Inactivation of mgrB gene regulator and resistance to colistin is becoming endemic in carbapenem-resistant Klebsiella pneumoniae in Greece: A nationwide study from 2014 to 2017

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Inactivation of *mgrB* gene regulator and resistance to colistin is becoming endemic in carbapenem-resistant *Klebsiella pneumoniae* in Greece: a nationwide study from 2014 to 2017

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Running title: Carbapenem and colistin resistant *Klebsiella pneumoniae*

Abstract

Introduction: In Greece, the spread of carbapenem-resistant Enterobacteriaceae in humans has led to the reintroduction of colistin as a therapeutic agent. Unfortunately, resistance to colistin has emerged with different mechanisms involved. The present work aims to determine the prevalence of carbapenem and colistin resistance and the corresponding mechanisms in *Klebsiella pneumoniae* clinical isolates from Greece.

Methods: From 2014 to 2017, 288 carbapenem-resistant *K. pneumoniae* clinical strains were collected among a collection of 973 isolates from eight different hospitals in Greece. Antibiotic susceptibility testing was performed using three different methods. Screening of carbapenem and colistin resistance genes was conducted using PCR amplification and sequencing.

Results: among the 288 (29.6 %) carbapenem-resistant isolates, 213 (73.9%) were colistin-resistant (MIC>2 mg / L). The KPC type was the most common carbapenemase gene (116; 40.3%), followed by VIM (41; 14.2%), NDM (33; 11.5%) and OXA-48 (22; 7.6 %). Moreover, 44 (15.3%) strains co-produced two types of carbapenemases. For colistin resistance, no *mcr* genes were detected, but rather mutations in chromosomal genes were found. These included inactivation of the *mgrB* gene for 148 (69.5%), including insertion sequences 94 (44.1%) as well as nonsense 4 (1.9%) and missense mutations 24 (11.3%). Moreover, PCR amplification of *mgrB* gene was negative for 26 (12.2%) strains. Finally, 65 (30.5%) colistin-resistant strains exhibited a wild-type *mgrB*, the mechanisms of which remain to be elucidated.

Conclusion: Our study shows that *K. pneumoniae* clinical strains in Greece are resistant to both carbapenems and colistin which becomes endemic and likely chromosomally encoded.

Keywords: *Klebsiella pneumoniae*; Greece; carbapenems; colistin resistance; *mgrB* gene.

1. Introduction

Carbapenems have long been considered the treatment of choice for human infections caused by multidrug-resistant Gram-negative bacteria (GNB), their use has increased due to the spread of extended spectrum β -Lactamases (ESBLs) among the Enterobacteriaceae family [1]. Nevertheless, their efficacy has been challenged by the emergence of carbapenem-resistant Enterobacteriaceae, which have spread worldwide and to Greece [2].

In Greece, a national outbreak of carbapenem-resistant *Klebsiella pneumoniae* carrying the VIM gene was reported in 2000s [3], followed by the emergence of KPC-producing *K. pneumoniae* isolated for the first time in August 2007 [4]. Since then, these KPC-producing strains have spread rapidly, causing initial outbreaks, evolving into an endemic situation [5]. A first isolate of NDM-1-producing *K. pneumoniae* was reported in 2012, since several outbreaks of NDM-producing Enterobacteriaceae have been reported in different hospitals in Greece [6]. The isolation of OXA-48 producing Enterobacteriaceae is still uncommon [3]. In 2017 the surveillance data from ECDC reports a carbapenem resistance rate of 64.7% in *K. pneumoniae* strains recovered from blood samples in Greece, the highest in Europe [7]. A European survey of carbapenemase-producing Enterobacteriaceae conducted from 2013 to 2014 in 35 European countries reported an average of 1.3 patients per 10,000 hospital admissions in Europe carrying carbapenemase [8]. In Greece, this incidence was 5.78 patients per 10,000 admissions, second only to Italy (5.96 / 10.000) [8]. Cassini et al. have estimated that 33,000 deaths/year in Europe could be attributable to multi-drug resistance bacteria (MDR) due to their high prevalence of resistance in GNB especially in Greece and Italy [9]. However, true deaths and attributability to antibiotic resistance is currently highly dubious [10]. The high prevalence of carbapenem resistance in Greece may lead to difficult-to-treat (DTR) infections [11].

