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Short Communication

Isolation of the first New Delhi metallo-ß-lactamase-1 (NDM-1)-producing and colistin-resistant *Klebsiella pneumoniae* sequence type ST15 from a digestive carrier in Albania, May 2018

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ABSTRACT

Objectives: Carbapenemases represent a public health threat, as they can spread through horizontal gene transfer and cause outbreaks. New Delhi metallo-ß-lactamase-1 (NDM-1) is a metallo-ß-lactamase that has spread rapidly in the last decade, causing worldwide alarm. This study aimed to describe the first isolate of NDM-1-producing and extensively drug resistant *Klebsiella pneumoniae* in Albania, its clinical context and genetic characterization.

Methods: Strain was isolated from both oral and rectal intensive care unit admission screening swabs of a 70-year-old male patient with no history of international travel in the previous 6 months. Sequencing was performed by Illumina NextSeq500 platform, with a paired-end run of 2 by 150 bp, after Nextera XT paired-end library preparation. Sequencing reads were assembled using SPAdes Genome (version 3.6.1) with accurate de novo settings. The assembled contigs were uploaded into the online tools: BIGSdb-Kp, ResFinder and PlasmidFinder.

Results: Isolate was resistant to all tested antibiotics but tigecycline and trimethoprim-sulfamethoxazole. Sequencing revealed the presence of acquired resistance genes conferring resistance to β-lactams (bla_{NDM-1}, bla_{CMY-6}, $bla_{CTX-M-15}$ *and* bla_{SHV-28}), aminoglycosides (*rmtC, aac(6′)-Ib3*), fluoroquinolones (*oqxA, oqxB, aac(6′)-Ib-cr*), fosfomycin (*fosA*) and sulfonamides (*sul1*). The bla_{NDM-1} gene was located on an IncA/C2 plasmid. Plasmid mediated *mcr-1* to *mcr-8* genes were absent in both isolates. Resistance to colistin was due to an amino acid substitution (Thr157Pro) in PmrB protein.

Conclusions: NDM-1-producing *Enterobacteriaceae* are spreading in the Balkans. Identification of NDM-1-producing and extensively drug resistant *K. pneumoniae* ST15 in Albania is a cause for serious concern. There should be a continuous national and Balkan multinational surveillance of bla_{NDM-1}-carrying isolates.

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1. Introduction

New Delhi metallo-ß-lactamase-1 (NDM-1) is a class-B carbapenemase that was first identified in 2008 from a Swedish patient of Indian origin; he was transferred from New Delhi, India where he had been hospitalized [1]. The NDM-1 gene bla_{NDM-1} makes bacteria resistant to all beta-lactams except monobactams. These genes have the ability to disseminate across bacterial species through horizontal gene transfer and this has caused worldwide alarm. NDM-1-producing organisms are a cause of major public health concern, as they have caused large outbreaks and are associated with high morbidity and mortality rates. The first Balkan-linked case of NDM-1 was described in 2010 [2] in an *Acinetobacter baumannii* strain isolated in 2007 in Germany from a patient who had been hospitalized in Serbia. The first Balkan

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NDM-1 outbreak, described in 2014 [3], involved 78 *Klebsiella pneumoniae* strains isolated in Greece in the period 2010–2013. The Balkan Peninsula is considered to be a hotspot for NDM-1 besides the Indian subcontinent, Arabian Peninsula and North African countries. There is strong evidence indicating the Balkans as a reservoir for carbapenemase-encoding genes, especially for NDM-1.

The aim of this study was to describe the first isolation of NDM-1 *K. pneumoniae* in Albania, and the clinical context and genetic characterization of the strain.

