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RESEARCH ARTICLE

Identification of NDM-1 producing and colistin resistant *Klebsiella pneumoniae* ST11: A highly drug-resistant strain detected in intensive care unit of a Greek tertiary care hospital

Maria Chatzidimitriou[1]*, Pandora Tsolakidou[2], Maria Anna Kyriazidi[3], Fani Chatzopoulou[1], Sotiris Varlamis[1], Maria Mavridou[1], Kallirhoe Kalinderi[1], Kyriazis Athanasios Kyriazidis[3] and Stella Mitka[1]

[1] Department of Biomedical Sciences, Faculty of Health Sciences, International Hellenic University, 57400 Thessaloniki, Greece

[2] Department of Microbiology, Hospital of Volos, 38222 Volos, Greece

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

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ABSTRACT

The spread of NDM-1-harboring *Klebsiella pneumoniae* is a worldwide concern. In this study the whole-genome sequence (WGS) of a carbapenem- and colistin-resistant *K. pneumoniae* 838Gr strain is presented. This strain was isolated from a urine sample of a patient in the Intensive Care Unit (ICU) at Volos Hospital, Greece. The initial assembly produced 224 contigs with a combined genome size of 5,561,803 bp and a GC content of 57.21%. The *K. pneumoniae* strain carried IncR, IncFIA, IncC, and repB (R1701) replicons. Multilocus sequence typing (MLST) analysis revealed that the isolate belonged to the sequence type 11 (ST11) and serogroup KL24 and O2a. The WGS analysis identified several beta-lactamase genes ($bla_{\text{TEM-1B}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{NDM-1}}$, $bla_{\text{OXA-1}}$, $bla_{\text{VEB-1}}$, $bla_{\text{OXA-10}}$, and $bla_{\text{SHV-11}}$) alongside resistance genes for other antibiotic classes, including *floR2, cmlA1, cmlA5, catB3, arr-3, aph(6)-Id, aadA2*. Colistin resistance was attributed to specific point mutations in *pmrB* (R256G, T140P). This is the first report of a carbapenem- and colistin-resistant *K. pneumoniae* ST11 strain in Greece. The findings of this study highlight the urgent need for increased surveillance and stringent infection control.

KEYWORDS

Klebsiella pneumoniae, NDM-1, ST11, whole-genome sequence, Greece

1. INTRODUCTION

Carbapenemases are enzymes produced by different bacteria, with *Klebsiella pneumoniae* being a notable producer. The genes responsible for the production of these enzymes are transferred via plasmids. New Delhi metallo-beta-lactamase (NDM) carbapenemase was initially identified in 2008 in a *K. pneumoniae* strain in India [1]. After the emergence of NDM-producing strains in the Indian subcontinent, these isolates have spread globally, with reports confirming their presence in the UK, other European countries, Canada, Australia, North and South America, New Zealand, Asia, and the Arabian Peninsula. The Balkans have been identified as another potential reservoir responsible for the spread of NDM-producing strains [2, 3]. In Greece, there have been multiple reports of outbreaks or sporadic cases of NDM-1-producing *K. pneumoniae* ST11 since 2011 [4–9].

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

Although a chromosomal location has been identified in some isolates, the NDM carbapenemase is predominantly associated with multiple independent acquisitions. These are mediated by plasmids of various sizes, belonging to a diverse array of incompatibility groups with both broad and narrow host ranges, such as IncF, IncA/C, IncL/M, IncH, IncN, and IncX3 [11–13].

Despite the growing body of research on NDM-1-producing *K. pneumoniae*, significant gaps remain in understanding of genetic diversity and evolutionary pathways of these strains, especially in specific geographic regions like Greece, where outbreaks have been sporadic but concerning. Previous studies have reported the presence of the ST11 clone of *K. pneumoniae* in Greece, but detailed phylogenetic analyses and comparisons with strains from other regions are still limited [4–9].

This study aims to fill this gap by employing Whole Genome Sequencing (WGS) to characterize an NDM-1-producing *K. pneumoniae* strain (838Gr) isolated from a patient in the Intensive Care Unit (ICU) of Volos Hospital in Greece. By identifying the complete resistome, virulome, and mobile genetic elements present in this strain, the study provides crucial data that can inform both local and global strategies for monitoring, controlling, and treating infections caused by these highly resistant organisms.