Under these circumstances, colistin has been reintroduced as last resort treatment for infections caused by multidrug-resistant carbapenemase producers [12]. However, the emergence of colistin resistance in GNB has been reported in several countries mediated via different genetic variations represented by chromosomal mutations in genes involved in lipopolysaccharide synthesis, namely *phoP / phoQ, pmrA / pmrB* or *crrA / crrB* as well as on the *mgrB* regulatory gene [12]. These mutations lead to the overexpression of these systems and to an increase in the synthesis of phosphoethanolamine (pEtN) and 4-amino-4-deoxy-L-arabinose (LAra4N) [12]. Moreover, colistin resistance can also be plasmid-mediated, the first colistin resistance gene involving a conjugative plasmid named *mcr*-1 (mobilized colistin resistance) has been reported by Yi-Yun Liu et al. in China in December 2015 [13]. The latter has been identified in GNB isolates of human, animal and environmental origin [13]. The location of the plasmid gives it the potential to be laterally transferable, which explains its wide diffusion confirmed by numerous studies [14]. Since then seven other *mcr* variants have been reported worldwide [15]. The use of an efflux pump and the formation of capsules may also be involved in the resistance to colistin [14].

Studies conducted in Greece on *K. pneumoniae* isolates from different clinical samples have reported a significant increase in colistin resistance from 3.5% (4/115) in 2010 to 20% (25/120) after 2010 [16], a recent study reported a 19% resistance rate to last-line antibiotics, including colistin in human clinical isolates [3]. This rapid increase, coupled with the lack of molecular data, motivated this study project, which consists of evaluating the prevalence of colistin and carbapenem resistance among a collection of *K. pneumoniae* clinical strains isolated between 2014 and 2017 from eight hospital sites in Greece, and to understand the molecular mechanisms involved.

2. Materials and methods

2.1 Bacterial isolates

Between 2014 and 2017, 973 *K. pneumoniae* clinical strains were collected from eight Greek hospitals, six in Athens, one in Thessalia (central Greece) and one in Peloponnese (Figure S1). Preliminary, the strains were tested against carbapenems (meropenem) by E-test (BioMérieux, France), the resistant strains with an MIC> 8 mg/L were selected and stored for further analysis. Identification at the species level was performed by Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry (Bruker Daltonik, Bremen, Germany) [17].

2.2 Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed on meropenem-resistant isolates using the disk diffusion method on a Mueller-Hinton agar (MHE BioMérieux, France) and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines except for cyclines that were interpreted according to CLSI recommendations. The imipenem MIC determination was performed by the E-test method (BioMérieux, France), data were interpreted according to the criteria of EUCAST. MICs of colistin were determined by broth microdilution method and the results were interpreted according to the guidelines described by the EUCAST.

DTR bacteria were defined as previously described [11], strains were considered as DTR when having an intermediate or resistant status to three antibiotic classes (β-lactams, carbapenems, and fluoroquinolones).

145 **2.3 Molecular characterization**

146 DNA extraction was performed on BioRobot EZ1 (Qiagen, Venlo, Netherlands) using a
147 commercial EZ1 DNA extraction kit (Qiagen) according to the manufacturer's instructions.

148

149 **Carbapenemase encoding genes**

150 All strains were screened for the presence of bla$_{KPC}$, bla$_{NDM}$, bla$_{VIM}$, bla$_{OXA-48}$, and bla$_{IMP}$ by
151 quantitative polymerase chain reaction (qPCR) (table S_1).

152

153 **Colistin resistance genes**

154 All strains were screened for the presence of plasmid-mediated colistin resistance genes
155 represented by *mcr-1, mcr-2, mcr-3, mcr-4*, *mcr-5* and *mcr-8* by PCR using specific primers
156 and probes (table S_1). Those strains were also investigated to detect possible genetic
157 alterations associated with colistin resistance in the *mgrB* genes. The *mgrB* genes were
158 sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster
159 City, California, United-States). Sequences of the colistin resistance genes were recovered
160 and compared to those of the reference strain *K. pneumoniae* MGH78578 carrying wild-type
161 genes (GenBank accession number NC_009648). The PROVEAN software
162 (http://provean.jcvi.org/index.php) was used to check whether amino acid sequence changes
163 could induce an alteration of the protein function [18]. In addition, the ISfinder database was
164 used to identify the insertion sequences types (https://www-is.biotoul.fr/) [19].

165

166 **2.4 Whole genome sequencing**

167 Genomic sequencing was performed with the MiSeq sequencer (Illumina Inc., San Diego,
168 CA, USA) in a pair-end in order to study certain atypical phenotypes. The genome was

169 assembled using the A5 pipeline, then reorganized by mapping on a reference genome *K.*

170 *pneumoniae* MGH785 using MAUVE.

