Abdominal abscess due to NDM-1-producing *Klebsiella pneumoniae* in Spain

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We describe a clinical case of an abdominal abscess due to NDM-1-producing *Klebsiella pneumoniae* in a 35-year-old Spanish patient after hospitalization in India for perforated appendicitis and peritonitis. The strain belonged to the MLST type 231 and had multiple additional antibiotic resistance genes such as *blaCTX-M-15*, *armA* methylase, *aac(6')-Ib-cr*, *dfrA12*, *sul1* and *qnrB* and lack of porin genes *ompK35* and *ompK36*. The patient was cured after abscess drainage.

**Introduction**

Acquired carbapenemases are increasingly reported in Gram-negative pathogens (Miriagou *et al.*, 2010). Metallo-β-lactamases belong to the class B carbapenemases and confer resistance to all β-lactams, including carbapenems, except aztreonam. Spread of metallo-β-lactamases among members of the *Enterobacteriaceae* represents a public health threat. Although VIM and KPC types are the most common carbapenemases in Europe, the class D OXA-48 carbapenemase is also spreading, and the New Delhi metallo-β-lactamase 1 (NDM-1) has been described in Sweden (Yong *et al.*, 2009) and the UK (Kumarasamy *et al.*, 2010), mostly in patients who had received health care in India. In a recent European survey, a total of 77 cases were reported from 13 countries from 2008 to 2010 (Struelens *et al.*, 2010).

We describe a clinical case of NDM-1-producing *Klebsiella pneumoniae* in Spain causing an abdominal abscess after surgery for perforated appendicitis. To the best of our knowledge, this is the first case of NDM-1-producing *K. pneumoniae* in our country.

**Case report**

A 35-year-old white male was admitted to a third level teaching hospital in Madrid (Spain) in August of 2010 due to acute abdominal pain in the right iliac fossa. In the previous 9 days, the patient had been hospitalized in India because of acute perforating appendicitis followed by peritonitis. The patient underwent a laparoscopic appendectomy and peritoneal cavity washing, *Pseudomonas aeruginosa* and *Escherichia coli* were cultured from the peritoneal fluid, both susceptible to levofloxacin and meropenem, and treatment with one unidentified antibiotic was prescribed for 5 days. The patient returned to Spain, and 2 days after completing antibiotic treatment started to have pain in the right iliac fossa and had a temperature of 38.8 °C. A haemogram revealed leukocytosis and an abdominal echography showed a pus collection in the right iliac fossa. Blood cultures were obtained, and antimicrobial therapy with amoxicillin/clavulanic acid (1 g t.i.d.) was prescribed. Three days later, leukocytosis continued, treatment was changed to meropenem (1 g t.i.d.) and the abscess was then drained. The purulent exudate was cultured onto conventional media for aerobic and anaerobic bacteria following standard methodologies. Growth was positive for *Enterococcus faecium* and carbapenem-resistant *K. pneumoniae* but anaerobic cultures were negative. Blood cultures were also negative.

Although the patient was not specifically treated with antibiotics active against carbapenem-resistant *K. pneumoniae*, his health condition improved over the next 2 weeks, probably due to the pus drainage. A control echography did not reveal any pus collection and the patient was discharged 16 days after pus drainage. No secondary clinical cases were observed.

**Methods**

Antibiotic susceptibility and bacterial identification were carried out by the MicroScan microdilution method (Siemens Healthcare...
Diagnosics) and the Etest method (bioMérieux). *K. pneumoniae* identification was confirmed by 16S rRNA gene sequencing. Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing guidelines (http://www.eucast.org/clinical_breakpoints/; last accessed January 2012). Carbapenemase production was tested by the modified Hodge test using an imipenem disc. Imipenem susceptibility alone and in combination with EDTA was also determined (Etest; AB Biodisk).

Standard PCR conditions were used to amplify several β-lactamase genes encoding carbapenemases (*blaKPC, blaIMP, blaNDM* and *blaCTX-M*), extended-spectrum β-lactamases (*blaTEM, blaSHV* and *blaCTX-M*), plasmid-borne AmpC (*blaCIT, blavONX, blaVIM, blaVHO, blaAAC and *blaKPC*), sulphonamide resistance genes (*sul1* and *sul2*), dihydrofolute reductase genes, aminoglycoside acetylase resistance genes (*aac(3)-Ia* and *aac(6’)-Ib*) and 16S rRNA methylases (*arrA, rmtA* and *rmtB*) (Oteo et al., 2008; Lee et al., 2001; Fritsche et al., 2008). The *qnr* subtypes were characterized as described by Jacoby et al. (2008). In addition, the entire sequences of the *ompK35* and *ompK36* genes were analysed by PCR and DNA sequencing (Kaczmarek et al., 2006).

Specific primers for PCR amplification and sequencing of *NDM-1* were designed according to GenBank (National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA) database entry FN396876 (NDM-F 5’-CCATGGGCGCGGT-ATGAGTGATTG-3’; NDM-3 5’-TCGCCAAGCTGACCGC-ATTAG-3’).