The novelty of this study lies in its detailed genomic analysis of a specific NDM-1-producing *K. pneumoniae* strain within the context of the ICU environment in Greece, offering valuable insights into the potential for endemic spread and the mechanisms behind this strain's multidrug resistance and pathogenicity. This study presents a comprehensive genomic analysis of a colistin-resistant bacterial isolate, focusing on the identification of antibiotic resistance genes, virulence factors, and mobile genetic elements.

2. MATERIALS AND METHODS

Ethical approval was not required as the clinical isolate was collected and stored as part of routine clinical care. Clinical isolates and patient records/information were anonymized and de-identified prior to analysis.

K. pneumoniae 838Gr strain was isolated on July 15th 2024 from the urine culture of a 21-year-old male patient hospitalized in the intensive care unit (ICU) at Volos Hospital, Greece. Susceptibility testing was performed using automated susceptibility testing with Vitek-2 (Biomerieux). Results were interpreted according to the clinical breakpoints of European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2024. In addition, the broth microdilution method was performed for colistin (MicronautS MRGN-Screening; Merlin Diagnostika GmbH, Berlin, Germany).

The *K. pneumoniae* strain was subjected to WGS on an Ion Torrent platform at a private laboratory in Greece. For the assembly and analysis of the results, the Galaxy server online tool was used (Table 1). Capsular KL and O antigen loci were accessed using Kaptive https://github.com/klebgenomics/Kaptive. ICEfinder (https://bioinfo-mml.sjtu.edu.cn/ICEfinder/ICEfinder.html) was used to identify horizontally transferred genetic elements.

Table 1. Galaxy server online tool

Create assemblies with Unicycler
Pipeline for bacterial genomes (Galaxy Version 0.5.1+galaxy)
Funannotate assembly clean
(Galaxy Version 1.8.15+galaxy5)
Quast
Genome assembly quality (Galaxy Version 5.2.0+galaxy1)
Multi locus sequence typing
Scans genomes against PubMLST schemes (Galaxy Version 2.22.0)
ABRicate
Mass screening of contigs for antimicrobial and virulence genes (Galaxy Version 1.0.1)
AMRFinderPlus
NCBI Antimicrobial Resistance Gene Finder (Galaxy Version 3.12.8+galaxy)
Plasmid identification in bacteria (Galaxy Version 2.1.6+galaxy1)
Insertion Sequence Element detection in prokaryotic genomes (Galaxy Version 1.7.2.3+galaxy1)
plasmidSPAdes
Extract and assembly plasmids from WGS data (Galaxy Version 3.15.5+galaxy2)
Integron Finder
A program that detects integrons in DNA sequences (Galaxy Version 2.0.5+galaxy0)

3. RESULTS

3.1. Susceptibility to antibiotics

The strain demonstrated resistance to penicillin, expanded-spectrum cephalosporins, and carbapenems, with an imipenem Minimum Inhibitory Concentration (MIC) >32 mg L^{-1}, meropenem MIC >32 mg L^{-1}, and ertapenem MIC >8 mg L^{-1}. It was also resistant to amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, tigecycline, and colistin (Table 2).

3.2. Results of the genomic analysis of *K. pneumoniae* 838Gr strain

The total length of the genome was 5561803 bp. The N50 value was 124,164, which indicates that the quality of the assembly was good (Table 3). Based on the genetic variation in *the gapA, infB, mdh, pgi, phoE, rpoB*, and *tonB* housekeeping genes, 838Gr was classified as an ST11 multilocus sequence type. The strain was designate to capsular type 24 (KL24) and O2a somatic antigen type (O2a) harboring the IncC, IncFIA (HI1), IncR, and repB (R1701) replicons.