2. Material and methods

2.1. Clinical description

In May 2018, a 70-year-old male patient, with no history of international travel in the last 6 months, was admitted to the central Intensive Care Unit (ICU) of University Hospital Center 'Mother Theresa' (UHCMT) in Tirana, Albania; he had acute pulmonary edema and generalized anasarca. He commenced non-invasive ventilation and conservatory treatment consisting of diuretics, anticoagulants, corticosteroids and intravenous ceftazidime (3 × 1 g). He was subsequently transferred to the Internal Medicine Ward of the same hospital, as his condition improved. Four days later, his condition worsened with hypercapnia and he was readmitted to the ICU where he was intubated and mechanically ventilated. He spent 10 days at UHCMT before being transferred to University Hospital 'Shefqet Ndroqi' (UHSN) for further evaluation of his pulmonary conditions. His admission diagnosis at UHSN was cor pulmonale with type 2 respiratory failure and severe tricuspid valve regurgitation. Computed tomography scan images revealed multiple bilateral pulmonary pneumatocele with discrete heterogenous densification of the bilateral pulmonary parenchyma and presence of pleural, pericardial and peritoneal sub-hepatic liquid. Intestinal ansae of colon transvers were extremely dilated with hydroaeric levels. His antibiotic therapy changed to intravenous meropenem (3 × 1 g). Because of severe respiratory acidosis on the same day the patient was transferred to the ICU where he was intubated and mechanically ventilated. Three screening samples were taken as part of the ICU admission routine (nasal, oral and rectal swabs). Both oral and rectal swabs yielded growth of *Klebsiella pneumoniae* strains resistant to all antibiotics tested but trimethoprim-sulfamethoxazole. The patient was put under contact precautions, and infection control measures were implemented in order to limit the spread. Five days later, the patient was extubated and afebrile, but his condition remained grave. On June 9, by written request of his family, he was taken home, where he died 3 days later.

2.2. Laboratory characterization

In Albania, species identification was performed with the BBL Crystal™ enteric/nonfermenter (E/NF) Identification (ID) Kit (Becton Dickinson, Sparks, MD, USA). Antimicrobial susceptibility testing (AST) was performed after European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion methodology and interpretation were performed according to EUCAST Clinical Breakpoint Tables version 8.1. KPC, metallo-ß-lactamase (MBL) and OXA-48 Confirm Kit (ROSCO Diagnostica A/S, Taastrup, Denmark) were used to identify carbapenemase activity. As growth was inhibited by dipicolinic acid, which is an MBL inhibitor, the organisms were suspected to be MBL producers and were shipped to Italy. In Italy, antimicrobial susceptibility tests were performed with Vitek 2 Compact (bioMérieux, Marcy-l'Étoile, France), except for tigecycline, which was performed with an ETEST® strip (bioMérieux, Marcy-l'Étoile, France), and for colistin, meropenem and piperacillin/tazobactam, which used Sensititre™ microdilution plates (Life Technologies, Monza, Italy).

2.3. Sequencing

Bacterial cultures were purified for deoxyribonucleic acid (DNA) extraction by two successive single colony selections after streaking onto blood agar medium incubated overnight at 37 °C (Becton Dickinson, Franklin Lakes, and NJ). DNA was extracted from a liquid suspension of the purified cultures using the Maxwell® 16 Cell DNA Purification Kit SEV in combination with a Maxwell® 16 Instrument to perform automated isolation of genomic DNA.

Both strains were sequenced by Illumina NextSeq500 platform, (Illumina Inc., San Diego, CA), with a paired-end run of 2 by 150 bp, after Nextera XT paired-end library preparation. Sequencing reads were assembled using SPAdes Genome (version 3.6.1) with accurate de novo settings. The assembled contigs were uploaded into the online tool BIGSdb-Kp http://bigsdb.pasteur.fr/perl/bigsdb/bigsdb.pl?db=pubmlst_klebsiella_seqdef_public. Core genome multilocus sequence typing (cgMLST) analysis was performed using the SeqSphere+ software (Ridom, Germany) exploiting a genome-wide allelic numbering schema named core genome Multilocus Sequence Typing (cgMLST). The strains were mapped on the reference genome NTUH-K2044 (NC_012731.1) by BWA software (v 0.6.2) and 2358 targets were extracted. All the targets were covered by at least five reads with a Phred value >30. A threshold of ≤4 allelic differences was used to define clusters. Whole-genome sequence data were evaluated through bioinformatics tools (ResFinder v.3.0 and PlasmidFinder v.2.0) available from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/).

3. Results

The antibiotic resistance profile of the two identified isolates can be observed in Table 1. Isolates were resistant to cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, colistin and fosfomycin, but were susceptible to tigecycline and trimethoprim-sulfamethoxazole. The assembled genomes and bioinformatic analysis revealed that the two *K. pneumoniae* isolates belonged to ST15 and showed the presence of the following acquired antimicrobial genes: *blaNDM-1, blaCMY-6, blaCTX-M-15, blaSHV-28*, *rmtC, aac(6')-Ib3*, *oqxA, oqxB, aac(6')-Ib-cr, fosA* and *sul1*, conferring resistance to beta-lactams, aminoglycosides, fluorquinolones, fosfomycin and sulphonamides [4]. In addition, mutations in nucleotide sequences of the quinolone resistance determining region of *gyrA* gene allele 20, *gyrB* gene allele 1, *parC* gene allele 10 and *parE* gene allele 1 were detected by using BIGSdb-Kp online

Table 1
Minimal inhibitory concentrations of *Klebsiella pneumoniae* isolates (n = 2).

