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Interhospital spread of NDM-7-producing *Klebsiella pneumoniae* belonging to ST437 in Spain

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ABSTRACT

This study describes an interhospital spread of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) producing NDM-7 carbapenemase that started in December 2013 in Madrid, Spain. NDM-7-producing CRKP were isolated from urine, rectal swabs or blood samples from seven patients admitted to three different hospitals (Hospital Universitario La Paz, Hospital de Cantoblanco and Hospital Central de la Cruz Roja). The isolates were resistant to all antimicrobials tested except colistin and fosfomycin. One blood isolate was susceptible to minocycline and tigecycline but was resistant to fosfomycin. All isolates were closely related by pulsed-field gel electrophoresis (PFGE) and DiversiLab® analysis and belonged to multilocus sequence typing (MLST) sequence type 437. In addition, bla_{NDM-7}, bla_{TEM-1}, $bla_{CTX-M-15}$ and *aac(3)-IIa* were identified. Family contacts of the index case were negative for NDM-producing bacteria. The outbreak occurred in two separate waves and the cases associated with Hospital de Cantoblanco had been admitted to the same room. Environmental samples from the trap of a sink and a shower in this room were positive for NDM-7-producing CRKP. To our knowledge, this is the first reported worldwide outbreak of NDM-7-producing CRKP. No relationship with the Indian continent, the Balkans or the Middle East could be established. Frequent transfer of aged or chronically ill patients between the facilities involved may have favoured the spread of NDM-7-producing CRKP. The spread of the second wave in Hospital de Cantoblanco probably occurred as a result of transmission from an environmental reservoir.

1. Introduction

New Delhi metallo-β-lactamase (NDM), a carbapenemase enzyme first described in Sweden in 2009 [1] in a patient who had received medical care in India, has since been detected and reported worldwide [2]. NDM carbapenemases are metallo-β-lactamases (MBLs) that have a broad substrate profile that includes carbapenems but not monobactams. Although NDM-producers have been described worldwide, they are mainly recovered from patients who have travelled or received medical care in the Indian subcontinent, and in some cases from patients epidemiologically linked with the Balkan states [2] and the Middle East [3]. In a European survey carried out from 2008 to 2010, 77 cases were reported from 13 countries [4]. Despite the concern generated by the worldwide spread of NDM-type carbapenemases, few cases have been detected in Spain [5–8]; all have been NDM-1 and had an established origin in India. The aim of this study was to describe the first hospital outbreak caused by NDM-7-producing *Klebsiella pneumoniae* with interhospital spread.

2. Materials and methods

2.1. Setting

Three hospitals of the Spanish National Public Health Service were involved in the outbreak. Hospital Universitario La Paz (HULP)

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is a teaching 1328-bed acute tertiary hospital. Attached to HULP is Hospital de Cantoblanco (HB), a 160-bed secondary centre for acute and rehabilitation care located 15 km from HULP. Both provide care for a population of 600 000 people in Madrid, Spain. Finally, Hospital Central de la Cruz Roja (HCRO) is a 168-bed secondary centre that gives medical and surgery support to the rest of the hospital network of Madrid. HCRO provides care to a large number of aging patients transferred from emergency departments of other hospitals.

2.2. Patients

Seven patients from three different hospitals were either colonised (three patients) or infected (four patients) by carbapenem-resistant *K. pneumoniae* (CRKP). Five were men and the median age was 78 years; two patients died. The median duration from admission to detection of CRKP in these seven patients was 32 days (range, 21–44 days). Table 1 shows the clinical and epidemiological characteristics of the CRKP cases. Patients 1, 4 and 6 were not treated because they were considered colonised. Patient 2 died before the results of the urine culture were available.

2.3. Bacterial isolates and determination of carbapenemase and extended-spectrum β-lactamase (ESBL) production

CRKP isolates were identified using a MALDI Biotyper (Bruker Daltonik, Bremen, Germany). Antibiotic susceptibility was determined using the Wider® system (Francisco Soria Melguizo, Madrid, Spain). Isolates were categorised as susceptible or resistant according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [9]. ESBL production was detected by the double-disk diffusion method with amoxicillin/clavulanic acid and aztreonam (Sensi-Disc™; Becton Dickinson, Heidelberg, Germany). Carbapenem minimum inhibitory concentrations (MICs) were confirmed by Etest (bioMérieux, Marcy-l'Étoile, France). All isolates were studied according to the algorithm for phenotypic carbapenemase detection recommended by EUCAST [10]. Carbapenemase production was tested by the modified Hodge test (MHT) using imipenem, meropenem and ertapenem disks, and by the colorimetric Carba NP method [11]. Inhibition tests with boronic acid and with ethylene diamine tetra-acetic acid (EDTA) were used to screen for the production of class A and class B carbapenemases, respectively. All study isolates were submitted to the antibiotic laboratory of the Spanish National Centre of Microbiology (Madrid, Spain), which acted as a reference laboratory for further molecular studies.

