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Genome Note

Emergence of *Klebsiella pneumoniae* ST11 co-producing NDM-1 and OXA-48 carbapenemases in Greece

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ABSTRACT

Objectives: *Klebsiella pneumoniae* is a well-known pathogen frequently implicated in serious life-threatening nosocomial infections. Here we present a *K. pneumoniae* isolate (AHEPA1046) co-harbouring $\textit{bla}_{\text{NDM-1}}$ and $\textit{bla}_{\text{OXA-48}}$ isolated from a blood sample of an inpatient in Thessaloniki, Greece.
Methods: Whole-genome sequencing (WGS) was performed using an Illumina MiniSeq Sequencing System. Multilocus sequence typing (MLST) was performed using a BLAST-based approach, and antimicrobial resistance genes and plasmid replicons were identified by ResFinder and PlasmidFinder, respectively. The Rapid Annotation using Subsystem Technology (RAST) v.2.0 server was used for genome annotation.
Results: WGS analysis revealed the complete resistome of *K. pneumoniae* AHEPA1046. The strain harboured $\textit{bla}_{\text{NDM-1}}$ and $\textit{bla}_{\text{OXA-48}}$ together with 16 additional antimicrobial resistance genes and was resistant to carbapenems, aminoglycosides, quinolones, macrolides, tetracyclines, trimethoprim, fosfomycin and phenicols. Moreover, it was classified as ST11.
Conclusion: This is the first report of a *K. pneumoniae* clinical isolate from Greece co-producing NDM-1 and OXA-48 carbapenemases and is one of a few reported worldwide.

Klebsiella pneumoniae is an important pathogen involved in serious hospital-acquired infections all over the world. Acquisition of a multidrug-resistant profile is often associated with carbapenem resistance and the predominance of certain sequence types (STs). In this regard, the most clinically significant types of carbapenemases include KPC, VIM and, recently, NDM and OXA-48. Here we report the resistome, multilocus sequence typing (MLST) and antimicrobial resistance pattern of a *K. pneumoniae* isolate harbouring six different β-lactamase genes, including $\textit{bla}_{\text{NDM-1}}$ and $\textit{bla}_{\text{OXA-48}}$ carbapenemase genes. To the best of our knowledge, this is the first NDM-1- and OXA-48-producing *K. pneumoniae* isolated in Greece.

K. pneumoniae strain AHEPA1046 was recovered on 31 March 2016 from a blood culture of a 30-year-old male patient during long-term hospitalisation following a car accident. The patient suffered from pneumothorax and several injuries, including multiple head trauma, and was hospitalised in the intensive care unit and surgical wards. During his hospitalisation he received various antimicrobials including ampicillin/sulbactam, amikacin, linezolid, cefoxitin, meropenem and tigecycline. He was treated with colistin plus gentamicin for a bloodstream infection and was finally discharged on 25 April 2016 with an improved medical condition and negative blood cultures.

Bacterial identification and initial antimicrobial susceptibility testing were performed using a VITEK®2 automated system (bioMérieux, Marcy-l'Étoile, France). In addition, the broth microdilution method was performed for several antimicrobial agents including β-lactams, colistin and tigecycline (Micronaut-S MRGN-Screening; Merlin Diagnostika GmbH, Berlin, Germany). Antimicrobial susceptibility test results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. The combination meropenem disk test was performed for phenotypic detection of carbapenemases.

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Table 1
Antimicrobial resistance genes found in *Klebsiella pneumoniae* AHEPA1046.

Resistance	Gene	% identity	Accession number
β-Lactams	*bla*$_{\text{NDM-1}}$	100.00	**FN396876**
	bla$_{\text{OXA-48}}$	100.00	**AY236073**
	bla$_{\text{CTX-M-15}}$	100.00	**AY044436**
	bla$_{\text{CTX-M-14b}}$	100.00	**DQ359215**
	bla$_{\text{SHV-182}}$	99.88	**KP050489**
	bla$_{\text{OXA-1}}$	100.00	**HQ170510**
Aminoglycosides	*aph(3')-Ia*	100.00	**V00359**
	aac(6')-Ib3	100.00	**X60321**
	aac(3)-IIa	100.00	**CP023555**
Fluoroquinolones	*oqxA*	99.23	**EU370913**
	oqxB	99.09	**EU370913**
	qnrS1	100.00	**AB187515**
	aac(6')-Ib-cr	99.65	**DQ303918**
Fosfomycin	*fosA*	99.27	**ACWO01000079**
Trimethoprim	*dfrA14*	100.00	**KF921535**
Macrolides	*mph(B)*	100.00	**D85892**
Phenicols	*catB4*	100.00	**AF322577**
Tetracyclines	*tet*(A)	100.00	**AJ517790**

