Short communication

NDM-1- and OXA-163-producing Klebsiella pneumoniae isolates in Cairo, Egypt, 2012

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1. Introduction

Infections caused by multidrug-resistant strains of Klebsiella pneumoniae are spreading widely all over the world [1]. These infections are associated with high morbidity and mortality, mainly in immunocompromised individuals such as cancer patients [2]. They have become highly prevalent in some geographic areas, including Mediterranean countries and the Middle East [1].

Carbapenems are first-line drugs for severe infections caused by extended-spectrum β-lactamase (ESBL)-producing K. pneumoniae strains. However, the worldwide spread of carbapenemase-producing clones is severely undermining the efficacy of these antimicrobials [1]. In Egypt, isolation of carbapenem-resistant K. pneumoniae has been reported in the last years [3].

Here we report two autochthonous cases of infection caused by two carbapenem-resistant K. pneumoniae isolates, producing NDM-1 and OXA-163, respectively, in two cancer patients in Cairo, Egypt, in 2012. The phenotypic and molecular characteristics of the two isolates are described.

2. Materials and methods

2.1. Patients and bacterial isolates

Case 1 was a 56-year-old male hospitalised on 6 July 2012 with a diagnosis of adenocarcinoma of the gastro-oesophageal tract. On 4 October 2012, he was admitted to the intensive care unit (ICU) owing to severe sepsis. A gastric fluid sample was taken that yielded imipenem-resistant K. pneumoniae (isolate 39). The patient started on therapy with levofloxacin and teicoplanin. Thereafter, he was submitted to oesophagectomy and gastric pull-up on 16 October 2012.

Case 2 was a 45-year-old male. He had a diagnosis of acute myeloid leukaemia on 3 November 2012. Because of cardiac and renal impairment with fever, he was admitted to the ICU on 26 November 2012 and was started on meropenem, vancomycin, amikacin and acyclovir. A blood culture was positive for carbapenem-resistant K. pneumoniae (isolate 181). The previous antibiotic treatment was interrupted and a combination of imipenem and levofloxacin was administered.

In both cases the treatment was reported as effective. Both patients were hospitalised at the National Cancer Institute (NCI) (Cairo, Egypt) and had never travelled outside of Egypt.

Identification and antibiotic susceptibility testing of the K. pneumoniae isolates was carried out at the microbiology laboratory...
of NCI using a MicroScan® system (Siemens Healthcare Diagnostics).

2.2. Antimicrobial susceptibility testing

The two carbapenem-resistant K. pneumoniae strains were sent to the molecular epidemiology laboratory of the Department of Sciences for Health Promotion and Mother–Child Care ‘G. D’Alessandro’, University of Palermo (Palermo, Italy) for confirmation and typing. Susceptibility testing to third-generation cephalosporins, carbapenems, gentamicin, fluoroquinolones, colistin and tigecycline was performed with Etest strips (bioMérieux, Marnes-la-Coquette, France). Results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [4].

2.3. Molecular characterisation

PCR screening for carbapenemase-encoding genes of classes A (blaKPC, blaGES), B (blaVIM, blaIMP, blaNDM) and D (blaOXA-48) and for ESBL-encoding genes blaTEM, blaOXA, blaSHV and blaCTX-M was performed as described previously [5,6]. PCR products were purified and sequenced using a BigDye® Terminator v.1.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK) and an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The nucleotide and deduced protein sequences were analysed with the software available from the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov).

Multiplex PCR for detecting 16S rRNA methylase genes was carried out according to Bergot et al. [7]. Plasmid-mediated quinolone resistance genes qnrA, qnrB, qnrC, qnrD, qnrS, qepA and aac(6’)-Ib-cr were also investigated by PCR as described previously [8]. The identity of resistance genes was confirmed by DNA sequence analysis. Outer membrane protein (OMP) gene amplification was conducted using ompK35-F and -R and ompK36-F and -R, and PCR products were sequenced using OMP primers according to Kaczmarek et al. [9]. Sequences were analysed by comparison with reported nucleotide sequences in GenBank. Expression levels of the ompK35 and ompK36 genes were not investigated.

To assess clonality, the two isolates were submitted to pulsed-field gel electrophoresis (PFGE) following XbaI DNA digestion. In addition, multilocus sequence typing (MLST) was performed on both isolates according to the protocol described on the K. pneumoniae MLST website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html). MLST results were compared with the international K. pneumoniae MLST database at the Pasteur Institute (Paris, France).

3. Results

The phenotypic and genetic antimicrobial drug resistance characteristics of the two K. pneumoniae isolates are summarised in Table 1. The isolates exhibited resistance to extended-spectrum cephalosporins, aztreonam and carbapenems. In addition, both isolates were resistant to ciprofloxacin and were susceptible to colistin and tigecycline. Isolate 39 was also resistant to gentamicin.