171

172 **2.5 Statistical analyses**

173 Statistical analyses were performed using the Chi-squared test. A p-value <0.05 was

174 considered as statistically significant.

175

3. RESULTS

3.1 Bacterial identification

The 973 *K. pneumoniae* strains were isolated from 196 (20.1%) blood, 569 (58.5%) urines, 153 (15.7 %) respiratory samples, and the remaining 55 (5.6%) were from other sources. The 288 meropenem-resistant *K. pneumoniae* strains were isolated from 148 (44%) blood, 89 (27%) urines, 44 (13%) respiratory samples and the remaining 7 (2.43%) were from other sources. The majority of strains (213, 74%) were isolated in 2017.

The MSP dendrogram of 288 meropenem-resistant *K. pneumoniae* strains did not reveal any distinct groups or clusters (year, origin, hospital), (phenotype of carbapenem or colistin resistance) (Figure 1).

3.2 Phenotypic profile of the bacteria studied

Antibiotic results from the 288 strains of *K. pneumoniae* reported a very remarkable resistance to the β-lactam family (97%) (Figure 2). *K. pneumoniae* strains were resistant to aminoglycosides with 57% and 69% for gentamicin and amikacin, respectively. Resistance to quinolones, sulfamides, nitrofurans and cyclines was observed in 96%, 78%, 75% and 74% of strains, respectively. However, fosfomycin was the most active antibiotic on the majority of strains tested (28%). Out of the 288 strains, 226 (78%) of the strains were DTR.

The imipenem E-test showed that out of 288 *K. pneumoniae* strains, 256 (88.8%) were resistant to imipenem with MICs ranging from 3 mg/L to > 32 mg/L: 156 (16%) strains had an MIC> 32 mg/L. The 32 remaining strains were resistant to both ertapenem and meropenem but not to imipenem. Colistin MIC revealed that out of 973 *K. pneumoniae* strains, 213 (21.9%) were resistant to colistin, with MICs ranged from 4 mg/L to >256 mg/L.

Statistical analyses showed that resistance to colistin was significantly associated with the resistance to carbapenems for the meropenem resistant strains (n=288) ($p<0.05$).

3.3 Molecular analyses

Molecular mechanisms of carbapenem resistance

Of the 288 meropenem-resistant *K. pneumoniae* strains, 256 (88.9%) carried a gene encoding a carbapenemase. 32 (11.1%) strains were carbapenem-resistant and negative for all tested carbapenemase genes in this study. The KPC enzyme was the most common carbapenemase and was identified in 116 strains (40.3%), followed by the VIM and NDM detected in 41 (14.2%) and 33 (11.5%) of the strains, respectively. OXA-48 enzymes were found in 22 strains (7.6%) (Table 1). Four OXA-48 positive strains remain susceptible to imipenem (MICs $\leq$ 2 mg/L). The IMP enzyme was not detected in any strain. On the other hand, 44 (15.3%) strains harboured two types of carbapenemases, with five combinations namely $bla_{KPC + NDM}$, $bla_{KPC + OXA-48}$, $bla_{KPC + VIM}$, $bla_{NDM + OXA-48}$, $bla_{NDM + VIM}$ (Table 1). Sequence analysis of the bla_{KPC}, bla_{NDM}, bla_{OXA-48} positive strains revealed that these strains belonged to the KPC-2, NDM-1, and OXA-48 variants. On the other hand, of the VIM positive strains, five different variants were identified namely VIM-1, VIM-2, VIM-19, VIM-52 and VIM. -55. The VIM 55 and VIM 52 being variants of VIM-1.

Molecular mechanisms of colistin resistance

Plasmid-mediated colistin resistance genes

PCR screening for plasmid-mediated colistin resistance genes (*mcr-1*, to *mcr-8*) was performed on the 288 *K. pneumoniae* strains. Results show that no positive *mcr* isolates were detected.

Genetic alterations of the *mgrB* gene

The entire *mgrB* gene of the 213 colistin-resistant *K. pneumoniae* strains was amplified by standard PCR and sequenced. Among these isolates, 187 were *mgrB* positive and 26 were

negative. Of the 187 positives, 94 (50.3 %) strains generated amplicons larger than the normal size of the *mgrB* gene, suggesting the presence of sequence insertions at the coding region for the *mgrB* gene.