MLST of *NDM-1*-producing *K. pneumoniae* was done according to the Institut Pasteur scheme (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html; last accessed January 2011). Plate mating was performed using *E. coli* HB101 (azide- and kanamycin-resistant) as a recipient and selecting on Mueller–Hinton agar plates containing kanamycin (100 mg l⁻¹), azide (160 mg l⁻¹) and cefotaxime (4 mg l⁻¹). Plasmids were typed as described by Carattoli et al. (2005). Plasmid sizes were determined by S1 nuclease digestion of whole genomic DNA and PFGE (Sánchez-Romero et al., 2012).

### Results and Discussion

The *K. pneumoniae* clinical isolate was resistant to all the tested antibiotics except colistin (MIC = 1 mg l⁻¹), tigecycline (MIC = 0.25 mg l⁻¹) and fosfomycin (MIC = 32 mg l⁻¹). The modified Hodge test and the imipenem/imipenem-EDTA Etest strips were positive, with an imipenem MIC > 256 mg l⁻¹ and an imipenem/EDTA MIC ≤ 1 mg l⁻¹ (Table 1).

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;16 (resistant)</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>&gt;16 (resistant)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>&gt;64 (resistant)</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>&gt;64 (resistant)</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>&gt;16 (resistant)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&gt;16 (resistant)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;128 (resistant)</td>
</tr>
<tr>
<td>Cefotaxime/clavulanic acid</td>
<td>&gt;16 (NA)</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>&gt;128 (resistant)</td>
</tr>
<tr>
<td>Cefpodoxime/clavulanic acid</td>
<td>&gt;32 (NA)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;16 (resistant)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8 (resistant)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&gt;8 (resistant)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;2 (resistant)</td>
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<tr>
<td>Cotrimoxazole</td>
<td>&gt;2 (resistant)</td>
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<tr>
<td>Nitrofurantoin</td>
<td>&gt;256 (resistant)</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>32 (susceptible)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.25 (susceptible)</td>
</tr>
<tr>
<td>Colistin</td>
<td>1 (susceptible)</td>
</tr>
</tbody>
</table>

**Table 1. Antibiotic susceptibility of *Klebsiella pneumoniae***

MLST type 231 co-producing NDM-1 and CTX-M-15

NA, Not applicable.

of them unrelated to ST231. Only information about six ST231 *K. pneumoniae* isolates is available at the Institut Pasteur MLST database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html; last accessed January 2012), four of them isolated from Vietnam (two meningitis and one sepsis), one from Africa (urinary tract infection) and one from India (peritoneal fluid). No antibiotic resistance data for these isolates are available at the website.

Conjugation experiments failed. PFGE after S1 nuclease digestion revealed that the NDM-1-producing *K. pneumoniae* harbourd two plasmids of ~120 and ~220 kb. *blaNDM-1* was detected in the ~120 kb plasmid by PCR amplification and sequencing of the corresponding DNA band. A PCR-based replicon-typing scheme demonstrated that the ~120 kb plasmid belonged to Inc group F (replicon FIB).

To the best of our knowledge, this is the first case of NDM-1-producing *K. pneumoniae* in Spain. Only one case of a member of the *Enterobacteriaceae* producing NDM-1 has been previously communicated in Spain; it was an *E. coli* isolate recovered from a stool specimen from a Spanish patient with travellers’ diarrhoea who had travelled to India (Solé et al., 2011).

We did not observe secondary nosocomial transmission of this extensively drug-resistant *K. pneumoniae*, or NDM-1
transmission to other bacterial species. However, there is a high potential risk of nosocomial secondary outbreaks following hospital admission of patients receiving health care in endemic countries. Surveillance measures, including screening colonization, should be enhanced upon admission of these patients. This patient was assigned to contact precautions, including the use of disposable gowns and gloves, and improvement of hand hygiene compliance by the use of alcohol rubs. The patient was not colonized by carbapenem-resistant \textit{K. pneumoniae}.

In northern Italy, NDM-1 was detected in \textit{K. pneumoniae} and \textit{E. coli} isolates obtained from six hospitalized patients. One of the patients had been hospitalized previously in India and may have been the source of the outbreak (Gaibani et al., 2011).

In Spain, several outbreaks caused by members of the \textit{Enterobacteriaceae} producing carbapenemases have been recently reported, mostly due to VIM-1 (Tato et al., 2010; Oteo et al., 2010; Sánchez-Romero et al., 2012), OXA-48 (Pitart et al., 2011) and KPC (Curiao et al., 2010).

Isolation of \textit{K. pneumoniae} co-producing NDM-1 and CTX-M-15, as previously described (Poirel et al., 2011; Sole et al., 2011) is a relevant epidemiological issue because CTX-M-15 and NDM-1 may share a common source in the Indian subcontinent. Effective measures to control the spread of NDM-1 and other carbapenemases are strongly needed.

Acknowledgements

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References


