and an *E. coli* J53 (sodium azide-resistant) strain as recipient. A transconjugant strain was selected on Luria agar supplemented with meropenem 2 µg/ml and sodium azide 100 µg/ml. We determined the presence of a common 101-kb plasmid in all isolates, typed IncFII, that was positive for *bla*_{NDM-1} hybridization. The *bla*_{CTX-M-15} gene was detected in a 195-kb plasmid in *E. cloacae* and in a 170-kb plasmid in *E. coli*. In the transconjugant strain, only the 101-kb plasmid harboring NDM-1 was detected.

Epidemiological investigation. Patients A, B, and C were admitted to the same ICU bed in consecutive order. Patient C was not a fecal carrier, and he was not infected with NDM-1 during this admission; however, he was later readmitted due to pneumonia caused by an NDM-1 *K. pneumoniae* infection with a pulso-type identical to that of the strain infecting patients B and C. After the initial two cases, all patients admitted to the ICU or in contact with patients carrying or infected by a CRE were screened weekly for CRE fecal carriage, but no other patients harboring NDM-1-producing isolates were detected. We evaluated all CRE isolates available from the previous 6 months for the presence of NDM, and no previous isolates carrying this enzyme were detected. Patient D, who was not treated in the ICU but was treated in another ward during the same period, was infected by a *K. pneumoniae* isolate producing NDM-1 that exhibited the same molecular fingerprint as those isolated from patients B and C. Hospital sanitation procedures for the ICU were reinforced after the first two cases, and hydrogen peroxide-based disinfection was performed on this bed as soon as patient C was found to be infected by a *K. pneumoniae*-producing NDM-1 isolate. No further cases were detected. Other clinical data and isolate information are summarized in Table 1.

Concluding remarks. This report underscores the enormous threat posed by NDM-1 CREs to hospitals and their patients because of the ability of *bla*_{NDM-1} to spread clonally and be transmitted between different species *in vivo*.

In this outbreak, patient A may have coharbored a nondetected NDM-1-producing *K. pneumoniae* isolate as enteric carriage, along with *E. coli* and *E. cloacae*, which contaminated this bed at the ICU and was transmitted to the other cases, even after standard sanitation measures were employed. Contamination of surfaces in the hospital environment is a well-recognized mechanism of patient-to-patient transmission of other hospital-acquired infections. Nevertheless, data on CRE are controversial; some researchers have demonstrated that the vicinity of patients colonized with CRE is often contaminated, whereas other studies have infrequently isolated these pathogens from the environmental surfaces in rooms of infected patients. This is believed to depend largely on the inoculum (13–15).

We believe that patient C was colonized during his first admission to this ICU bed but developed a pulmonary infection by the outbreak strain later, which indicates that colonization may persist as long as 3 months (16). We were unable to establish a direct epidemiological relationship between patient D and the others. We believe that transmission may have occurred via the hands of medical or nurse personnel because the ICU does not have dedicated personnel. However, we were not able to confirm this because we did not screen hospital personnel for carriage (17). An ST22 *K. pneumoniae* isolate was recently reported as an NDM-1 carrier in a pediatric case in Mexico and may represent a circulating strain in our region (18). Therefore, we believe that these

strains have been present in our region but remained unrecognized given the insufficient screening done until now.

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