ISK*pn*26 of the IS5 family was the most common insertion sequence found in *mgrB* and was identified in 42 (19.7%) isolates, followed by IS*Ec68* (19 strains, 8.9%), ISK*pn*14 (13; 6.1%), IS1R (10; 2.3%), ISK*pn*25 (6; 2.8%), IS903 (2; 0.9%) and IS5 (2; 0.9%) (Table 2). The ISK*pn*26 and IS*Ec*68 belonging to the IS5 family had a length of 1,200 bp and 1,199 bp, respectively (Figure 3). These ISs were inserted at the same position, between nucleotides 69 and 70. In contrast, an IS*E*c68 was inserted between nucleotide 141 and 142, with a length of 881 bp. An ISK*pn*14 belonging to the IS1 family was found inserted at two different positions with sequence lengths of 780 bp and 778 bp (Figure 3). The IS1R of the IS1 family were identified at different positions in the gene but at the promoter of the *mgrB* gene in one isolate. An IS903 was also found inserted at the level of the gene promoter in a strain that also had a 4-base insertion (GTGC) between nucleotides 80 and 81 (Figure 3). A 394 bp ISK*pn*25 belonging to the ISL3 family was detected between nucleotides 132 and 133.

***MgrB* gene sequence changes by nonsense mutations.** In 4 (1.9 %) strains, a single mutation induced the appearance of a premature stop codon (Table 2), which leads to the replacement of a glutamine by a stop codon at position 30 (Q30Δ). The resulting protein had only 29 amino acids long instead of a non-mutated protein of 47 amino acids (Figure 4. A).

***MgrB* gene sequence change by missense mutations.** 24 *K. pneumoniae* colistin-resistant strains had a single nucleotide substitution in the *mgrB* gene (Figure 4. A). Mutations induced the replacement of phenylalanine (F35I) by isoleucine at position 35 in one isolate and glycine by serine (G37S) at position 37 in three isolates. Substitution of lysine with valine at

position 2 (K2V) was the most frequently encountered mutation (20 isolates) (Table 2). All these substitutions have been reported deleterious by the PROVEAN software. One isolate had a four-base insertion (TGCG) between nucleotides 85 and 86, resulting in frame shift and non-functional truncated protein of 27 amino acids in length.

Partial and total deletion of the *mgrB* gene. For DNAs of 26 (12.2%) colistin-resistant *K. pneumoniae* strains that could not be amplified by standard PCR, additional amplification using internal primers of the *mgrB* gene was performed (Figure 3). The latter made it possible to obtain amplicons for 5 of the 26 strains. The five strains had a partial deletion of the gene as well as significant alterations in the protein sequence of the *mgrB* gene (Figure 4.B). For the remaining 21 strains, no sequence was obtained, suggesting a complete absence of the *mgrB* locus.

Genomic sequencing of one of those strain revealed that the *mgrB* gene was truncated by an IS, and the strains lacked a region of 500 bp containing 3 genes including a part a *mgrB* gene (57 pb). A BLAST search along 7kb region containing the *mgrB* gene against *K. pneumoniae_MGH 78578* revealed a conserved chromosomal location and genetic environment of this gene (Figure 5).

4. DISCUSSION

In Greece, since the first report of *K. pneumoniae* producing VIM in 2001, the prevalence of carbapenem-resistant *K. pneumoniae* has increased and is currently considered endemic [20].

In the present study we found a prevalence of carbapenem resistance in *K. pneumoniae* clinical strains of about 30% in 8 hospitals Greece. The KPC type (40.3%) remains the most prevalent carbapenemase encoding gene followed by VIM (12.5%), the latter are also known for their endemicity in Greece [21].

These results are consistent with a previous national surveillance study carried out between January 2011 and June 2012, in 119 hospitals in Greece that showed that in *K. pneumoniae* strains, the KPC enzyme was the most prevalent mechanism of resistance to carbapenems (82.6%), followed by the VIM in second position (9.7%) [22]. However, a recent study conducted in a European survey of carbapenemase-producing Enterobacteriaceae from 2013 to 2014 showed for the first time that out of 86 carbapenem-resistant *K. pneumoniae* strains isolated in Greece, the NDM-type enzyme was the second most prevalent carbapenemase with a rate of 14% after KPC (10.5%) [8].