Genomic DNA was extracted from bacterial colonies using a QIAamp® DNA Mini Extraction Kit (QIAGEN) and then 1 ng of DNA was used for library preparation using a Nextera XT DNA Library Prep Kit (Illumina Inc., San Diego, CA, USA). DNA libraries were cleaned up with AMPure XP Beads (Beckman Coulter Inc.), were quantified using a Qubit$^{\text{TM}}$ 3.0 fluorometer and were loaded on an Illumina MiniSeq platform (Illumina Inc.). Four de novo assemblers were used, namely SPAdes 3.13.1, Velvet 1.2.10, IDBA and Abyss 2.0, and the contigs of all assemblies were integrated using CISA 1.2. MLST was performed using a BLAST-based approach, and antimicrobial resistance genes were identified by ResFinder provided through the Bacterial Analysis Pipeline v.1.0.0 (threshold for %ID, 90%; minimum length, 60%). The presence of plasmid replicons was investigated by applying PlasmidFinder 1.3 (https://cge.cbs.dtu.dk/services/ResFinder/) on the assembled contigs.

K. pneumoniae AHEPA1046 was resistant to all cephalosporins (including ceftazidime, cefoxitin, ceftriaxone and cefepime), piperacillin/tazobactam, aztreonam, amikacin and ciprofloxacin. It also exhibited resistance to imipenem and meropenem with minimum inhibitory concentrations (MICs) of 8 mg/L and 32 mg/L, respectively. It remained susceptible in vitro to gentamicin, colistin, tigecycline and fosfomycin at 1, 2, 0.5 and 32 mg/L, respectively.

The total genome size of the isolate was 5 982 967 bp, consisting of a chromosome and eight plasmid replicons [ColRNAI, IncA/C2, IncFIB(K), IncFIB(Mar), IncFII(K), IncFII(pKPX1), IncHI1B and IncR, with percentage of identity 100%, 100%, 100%, 99.09%, 97.97%, 100%, 99.82% and 100% respectively]. The G + C content was 56.59%. A total of 6175 genes were identified, including 5822 coding genes, 18 rRNAs, 81 tRNAs, 14 non-coding RNA genes and 240 pseudogenes. The resistome included genes conferring resistance to β-lactams, aminoglycosides, quinolones, macrolides, tetracyclines, trimethoprim, fosfomycin and phenicols (Table 1). Based on genetic variation of the *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB* housekeeping genes, isolate AHEPA1046 was classified as ST11.

ST11 is a common NDM-positive lineage found in several countries worldwide. Although NDM-1 is spread on all continents and OXA-48 is endemic in Turkey and the Middle East, isolates bearing both carbapenemases are rare worldwide. The first two reports were from China [1] and Turkey [2] in 2017, followed by clonal dissemination of ST11 and ST893 *K. pneumoniae* co-harbouring *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{OXA-48}}$ in an Iranian hospital in 2018 [3]. Lately, two novel sequence types (ST3366 and ST3367) have emerged in Italy [4], and a case of successful treatment and digestive decolonisation of a patient with osteitis caused by a *K. pneumoniae* harbouring both enzymes was reported from France [5]. To the best of our knowledge, this is the first time such a combination is reported in Greece where KPC and VIM carbapenemases prevail and approximately 5% of carbapenemase-producing *K. pneumoniae* isolated in hospitals are KPC + VIM-producers.

It is true that not all carbapenemases inactivate all β-lactams, leaving in certain cases some limited treatment options. More carbapenemases may, however, provide a wider overall hydrolytic spectrum, further restricting treatment alternatives. Another approach for carbapenemase-producers is the combined administration of a carbapenem with an aminoglycoside, colistin or tigecycline, especially in cases of low carbapenem MICs. This obviously could be less effective in the presence of two enzymes that contribute to carbapenem resistance.

No other isolate was found to carry NDM-1 and OXA-48 carbapenemases since then in our hospital, therefore further studies are needed in order to assess whether silent dissemination of these double carbapenemase-producers has occurred in Greece.

Nucleotide sequence accession no

The whole genome shotgun project of *K. pneumoniae* isolate AHEPA1046 has been deposited in GenBank under the accession no. **VEVL00000000**. The version described in this paper is **VEVL00000000.1**.

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Competing interests

None declared.

Ethical approval

Not required.

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