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RESEARCH ARTICLE

Characterization of a carbapenemase-producing *Klebsiella pneumoniae* isolate of a patient in an intensive care unit in Greece: A study of resistome, virulome, and mobilome

Pandora Tsolakidou[1], Aikaterini Papadimitriou[2], Kyriazis Athanasios Kyriazidis[3], Pessach Ilias[4], Stella Mitka[4] and Maria Chatzidimitriou[4*]

[1] Hospital of Volos, Polymeri 134, 38222, Volos, Greece

[2] General Hospital Papageorgiou, Thessaloniki, Greece

[3] Medical School, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece

[4] Department of Biomedical Sciences, School of Health Sciences, International Hellenic University, 5400, Thessaloniki, Greece

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ABSTRACT

Klebsiella pneumoniae is a major pathogen associated with hospital-acquired infections, particularly those involving multidrug-resistant strains. Carbapenem resistance, often driven by carbapenemases such as KPC, VIM, OXA-48, and NDM, poses a significant challenge in clinical settings. This study reports on *K. pneumoniae* strain A165, isolated from a blood culture of a 51-year-old female patient hospitalized for respiratory distress post-SARS-CoV-2 infection. This *K. pneumoniae* strain exhibited resistance to several antibiotics, including carbapenems, cephalosporins, aminoglycosides, and fluoroquinolones, but remained susceptible to gentamicin, colistin, and trimethoprim-sulfamethoxazole. Next-generation sequencing was performed on Ion torrent platform, that revealed a genome size of 5,676,404 bp, including a chromosome and six plasmids. The strain was classified as sequence type 11 (ST11), a high-risk lineage associated with carbapenem resistance. The resistome of A165 included multiple β-lactamase genes, such as $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$, as well as genes conferring resistance to other antibiotic classes. The virulome analysis identified genes involved in iron acquisition (yersiniabactin operon genes: *ybtE*, *ybtT*, *irp1*, *irp2*; aerobactin receptor: *iutA*), adhesion (*mrkA-J*, *fimA-K*), capsule and biofilm formation (*rcsA*, *rcsB*, *ompA*) and resistance to complement (*traT*) contributing to its pathogenic potential. The mobilome analysis revealed nine insertion sequences, including *IS*Kpn1, *IS*Kpn18, *IS*Kpn43, *IS*Kpn28, *IS*Kpn14, *IS*Ecp1, and *IS*6100. The strain also harbored six replicons: Col440II, ColRNAI, IncFIA(HI1), IncFIB(K), IncFII(K), and IncR, which are associated with the horizontal transfer of resistance and virulence genes. Comparative analysis with global isolates demonstrated the widespread dissemination of carbapenemase-producing *K. pneumoniae*, with notable occurrences in Europe, Asia, and the Americas. This study highlights the growing concern of multidrug-resistant *K. pneumoniae* in hospital settings and emphasizes the need for robust surveillance and infection control measures.

KEYWORDS

Klebsiella pneumoniae, NDM-1, OXA-48, beta-lactamase inhibitors

*Corresponding author.
E-mail: mchatzid952@gmail.com

INTRODUCTION

Klebsiella pneumoniae is a major pathogen responsible for severe hospital-acquired infections globally. The development of multidrug resistance (MDR) in *K. pneumoniae* is frequently

linked to carbapenem resistance and to specific sequence types. Notably, the most significant carbapenemases involved in this resistance include KPC, VIM, OXA-48, and NDM. The first report of *K. pneumoniae* ST11 co-producing NDM-1 and OXA-48 in Greece was documented in 2019 by Protonotariou et al. [1].

Here we report the resistome, the virulome and the mobilome of a *K. pneumoniae* isolate harboring different beta-lactamase genes including $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$ carbapenemase genes. The A165 strain of *K. pneumoniae* represents a particularly concerning case of antibiotic resistance, exhibiting resistance to cephalosporins, new beta-lactamase inhibitors (such as ceftazidime/avibactam, imipenem/relebactam, and meropenem/vaborbactam), as well as aminoglycosides and fluoroquinolones. Interestingly, this strain remained susceptible to certain antibiotics, including gentamicin, colistin, and trimethoprim-sulfamethoxazole. Understanding the resistance profile of *K. pneumoniae* A165 is crucial for guiding effective treatment strategies and monitoring the spread of MDR pathogens in clinical settings. This article explores the antibiotic resistance mechanisms of the *K. pneumoniae* A165 strain, highlighting its implications for infection management and the broader issue of antimicrobial resistance.