The resistome of *K. pneumoniae* 838Gr strain consisted of 29 genes. Resistance genes were divided into subcategories: genes conferring resistance to antibiotics, point mutations in genes, virulence genes and genes conferring resistance to antiseptics (Table 4). The resistome consisted of genes conferring antimicrobial resistance to beta-lactams

Table 2. Antimicrobial Susceptibility Testing of *K. pneumoniae* 838Gr strain

Antimicrobial	MIC (mg L^{-1})	Interpretation
Ertapenem	≥8	Resistant
Ampicillin	≥32	Resistant
Amoxicillin/Clavulanic Acid	≥32	Resistant
Ampicillin/Sulbactam	≥32	Resistant
Ticarcillin/Clavulanic Acid	≥128	Resistant
Piperacillin/Tazobactam	≥128	Resistant
Cefalothin	≥64	Insufficient Evidence
Cefuroxime	≥64	Resistant
Cefixime	≥4	Resistant
Cefotaxime	≥64	Resistant
Ceftazidime	≥64	Resistant
Ceftriaxone	≥64	Resistant
Cefepime	≥64	Resistant
Aztreonam	≥64	Resistant
Imipenem	≥16	Resistant
Meropenem	≥16	Resistant
Amikacin	≥64	Resistant
Gentamicin	≥16	Resistant
Tobramycin	≥16	Resistant
Nalidixic Acid	≥4	Resistant
Ciprofloxacin	≥4	Resistant
Levofloxacin	≥8	Resistant
Moxifloxacin	≥8	Resistant
Ofloxacin	≥8	Resistant
Tigecycline	≥8	Resistant
Chloramphenicol	≥64	Insufficient Evidence
Colistin	≥16	Resistant
Trimethoprim/Sulfamethoxazole	≥320	Resistant

Table 3. Genome statistics of *K. pneumoniae* 838Gr strain

Parameter	Value
Genome Length	5561803 bp
Contigs	179
N50	124164
GC (%)	57,21

(*bla*$_{TEM-1B}$, *bla*$_{CTX-M-15}$, *bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-10}$, *bla*$_{VEB-1}$, *bla*$_{SHV-11}$), aminoglycosides *(rmtB, aph(6)-Id, aph(3″)-Ib, aadA1, aadA2, aac(3)- IIa, aph(3′)-Ia, ant(2″)-Ia, oqxA, oqxB)*, chloramphenicol and its derivative florfenicol (*floR2, cmlA1,cmlA5, catB3)*, fosfomycin (*fosA6*), sulfonamide (*sul*2), trimethoprim (*dfr*A12, *dfr*A14), bleomycin (*ble*), and the disinfectant quaternary ammonium (*qacEdelta1*). In addition, point mutations in *gyrA* and *parC*, conferring resistance to fluoroquinolones, and point mutations in *pmrB* gene, conferring resistance to colistin, were detected.

Virulence genes detected were genes for type 3 fimbriae (*mrkA-H*), type I fimbriae (*fimA-K*), aerobactin (*iutA*), enterobactin (*entA-S, fepA-G, fes)*, Salmochelin (*iroE, iroN*), yersiniobactin (*fyuA, irp1, irp2, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX*), for regulation of Capsule Synthesis (*rcsB, rcsA*), and for Type VI Secretion System I, II and III (T6SS-I, T6SS-II,T6SS-III) (Table 4).

The analysis of the *Klebsiella pneumonia* 838Gr strain revealed the presence of many insertion sequences (ISs), which play a crucial role in the genetic adaptability and evolution of bacterial genomes (Table 5). Also Tn5403 was identified. In addition, three integrons were identified (Fig. 1) as mediators of antibiotic resistance. One integron contained the *bla*$_{OXA-10}$, *cmlA5*, and *ant-2* genes, which confer resistance to beta-lactams, chloramphenicol, and aminoglycosides, respectively, along with four *attC* sites. Another integron harbored the *dfrA2* and *aadA2* genes, while a third integron carried the *catB3* and *bla*$_{OXA-1}$ genes.