Although the OXA-48 enzyme has been reported in sporadic cases [3], we have noted the emergence of strains carrying OXA-48 gene. The true prevalence of this enzyme is poorly known in Greece [23]. Usually, OXA-48 confers a lower resistance than other carbapenemases and is not always detected in antibiotic susceptibility testing [24]. Interestingly we found OXA-48 positive *K. pneumoniae* strains in our study that were sensitive to carbapenems. We were not able to detect the IMP enzyme in any of the isolates; indeed, in Greece there are no data regarding its isolation [25]. The co-production of different carbapenemases by the same strain is more frequently reported in the latest studies in several countries (India, Egypt, China and Greece) [23,24,26–29]. In our strain collection, we identified 44 (15.3%) strains of *K. pneumoniae* co-producing two types of carbapenemases. Recent studies in Greece have reported the

294 presence of *K. pneumoniae* strains producing both KPC-2 and VIM-1 as well as NDM-1 and
295 VIM-1 [28,29]. The KPC-2 and NDM-1 variants remain the most prevalent carbapenemases
296 among our strains, confirming the previous Greek estimates [21,29]. For the VIM type genes
297 analysed, several variants were detected. VIM-1, VIM-2 and VIM-19 have already been
298 described in Greece [21,29], in contrast to VIM-52 and 55.
299 Colistin has regained a significant part of the therapeutic regimen for the treatment of
300 carbapenem-resistant bacterial infections. However, it was rapidly followed by the emergence
301 of resistance. In our strain collection, among the 973 *K. pneumoniae* strains we reported an
302 overall colistin resistance rate of 21.9% and a rate of 73.9% of resistance among carbapenem-
303 resistant *K. pneumoniae* strains. The fact that none of the *mcr* genes tested were detected
304 confirms the data in the literature that show that the prevalence of *mcr* genes worldwide is
305 much higher in strains of animal origin than in human ones [30]. This assumes that their
306 reservoir is at least in animals and the environment, following the important use of colistin in
307 animal production and in agriculture in general [30]. Unlike other countries, in Greece, the
308 massive use of colistin in clinical practice following the spread of EPCs has led to the
309 selection of multidrug-resistant bacteria in hospital settings. According to data available on
310 ECDC, the consumption of colistin was 0.004 DDDs (daily defined dose of antibiotics) / 1000
311 patients per day in 2000, 0.071 in 2010 and 0.135 in 2016 [7].
312 In the present study, out of the 213 colistin-resistant *K. pneumoniae*, 148 (69.5%) had an
313 inactivated *mgrB* gene, mediated either by sequence insertions, absence or point mutations of
314 *mgrB* gene. The *mgrB* gene is a conserved 144 nucleotide gene encoding a small 47 amino
315 acid transmembrane protein, a strong negative feedback from the *PhoQ / PhoP* regulatory
316 system [14]. Inactivation by sequence insertions was the predominant cause of colistin resistance
317 and the ISK*pn*26 element was the most prevalent.

The interruption of the *mgrB* gene by ISK*pn*25, IS903 and IS*C*s68, *IS5*, or ISK*pn*14 has already been reported [31–35], but has not been described so far in Greece. Avgoulea et al. reported that insertional inactivation of the *mgrB* gene conferred resistance to colistin in all isolates tested [31]. The F35I and G30Del substitutions in the *mgrB* gene have been reported, mutations in the same amino acid position have also been reported in colistin-resistant *K. pneumoniae* isolates by several other studies [36]. This reinforces the hypothesis that these substitution in the *mgrB* protein is a critical region that is likely to mutate upon the emergence of resistance. In addition, new amino acid changes in the *mgrB* gene were observed and probably led to a predicted truncated and non-functional *mgrB* protein are also reported.

The prevalence of colistin resistance remains low in many countries unlike Greece. Overall, this resistance is closely related to the dissemination of carbapenem-resistant bacteria. Thus, our statistical analyses have shown that resistance to colistin was significantly associated with the carbapenem resistance phenotype and correlated with the production of KPC and NDM carbapenemase genes in *K. pneumoniae,* contributing to their multi-resistant phenotypes.

In conclusion, our study reported a high rate of resistance to colistin (70%) in carbapenem-resistant *K. pneumoniae* clinical isolates likely due to chromosomal mutations of target genes, especially inactivation of the *mgrB* gene by sequence insertions and not by spreading of plasmid-mediated *mcr* colistin resistance genes. This finding clearly supports the notion that colistin selection pressure in humans and in animals led to the selection of different bacterial clones in colistin resistant bacteria with *mcr* variants in animals and environment and specific clones with chromosomal mutations in humans. However, the observed resistance could not be explained in a large proportion of samples (approximately 31% that harboured a wild type *mgrB* gene and in about 10% where the *mgrB* gene could not be amplified), thereby limiting the conclusions that might be drawn. Finally, our study suggests that colistin resistance is becoming endemic in Greece in carbapenem-resistant *K. pneumoniae* human isolates. The

prevalence of resistance to carbapenems and colistin in Greece should be surveyed and new therapeutic strategies including old drugs should be evaluated and used in Greece. Among the different antibiotics tested Fosfomycin may be a valid alternative to treat carbapenems and colistin resistant bacteria in Greece.