MATERIALS AND METHODS

The *K. pneumoniae* strain A165 was isolated on 16 August 2024 from the blood culture of a 51-year-old female patient, who was hospitalized following a SARS-CoV-2 infection and had experienced respiratory distress. Bacterial identification and initial antimicrobial susceptibility testing were carried out using the Vitek-2 automated system (bioMérieux, Marcy-l'Étoile, France). Additionally, colistin susceptibility was tested using the broth microdilution method (Liofilchem, Italy). Antimicrobial susceptibility results were interpreted based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. Carbapenemase production was phenotypically detected through an immunochromatography test (NG-Test® CARBA-5, NG Biotech, France).

Next-generation sequencing (NGS) was performed at a private laboratory in Greece, using the Ion Torrent platform. The data were assembled with Unicycler assembler version 0.1.5. Multilocus sequence typing (MLST) was carried out using Galaxy Version 2.22.0. Antimicrobial resistance genes, point mutations, and replicons were identified using the staramr tool, which scans genome assemblies against the ResFinder, PlasmidFinder, and PointFinder databases to detect AMR genes (Galaxy Version 0.10.0+galaxy1).

RESULTS

The *K. pneumoniae* A165 strain exhibited resistance to carbapenems, to cephalosporins, to new beta lactamase inhibitors such as ceftazidime/avibactam, imipenem/relebactam, meropenem/vaborbactam, to aminoglycosides, and to fluoroquinolones, while remaining susceptible to gentamicin, colistin, and trimethoprim-sulfamethoxazole. The genome size was 5,676,404 bp and GC content was 57.08% (Table 1). Based on the genetic variation of the housekeeping genes, strain A165 was classified as ST11.

The resistome included genes responsible for resistance to β-lactams, aminoglycosides, fluoroquinolones, trimethoprim, and fosfomycin. Point mutations conferring fluoroquinolone resistance were detected in *gyrA* (D87A and S83F), *parC* (S80I), and colistin resistance in *pmrB* (R256G) (Table 2). The mobilome contained nine insertion sequences: *IS*Kpn1, *IS*Kpn18, *IS*Kpn43, *IS*Kpn28, *IS*Kpn14, *IS*Ecl1, *IS*Ec9/*IS*Ecp1/*IS*Ecp1B, *IS*5, *IS*6100/*IS*6100R/*IS*6100L (Table 3). Analysis of chromosome detected six replicons: Col440II (96.81%), ColRNAI (100%), IncFIA(HI1) (98.45%), IncFIB(K) (100%), IncFII(K) (97.32%), and IncR (100%) (Table 3). Capsular typing identified the strain as K24 using the Kaptive system. The virulome included the yersiniabactin operon (Table 4). The virulome also included genes associated with adhesion regulation (*mrkA-J*, *fimA-K*), capsule and biofilm formation, and iron acquisition (Table 4).

Table 1. Statistics of *K. pneumoniae* A165 strain

Statistics without reference	20240924NGS01_assembler_spade…
# contigs	134
# contigs (≥ 0 bp)	305
# contigs (≥ 1,000 bp)	109
Largest contig	519,402
Total length (≥ 0 bp)	5,639,919
Total length (≥ 1,000 bp)	5,676,404
Total length	5,620,817
N50	207,076
N90	27,161
auN	224,644
L50	9
L90	35
GC (%)	57.08
Mismatches	
#N's per 100 kbp	0
#N's	0

Table 2. Resistome of *K. pneumoniae* strain A165

Resistance to	Genes
Beta-lactams	$bla_{\text{NDM-1}}$, $bla_{\text{OXA-48}}$, $bla_{\text{SHV-182}}$, $bla_{\text{CTX-M-14b}}$
Fosfomycin	*fosA6*
Aminoglycosides	*aac(6′)-Ib*
Trimethoprim	*dfrA14*
Fluoroquinolones	*oqxB*
	qnrB1
Tetracycline	*tet(A)*
Bleomycin	*ble*
Point mutations	*gyrA_D87A*, *gyrA_S83F*, *pmrB_R256G*, *parC_S80I*

Table 3. Mobilome of *K. pneumoniae* strain A165

Category	Elements identified
Replicons	Col440II (96.81%), ColRNAI (100%), IncFIA(HI1) (98.45%), IncFIB(K) (100%), IncFII(K) (97.32%), IncR (100%)
Insertion Sequences (IS)	*IS*Kpn1, *IS*Kpn18, *IS*Kpn43, *IS*Kpn28, *IS*Kpn14, *IS*Ecl1, *IS*Ec9/*IS*Ecp1/ *IS*Ecp1B, *IS*5, *IS*6100/*IS*6100R/*IS*6100L