Three integrative conjugative elements (ICE), identified as putative mobile elements, were found in the genome of *K. pneumoniae* 838Gr strain (Fig. 2). One integrative and conjugative element (ICE) was found to be located on the chromosome, carrying the VirB component of the Type 4 secretion system (T4SS). T4SS is encoded by the ICE ICE-EcoED1a-1, which is thought to contribute to the transfer of this element [13]. This finding highlights the pathogenicity of *K. pneumoniae* 838Gr strain. ISs are short DNA elements capable of moving within the genome, often carrying antibiotic resistance genes and contributing to the dissemination of these traits among bacterial populations. The presence of these ISs not only facilitates the horizontal transfer of resistance genes but also promotes genetic rearrangements, which can enhance the bacterium's ability to resist a wide range of antimicrobial agents. This highlights the dynamic nature of the bacterial genome and its capacity to evolve in response to antibiotic pressure, posing as significant challenge to infection control and treatment efforts.

4. DISCUSSION

High-risk international clones of *K. pneumoniae*, such as ST258, ST147, ST307, and ST11 [16], are characterized by their ability to acquire resistance determinants. ST11 belongs to the clonal group CG258. ST11-K64 is a hypervirulent carbapenem resistant clone [14] In Greece, the dominant clone is ST258 KPC-producing *K. pneumoniae* [18]. However, sporadic cases or outbreaks of ST11 NDM *K. pneumoniae* have been reported since 2014 [4, 5].

ST11 *K. pneumoniae* is a highly concerning strain owing to its widespread resistance to multiple antibiotics, particularly carbapenems. This strain has become a dominant clone of carbapenem-resistant *K. pneumoniae* worldwide, notably in regions, such as China, South America, and Europe [15].

ST11 is particularly problematic because it frequently carries genes encoding carbapenemases, such as KPC, NDM, and OXA-48 which confer resistance to carbapenems, often considered last-resort antibiotics. In addition to carbapenem resistance, ST11 strains are commonly resistant to other classes of antibiotics, including aminoglycosides, fluoroquinolones, and polymyxins, complicating treatment options [17].

Recent studies have shown that the ST11 lineage is a hypervirulent strain [14]. The lineage has diversified into various sublineages, including ST11 KL47 and ST11 KL64, with the latter becoming increasingly prevalent in regions

Table 4. Resistance-associated genes in *K. pneumoniae* 838Gr strain

Gene	Resistance
bla$_{\text{TEM-1B}}$	Amoxicillin; Ampicillin; Cephalothin; Piperacillin; Ticarcillin
bla$_{\text{CTX-M-15}}$	Amoxicillin; Ampicillin; Aztreonam; Cefepime; Cefotaxime; Ceftazidime; Ceftriaxone; Piperacillin; Ticarcillin
bla$_{\text{NDM-1}}$	Amoxicillin; Amoxicillin+Clavulanic acid; Ampicillin; Ampicillin+Clavulanic_acid; Cefepime; Cefixime; Cefotaxime; Cefoxitin; Ceftazidime; Ertapenem; Imipenem; Meropenem; Piperacillin; Piperacillin+Tazobactam
bla$_{\text{OXA-1}}$	Amoxicillin; Amoxicillin+Clavulanic acid; Ampicillin; Ampicillin+Clavulanic_acid; Cefepime; Piperacillin; Piperacillin+Tazobactam
bla$_{\text{OXA-10}}$	Amoxicillin; Ampicillin; Aztreonam; Piperacillin; Piperacillin+Tazobactam
bla$_{\text{VEB-1}}$	Amoxicillin; Ampicillin; Aztreonam; Cefepime; Cefotaxime; Ceftazidime; Ceftriaxone; Piperacillin; Ticarcillin
bla$_{\text{SHV-11}}$	Amoxicillin; Ampicillin; Aztreonam; Piperacillin; Piperacillin+Tazobactam
floR2	Chloramphenicol; Florfenicol
cmlA1, cmlA5, catB3	Chloramphenicol
arr-3, *arr*-2	Rifampicin
aph (6)-Id, *aph* (3″)-Ib, *aadA1, aad*A2	Streptomycin
aac (3)-IIa	Gentamicin; Tobramycin
aph (3′)-Ia	Kanamycin
ant (2″)-Ia	Gentamicin; Tobramycin
*oqx*A	Nalidixic acid; Ciprofloxacin
*oqx*B	Trimethoprim; Chloramphenicol; Cetylpyridinium Chloride; Benzalkonium Chloride; Ciprofloxacin; Nalidixic Acid
rmtB	Amikacin; Gentamicin; Tobramycin
sul2	Sulfamethoxazole
*dfr*A12, *dfr*A14	Trimethoprim
*fos*A6	Fosfomycin
tet(G)	Doxycycline; Tetracycline
ble	Bleomycin
Point mutations	
*parC*_S80I	Fluoroquinolone
*gyrA*_D87A, *gyrA*_S83F	Fluoroquinolone
pmrB_R256G, pmrB_T140P	Colistin
ompK36_D135DGD	Carbapenems
Resistance to antiseptics	**Antiseptic**
qacEdelta1	Quaternary Ammonium
qacE	Ethidium Bromide; Chlorhexidine; Cetylpyridinium Chloride; Benzylkonium Chloride
Virulence genes	**Associated function**
iroE iroN	Salmochelin
entA, entB, entC, entD, entE, entF, entS, fepA, fepB, fepC, fep, D, fepG, fes	Ent siderophore
iutA	Aerobactin
fyuA, irp1, irp2, ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX	Yersiniabactin
rcsA, rcsB	RcsAB
mrkA, mrkB, mrkC, mrkD, mrkF, mrkH, mrkImrkJ	Type 3 fimbriae
fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI, fimK	Type 1 fimbriae
acrA, acrB	AcrAB-TolC Efflux Pump
clpV/tssH, dotU/tssL, hcp/tssD, icmF/tssM, impA/tssA, ompA, sciN/tssJ, tle1, tli1, tssF, tssG, vasE/tssK, vgrG/tssI, vipA/tssB, vipB/tssC	T6SS-I
clpV, dotU, icmF, impF, impH, impJ, ompA, sciN, vasA/impG, vgrG	T6SS-II
dotU, icmF, impA, impF, impG, impH, impJ, lysM, ompA, sciN	T6SS-III