2.4. Surveillance and environmental studies

Rectal swabs were cultured directly on MacConkey agar plates containing 4 mg/L cefotaxime (Tec-Laim, Madrid, Spain). Stool samples of the family contacts were collected and were cultured on MacConkey agar plates containing 4 mg/L cefotaxime and in 10 mL of thioglycollate broth (Tec-Laim) containing one 10 μg imipenem disk per tube (Sensi-Disc™). After 24 h, a subculture of thioglycollate broth was made on MacConkey agar and MacConkey agar supplemented with 4 mg/L cefotaxime. Sampling of the sink and shower traps was performed by introducing a urinary catheter through the strainer and extracting water from the trap with a syringe. The water was centrifuged at 3000 rpm for 5 min and the precipitate was inoculated in brain–heart infusion broth (Tec-Laim), incubated overnight at 37 °C, and then plated on MacConkey agar with 4 mg/L cefotaxime. The colonies grown on MacConkey agar were analysed by the MHT and disk diffusion, and bla_{NDM} was confirmed by PCR.

2.5. Molecular characterisation of antibiotic resistance genes

All of the isolates in which carbapenemase production had been confirmed by phenotypic methods were further characterised by molecular methods. Multiplex real-time commercial PCR was used for identification of the carbapenemase genes bla_{KPC}, bla_{VIM} and bla_{OXA-48} (Progenie Molecular, Valencia, Spain); an in-house assay was performed for the detection of bla_{IMP} and bla_{NDM} [5,12] and the β-lactamase-encoding genes bla_{TEM}, bla_{CTX-M}, bla_{OXA-1} and bla_{AmpC} [13–15]. The presence of bla_{NDM-7} and $bla_{CTX-M-15}$ was finally confirmed by DNA sequencing using the dideoxynucleotide chain termination method. Screening was also undertaken for the *qnrA*, *qnrB* and *qnrS* genes encoding resistance to quinolones, for the aminoglycoside acetyltransferase resistance genes *aac(3)-IIa* and *aac(6′)-Ib* and for the 16S rRNA methylase genes *armA*, *rmtA* and *rmtB* using published methods [16,17]. In-house positive controls provided by the Spanish National Center for Microbiology were included in all PCR assays. Furthermore, the genetic environment of bla_{NDM-7} was analysed using specific primers previously reported [18] plus other primers designed in this study according to GenBank (National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD) database entry **NG_041649** (PRE-NDM-F, 5′-TCCAGGGATAACCGGGCAAACA-3′; PRE-NDM-R, 5′-GAGGATCTGGGCGGTCTGGTCA-3′; POS-NDM-F, 5′-TCTGGCAGCACACTTCCTATCTCG-3′; and POS-NDM-R, 5′-GCTCCCGCCGCGTTCACCAAT-3′).

2.6. Molecular typing

The genetic relationships between the CRKP isolates were determined by pulsed-field gel electrophoresis (PFGE) after total chromosomal DNA digestion with *Xba*I and automated repetitive-sequence-based PCR using the DiversiLab® system (bioMérieux). The multilocus sequence typing (MLST) sequence type was determined according to the Institute Pasteur scheme (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html; accessed December 2014). Clone-specific real-time PCR for *K. pneumoniae* ST11 and ST405 was used for rapid typing [19].

3. Results

3.1. Susceptibility testing

All CRKP isolates were resistant to all β-lactams tested, including imipenem, meropenem and ertapenem, aminoglycosides, fluoroquinolones and trimethoprim/sulfamethoxazole; they remained susceptible to fosfomycin and colistin. The isolate recovered from blood in Patient 5 was also susceptible to minocycline and tigecycline but was resistant to fosfomycin. Carbapenem MICs were >32 mg/L for imipenem, meropenem and ertapenem as further confirmed by Etest. The MHT, Carba NP and the inhibition test of carbapenemase activity with EDTA were positive, indicating production of a MBL. The double-disk diffusion method with amoxicillin/clavulanic acid and aztreonam was positive, indicating the production of an ESBL.

3.2. Mechanisms of antibiotic resistance and molecular epidemiology

PCR was positive for bla_{NDM}; its DNA sequence showed 100% homology with bla_{NDM-7} (GenBank sequences KC567147.1 and JX262694.1), and the predicted protein sequence differed from