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Declarations

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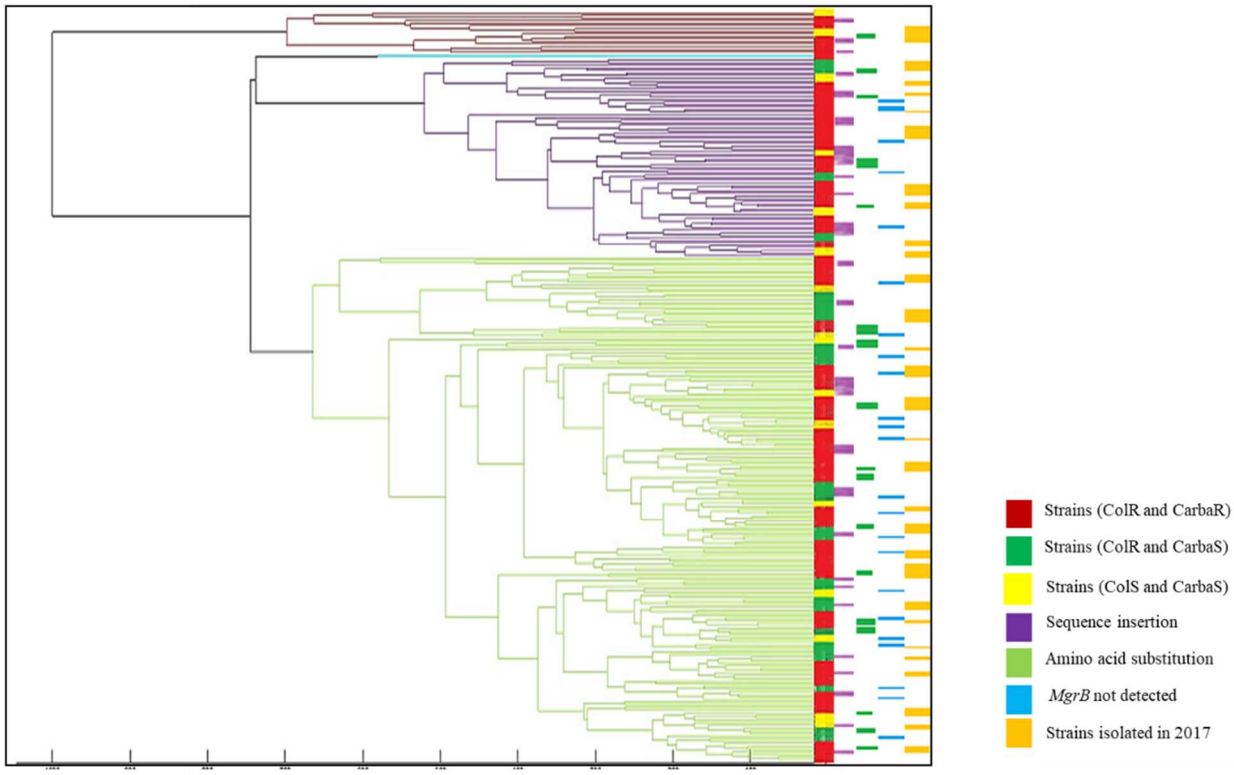

Figure 1. MALDI-TOF MS Dendrogram of 288 meropenem-resistant *K. pneumoniae* clinical strains, 2014 to 2017.