Table 5. Insertion sequences (ISs) of *K. pneumoniae* strain 838Gr

Family	Insertion sequence name	Cluster
IS1	ISKpn14	IS1_316
IS3	ISKpn18	IS3_369, IS3_506, IS3_204, IS3_176, IS3_61
IS5	IS5	IS5_112, IS5_222
IS4	–	IS4_157
IS6	IS6100	IS6_292
ISL3	ISAeme19	ISL3_160
IS21	–	IS21_259
ISNCY	ISKpn21	ISNCY_229, ISNCY_86
IS66	ISKpn24	IS66_393, IS66_46
IS1380	–	IS1380_141
IS110	–	IS110_208
IS91	–	IS91_365, IS91_50

like China and Taiwan. The genetic evolution within these sublineages often involves the acquisition of additional resistance genes and plasmids, leading to enhanced virulence and antibiotic resistance [14]. This strain's ability to cause severe hospital outbreaks, particularly in ICUs, highlights its adaptability and the challenges it poses for infection control [4, 5, 9]. As a result, managing infections caused by ST11 *K. pneumoniae* requires careful consideration of the limited choice of effective antibiotics and a heightened focus on infection prevention strategies.

The genomic analysis of the carbapenem-resistant *K. pneumoniae* strain 838Gr, isolated from a patient in the ICU at Volos Hospital, Greece, underscores the critical challenges posed by multidrug- resistant organisms in clinical settings. The study strain isn't an hypervirulent strain but a multidrug resistant. The detection of multiple beta-lactamase genes, including $bla_{\text{NDM-1}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{CTX- M-15}}$, and $bla_{\text{OXA-1}}$, highlights the extensive resistance profile of this strain, making it resilient to a broad spectrum of beta-lactam antibiotics, including carbapenems, which are often considered last-resort treatments. The presence of additional resistance genes, such as *rmtB* (aminoglycosides), *tet*(G) (tetracyclines), and *oqx*AB (fluoroquinolones), and point mutations in genes like *pmr*B, facilitate resistance to colistin further complicates treatment options, indicating a high level of that multidrug resistance. For treating NDM-producing *K. pneumoniae* infections, it is advisable to avoid newer combinations, such as ceftolozane/tazobactam, ceftazidime/avibactam, meropenem/vaborbactam, and imipenem/relebactam. This significantly restricts treatment options, often leaving only newer alternatives, such as cefiderocol and the aztreonam/avibactam combination. Furthermore, resistance to antiseptics that are plasmid-transmitted, such as *qacE,* makes infection control difficult [9].