Table 4. Virulome of *K. pneumoniae* strain A165

Operon	Associated genes
ybt (Yersiniabactin)	*ybtE, ybtT, ybtU, ybtA, ybtP, ybtQ, ybtX, ybtS*
irp (Iron-repressible)	*irp1, irp2*
Siderophore receptor	*fyuA*
ecp (*E. coli* common pilus)	*yagV/ecpE, yagW/ecpD, yagX/ ecpC, yagY/ecpB, yagZ/ecpA, ykgK/ecpR*
Enterobactin	*entA, entB, entC, entD, entE, entF, entS, fepA, fepB, fepC, fepD, fepG, fes*
ompA (Outer membrane protein A)	*ompA*
Aerobactin receptor	*iutA*
Iron/manganese transport (Escherichia)	*sitA sitD*
Regulation of capsular synthesis	*rcsA, rcsB*
Type 1 and type 3 fimbriae	*mrkA, mrkB, mrkC, mrkD, mrkF, mrkH, mrkI, mrkJ, fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, fimK'*
Resistance to complement	*traT*

DISCUSSION

ST11 is a prevalent high-risk NDM-positive lineage of *K. pneumoniae*, that was identified in many countries. Isolates harboring both carbapenemase genes, $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$, have been documented in various regions. In Europe, until 2019 sporadic cases were reported from different hospitals due to strain transmission from countries outside the continent. For instance, in the UK, two *K. pneumoniae* strains carrying both $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$ were reported in 2014, while in France, similar strains emerged in 2018. A European survey conducted in 2019 involving sequencing data from 143 $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$-positive *K. pneumoniae* isolates from 13 European national collections and the public domain revealed the discovery of 15 multi-country transmission clusters [2]. Similar findings were noted globally, with reports from China in 2017 [3], Turkey in 2017 [4], and Iran in both 2018 and 2023 [5, 6], which identified $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$-producing *K. pneumoniae* clinical isolates, particularly ST11 and ST893.

In Italy, NDM-1 and OXA-48 co-producing *K. pneumoniae* isolates were reported in 2019 in ST101 K17 serotype and in 2020 in ST101 [7, 8]. In Germany, the first significant outbreak was documented in 2019 with *K. pneumoniae* strains ST307 [9], followed by further outbreaks in 2022 involving ST147, ST307, ST395, and ST23 [10]. In the United States, between 2016 and 2022, California reported cases of *K. pneumoniae* isolates carrying both $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$, including strains from ST14, ST16, ST67, ST437, and ST2096 [11]. Similarly, in Pakistan, co-producing *K. pneumoniae* strains were reported in 2020 from ST147 [12].

K. pneumoniae strain A165 in this study is different from the strain that was described by Protonotariou et al. in 2019 in Greece [1]. That earlier reported strain had IncA/C2 replicon and it didn't have Col440II replicon. The strain A165 demonstrates a concerning interplay of antimicrobial resistance, virulence, and genomic adaptability. The strain harbors multiple replicons, including IncF, IncR, and IncHI2, which are frequently associated with the acquisition and dissemination of resistance and virulence genes. The presence of these replicons suggests a high propensity for horizontal gene transfer, enabling the strain to rapidly adapt to selective pressures in the clinical environment. Specifically, IncF replicons are known for their association with virulence plasmids, facilitating the integration of siderophore systems (yersiniabactin, aerobactin, and enterobactin) and adhesion-related genes (*fimH*, *mrkD*), enhancing colonization and survival within host tissues.

In addition to its replicon profile, the strain carries resistance determinants such as $bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$, and $bla_{\text{CTX-M-14}}$, which confers broad-spectrum resistance to beta-lactams, including carbapenems. The co-existence of these resistance genes alongside virulence factors emphasizes the multifaceted threat posed by A165. The combination of biofilm-associated genes (*ompA*, *wza*, *rcsA/B*) and replicons known for plasmid stability further highlights its potential to persist in hospital environments and medical devices. This genomic complexity underscores the urgent need for vigilant monitoring and tailored antimicrobial stewardship strategies. The role of plasmid-mediated gene transfer in enhancing the adaptability of A165 warrants further investigation, as understanding these mechanisms could aid in curbing the spread of such high-risk clones.

CONCLUSION

In conclusion, the *K. pneumoniae* A165 strain isolated from a Greek Intensive care Unit patient exemplifies the growing global threat of MDR, carbapenemase-producing pathogens in healthcare settings. The strain's resistance to multiple antibiotic classes, including the presence of $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$ carbapenemase genes, along with its high-risk sequence type (ST11), highlights the potential for widespread dissemination and the challenges posed by these pathogens. The strain's virulome, which includes iron uptake systems and genes associated with adhesion and biofilm formation, further underscores its pathogenic potential. This study emphasizes the need for continued surveillance, rapid

detection, and stringent infection control measures to mitigate the spread of these dangerous pathogens in hospitals worldwide. The findings also reinforce the importance of addressing the genetic mobility of resistance and virulence factors, which facilitate the adaptability of these bacteria. As MDR *K. pneumoniae* continues to emerge, a global collaborative effort is required to limit its impact on public health.

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