For both isolates, positive PCR results were obtained for the blaTEM, blaSHV and blaCTX-M genes (Table 1). The ESBL CTX-M-15 was detected in both isolates. Moreover, PCR results showed that isolate 39 was positive for blaNDM and isolate 181 was positive for blaOXA-48-like. Sequencing identified the blaNDM as NDM-1 and the blaOXA-48-like as OXA-163 (Table 1). Both isolates tested positive for aac(6’)-Ib-cr and qnrB1. The ompK35 and ompK36 genes of isolate 181 were amplified, sequenced and aligned with those of OMP gene sequences of K. pneumoniae MGH78578. The ompK35 gene of the isolate showed an indistinguishable sequence from that of a previously reported OXA-163-carrying K. pneumoniae isolate [3]. Comparison of the ompK36 gene sequence with the wild-type sequence revealed an insertion of three nucleotides (5’-GAC-3’) at positions 406–408 owing to duplication of the adjacent region at nucleotide positions 403–405. Sequencing also detected a nine nucleotide deletion at positions 550–558, a C insertion at nucleotide position 583, and a four nucleotide insertion (5’-GGC-3’) at position 924. Moreover, 43 nucleotide substitutions starting from position 210 to 969 were detected. The ompK36 sequence of isolate 181 was assigned accession no. KC977458 in the GenBank database.

PFGE showed that the two isolates were genetically unrelated to each other. In addition, MLST attributed to isolates 39 and 181 two different sequence types (STs), ST11 and ST16, respectively.

4. Discussion

The endemic presence of multiresistant enterobacterial strains carrying the blaNDM-1 gene in India and Pakistan along with isolation of these strains in other countries, such as the UK, Australia, USA and France, with epidemiological links with the Indian subcontinent has been repeatedly reported [1,6,10]. Recently, several reports have also described isolation of NDM-1-producing enterobacterial strains in Morocco, Oman, United Arab Emirates and Iran [11–14]. Of interest, the majority of the cases could not be directly linked to the Indian subcontinent nor had a history of foreign travel [9–12].

To the best of our knowledge, this is the first report of NDM-1-carrying K. pneumoniae in Egypt. This finding of an additional case without an apparent epidemiological link to an endemic area reinforces the hypothesis of an autochthonous presence of the blaNDM-1 resistance determinant in the Middle East and North African area. This is further supported by the identification in July 2011 of an NDM-1-producing Acinetobacter baumannii in a Czech patient repatriated from Egypt [15]. The NDM-1-producing K. pneumoniae isolate identified in the current study was shown to belong to ST11. This pandemic ST has been previously associated with NDMs in endemic areas [10] as well as with CTX-M-15 and class A (KPC) and class D oxacillines [10].

<table>
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<th>Table 1</th>
<th>Phenotypic and genetic characteristics of the two Klebsiella pneumoniae isolated in Cairo, Egypt, 2012.</th>
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<td>K. pneumoniae strain</td>
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ST, sequence type; PMQR, plasmid-mediated quinolone resistance; MIC, minimum inhibitory concentration; CAZ, ceftazidime; CRO, cefotaxime; FEP, cefepime; LEV, levofloxacin; CIP, ciprofloxacin; GEN, gentamicin; MEM, meropenem; IPM, imipenem; ETP, ertapenem; COL, colistin; TIG, tigecycline.∗MICS assessed by Etest.
OXA-48-like carbapenemases, which include OXA-163, have been identified mainly from North African countries, the Middle East, Turkey and India, but their occurrence in European countries is now well documented, with some reported hospital outbreaks [10,16]. More recently, OXA-48-like carbapenemases have also been described in North America [10]. OXA-163, in particular, has previously been identified in two K. pneumoniae isolates belonging to ST20 and ST37, respectively, isolated from two patients staying in the same cancer hospital in Cairo, Egypt [3]. The present report about a third OXA-163-carrying K. pneumoniae isolate belonging to ST16 confirms the endemic presence in this healthcare setting of this OXA-48-like determinant and proves the involvement of an additional clone. K. pneumoniae ST16 was responsible in the 2000s for nosocomial outbreaks of CTX-M-15-producing K. pneumoniae in Europe [17]. This ST has also been reported as carrying KPC-2 in Brazil, OXA-48 in Spain and NDM-1 in Canada in a patient imported from India [10,17]. Of interest, isolate 181 had high minimum inhibitory concentrations for carbapenems and, in this case as well as in the two previously reported isolates from Egypt, outer membrane permeability defects were likely contributing to resistance [3].

Dissemination of carbapenemase-producing clones of K. pneumoniae and genetic determinants of resistance to carbapenems appears increasingly wide. Attempts to map their distribution on a geographical basis appears to be doomed to a continuous update and, eventually, to failure owing to the inconsistency of surveillance and the complex worldwide trading network of resistance determinants and resistant strains. A further element needs to be added in the light of the recent developments, i.e., international travellers, such as tourists, foreign workers, immigrants or refugees. The Middle East is an area of special interest in this respect: indeed, in some countries, such as Saudi Arabia or United Arab Emirates, approximately one in three residents are foreign workers, mostly from Egypt, India, Yemen, Pakistan and the Philippines.

Global antimicrobial resistance surveillance systems supported by molecular typing, including resource-limited countries where emergence and spread of new strains and resistance mechanisms are more likely to occur, are urgently required.

Funding

None.

Competing interests

None declared.

Ethical approval

Not required.