Antibiotic tested	MIC (mg/L)	Interpretation
Amoxicillin-clavulanic acid	>16	R
Piperacillin-tazobactam	>64	R
Cefepime	>16	R
Cefotaxime	>32	R
Ceftazidime	>32	R
Imipenem	>8	R
Meropenem	>8	R
Ciprofloxacin	>2	R
Amikacin	32	R
Gentamicin	>8	R
Tigecycline	1	S
Colistin	>8	R
Fosfomycin iv	64	R
Trimethoprim-sulfamethoxazole	≤2	S

MIC, minimum inhibitory concentration.

tool (http://bigsdb.pasteur.fr/perl/bigsdb/bigsdb.pl?db=pubmlst_klebsiella_seqdef_public). The bla_{NDM-1} and bla_{CMY-6} beta-lactamase genes were located on an IncA/C2 plasmid JN157804, previously reported in a *Klebsiella pneumoniae* isolate from Kenya [5]. The $bla_{CTXM-15}$ and bla_{SHV-28} genes were identified on the chromosome, as previously reported [6].

Although strain was resistant to colistin, plasmid mediated *mcr-1* to *mcr-8* genes were absent in both isolates. No amino acid substitutions were identified in the PmrA, PhoP, PhoQ and MgrB proteins in the isolates compared to sequences obtained from wild-type isolates. PmrB protein differed from the wild type by a single threonine to proline amino acid substitution at position 157. A Thr residue was identified at position 157. Thus, it was found that amino acid substitution Thr157Pro is responsible for the acquired resistance to colistin, as previously reported [7].

PlasmidFinder online tool [8] revealed that both strains carried an IncA/C2 type plasmid (100% similarity with JN157804 plasmid), and an IncFIB type plasmid (99.82% similarity with plasmid pKPHS1 CP003223, previously described in Shanghai, China) [9].

The resulting minimum spanning tree identified by cgMLST analysis showed that the two NDM-positive isolates clustered closely together with a maximum of four alleles difference between neighboring isolates. The isolates were extensively drug resistant (XDR), as per the definition by Magiorakos et al. [10].

Whole-genome shotgun projects have been deposited in Genbank (BioProjects PRJNA505478 for *K. pneumoniae*).

4. Discussion

It is believed that this is the first documented case of an NDM-1-producing *K. pneumoniae* in Albania. A European prospective study by Grundmann et al. found no NDM-producing isolates in Albania and neighboring Macedonia and Kosovo, but did find 33, 12, 10, 5, 2 and 1 NDM-producing *K. pneumoniae* in Serbia, Greece, Montenegro, Romania, Bulgaria and Slovenia, respectively, as well as 8, 5 and 1 NDM-producing *Escherichia coli* in Bulgaria, Serbia and Croatia [11]. *K. pneumoniae* ST15 is a major clone among CTX-M-15-producing isolates. ST15 harboring blaNDM−1 has been reported in Spain, Croatia, Thailand, Canada, China, France and Morocco [12]. The first polyclonal outbreak of NDM-producing *K. pneumoniae* in the Balkans was recently described in Bulgaria [13]. Therefore, NDM-producing bacteria are spreading in the Balkans and this constitutes a major public health problem. The only confirmed carbapenemase detected in Albania is a *K. pneumoniae* carbapenemase (KPC-3) [14], which was published in a report in 2015. It is unclear whether the spread of carbapenemase-producing strains in Albania is contained or whether the laboratory capacities to detect carbapenemases are limited and number of samples submitted to microbiology laboratories is low (or both). According to Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR) Annual Report 2017 [15] there is no antimicrobial resistance surveillance system established in the country. As stated elsewhere [16], the contribution of active investigators is of utmost importance in this regard. More research is required to generally assess the prevalence of carbapenemases and especially that of NDM enzymes. Furthermore, there should be a continuous multinational surveillance of bla_{NDM-1}-carrying isolates in the Balkan states. Collaboration links should be established to appropriately control and stop spread of these dangerous organisms.

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Conflict of interest

None declared.

Ethical approval

Not required.

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