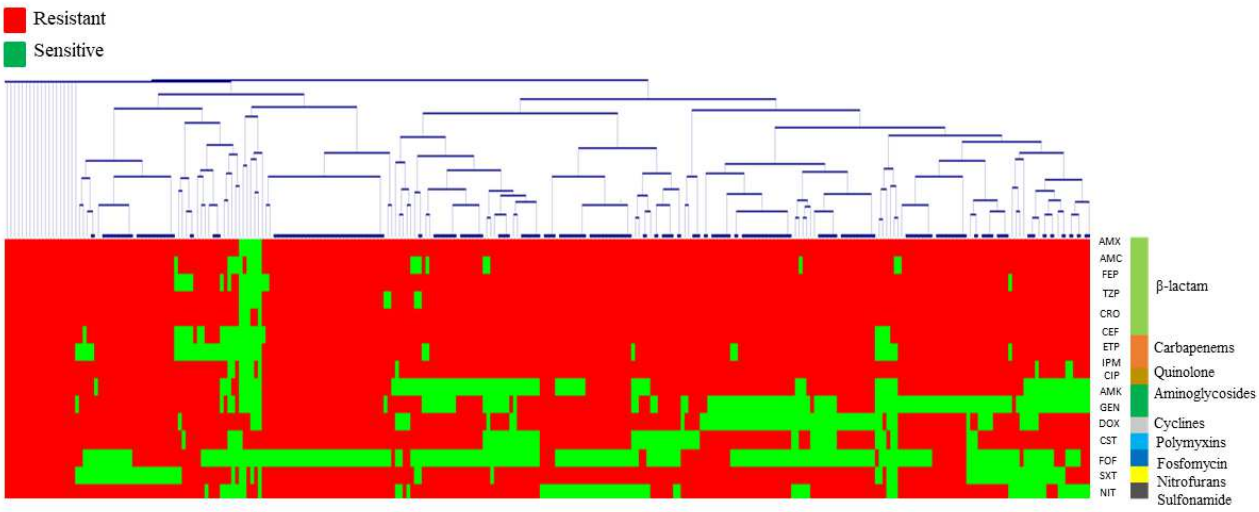

Figure 2. Hierarchical clustering of antibiotic susceptibility profiles (disk diffusion method) representing the activity of the 16 antibiotics tested against the 288 meropenem-resistant *K. pneumoniae* strains isolated in this study. AMX: Amoxicillin; AMC: Amoxicillin + clavulanic acid; FEP: Cefepime; TPZ: Piperacillin + Tazobactam; CRO: Ceftriaxone; CEF: Cefalotin; ERT: Ertapenem; IMP: Imipenem; CIP: Ciprofloxacin; AMK: Amikacin; GEN: Gentamicin; DOX: Doxycycline; CST: Colistin; FOF: Fosfomycin; SXT: Trimethoprim / sulfamethoxazole; NIT: Nitrofurantoin.

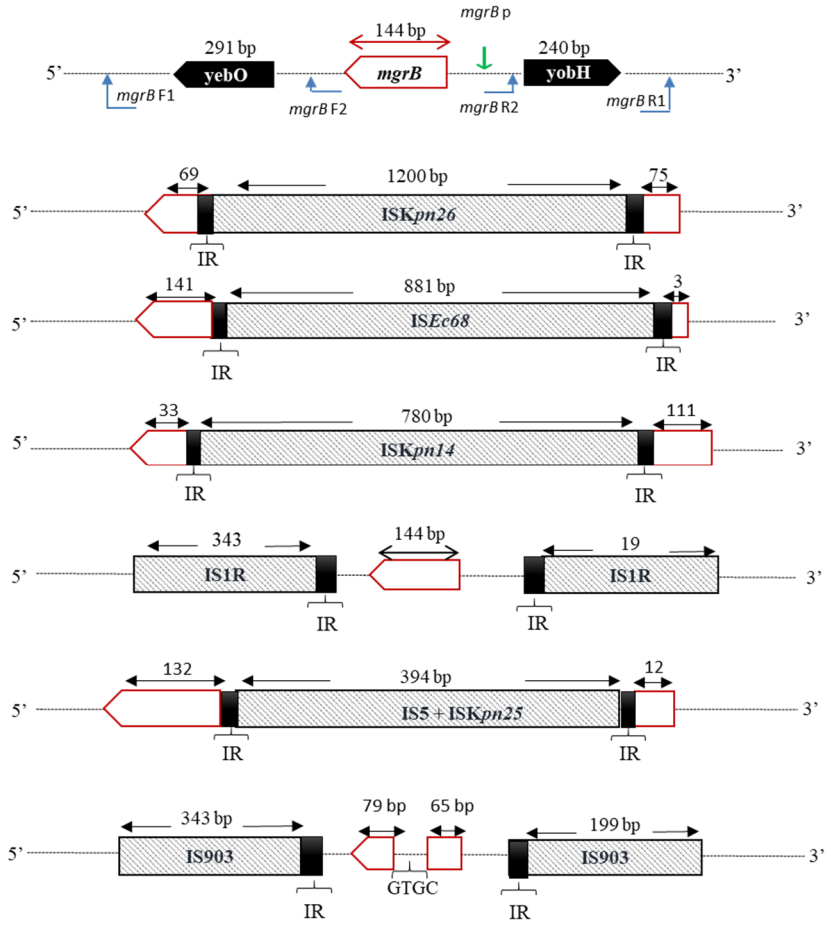

Figure 3. Schematic representation of the *mgrB* gene of *K. pneumoniae* strains resistant to colistin, showing the different integration sites of sequence insertions (IR: inverted repeat; *mgrB* p: *mgrB* promotor).