Moreover, the identification of virulence factors, such as *iut*A and *fyu*A, which are associated with iron acquisition, and the VirB component of the Type IV Secretion System, suggests that this strain not only poses a challenge because of its resistance but also possesses enhanced pathogenicity, potentially leading to more severe infections. The presence of mobile genetic elements, including various ISs, ICEs, integrons and transposons indicates the potential for horizontal gene transfer, facilitating the spread of resistance genes within bacterial populations. The number and the variety of replicons by the understudy strain IncR, IncFIA, IncC, and repB (R17010) points to horizontal transfer of genes. These findings emphasize the need for vigilant surveillance and stringent infection control measures in healthcare settings to combat the spread of such highly resistant and virulent strains. In particular they emphasize the need for constant molecular surveillance.

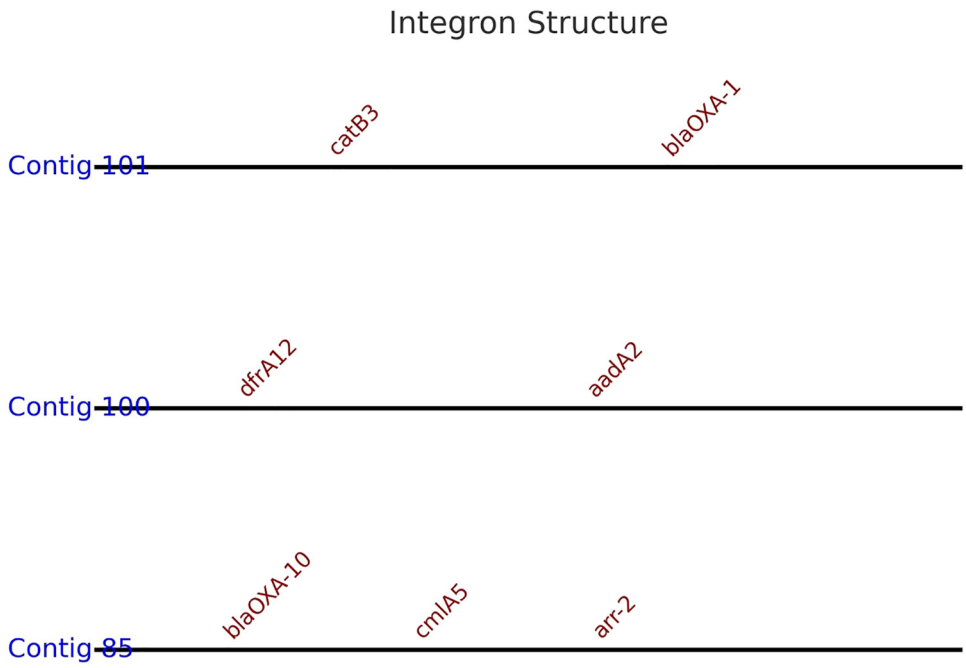

Fig. 1. Integrons of *K. pneumoniae* 838Gr strain

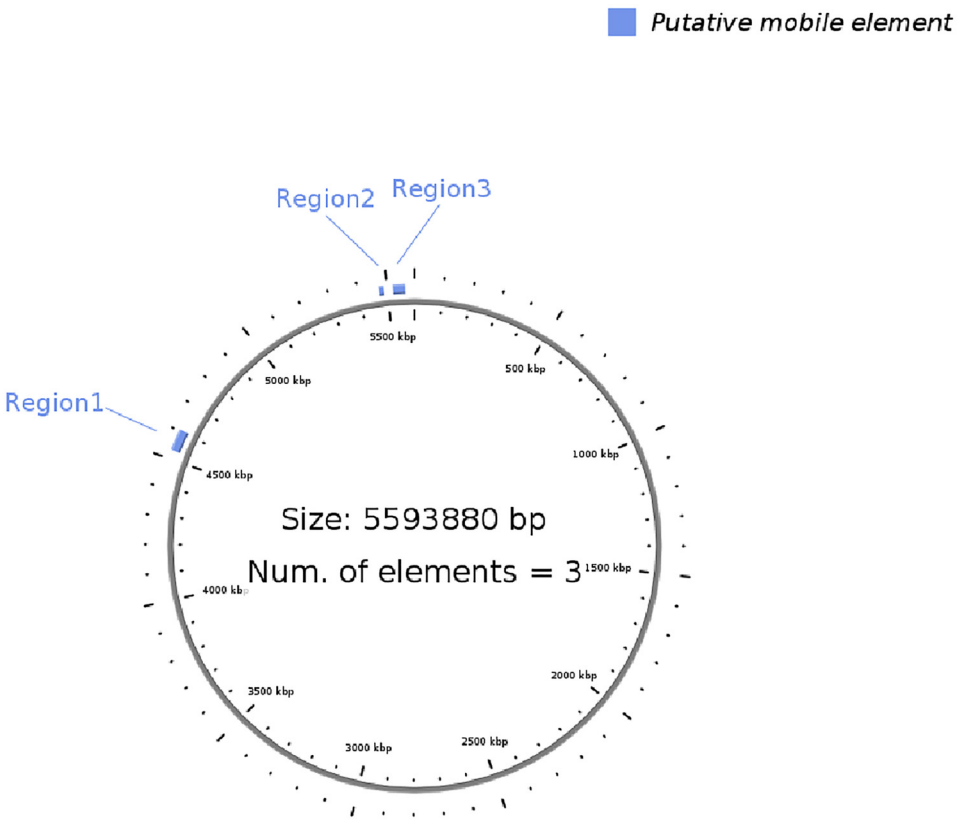

Fig. 2. Genome map of *K. pneumoniae* 838Gr strain showing the identified integrative conjugative elements (ICE). These elements, labeled as 'putative mobile elements,' are located at regions 1, 2 and 3 in the genome

To our knowledge it is the first time that a ST11, KL-24, O2a colistin resistant *K. pneumoniae* strain is reported in Greece. Using WGS data, we identified multiple resistance genes, including $bla_{\text{NDM-1}}$ for carbapenems, $bla_{\text{CTX-M-15}}$ for broad-spectrum cephalosporin, $bla_{\text{TEM-1B}}$ for beta-lactam antibiotics, and *rmt*B associated with aminoglycoside resistance. The $bla_{\text{NDM-1}}$ gene was located near transposon *Tn*5403. Virulence genes, such as *iut*A and *irp*2, linked to iron uptake systems, were also detected. Additionally, various insertion sequences, including *ISKpn*21 and *IS*5, were identified, indicating the potential for horizontal gene transfer. These findings underscore the genetic complexity and adaptability of pathogenic bacteria, highlighting the challenges in combating antimicrobial resistance.

4.1. Study limitations

Phylogenetic correlation of the stain 838Gr with other *K. pneumoniae* strains from the hospital in Volos was not performed due to the absence of comparative genomic analysis, inclusion of local strain sequences, and the lack of phylogenetic methods applied in the study.

5. CONCLUSION

In conclusion, *K. pneumoniae* 838Gr strain isolated at Volos Hospital highlights the serious threat posed by multidrug-resistant organisms in clinical environments. The strain's extensive resistance to a broad range of antibiotics, including last-resort treatments, such as carbapenems and colistin, underscores the challenges in managing infections caused by such pathogens. The presence of numerous resistance genes, virulence factors, and mobile genetic elements suggests not only a high level of resistance but also the significant potential for the spread of these traits. These findings emphasize the urgent need for novel therapeutic strategies and rigorous infection control measures to address the growing issue of antimicrobial resistance.

Author contributions: CM: conceptualization methodology and design of the study, resources, data curation, writing—original draft preparation, writing—review and editing. TP: investigation, acquisition of data, analysis and interpretation of data, writing. KMA: drafting the article or revising it critically for important intellectual content, writing. CH F: Laboratory testing. VS: software, validation, formal analysis. MM: Laboratory testing. KK: Laboratory testing. KKA: drafting the article or revising it critically for important intellectual content, writing. MS: final approval of the version to be submitted.

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