Figure 4. Alignment of the protein sequences of the *mgrB* gene of *K. pneumoniae* colistin resistant strains (a): using the external primers (the underlined areas indicate the presence of a mutation); (b): using the internal primers; KP: *Klebsiella pneumoniae*.

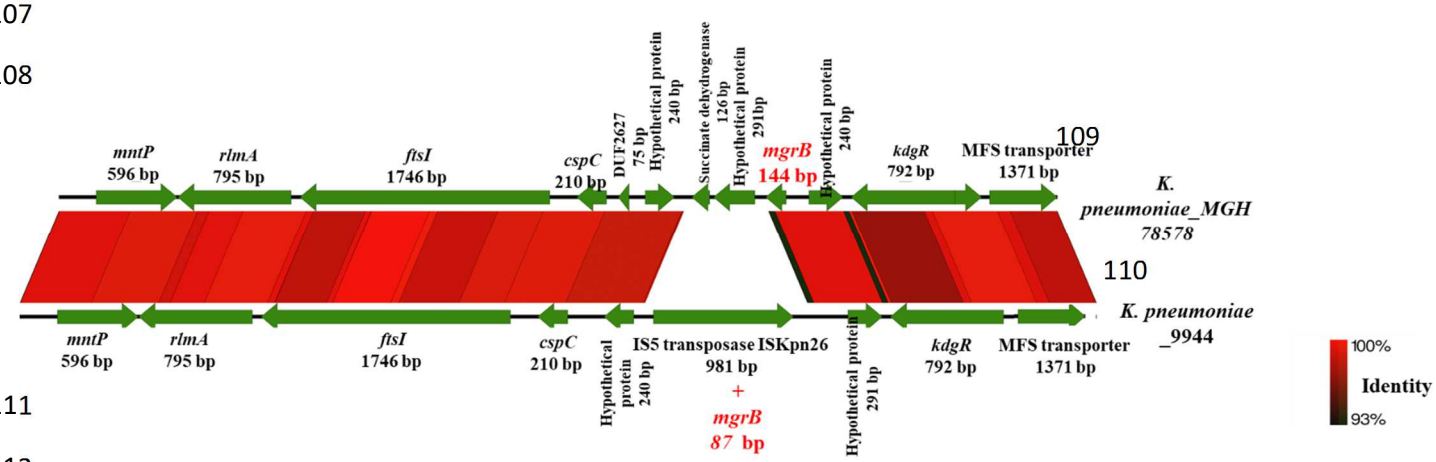

Figure 5: Genetic location of the 7kb containing the *mgrB* gene of the reference *K. pneumoniae_MGH 78578* and *K. pneumoniae 9944* using the EasyFig software. The arrows indicate the positions and directions of the ORFs, direction indicate the gene orientation.

Table 1: Carbapenemases detected among carbapenem-resistant *K. pneumoniae* strains isolated from Greek hospitals, 2014 to 2017. (* Several isolates contain more than one carbapenemase). KPC: *Klebsiella pneumoniae* carbapenemase; NDM: New Delhi metallo-beta-lactamase; VIC: Verona integron-mediated metallo-beta-lactamase; OXA-48: Oxacillinase-48.

Species Imipenem R (MIC >2mg/L	Carbapenem resistance genes	Positive results for carbapenemases N (%)		MIC imipenem Median value
		N	Percentage (%)	
Klebsiella pneumoniae	bla_{KPC}	116	40.3	32
	bla_{NDM}	33	11.5	
	bla_{OXA-48}	22	7.6	8
	bla_{VIM}	41	14.2	12
	$bla_{KPC+NDM}$	12	4.2	32
	$bla_{KPC+OXA-48}$	9	3.1	12
	$bla_{KPC+VIM}$	10	3.5	32
	$bla_{NDM+OXA-48}$	6	2.1	
	$bla_{NDM+VIM}$	7	2.4	
	Negative	32	11.1	
	Total	288*	100	32

145 **Table 2:** Table summarizing the different genetic alterations of the *mgrB* gene in the 213 *K.*

146 *pneumoniae* colistin resistant strains isolated from Greek hospitals, 2014 to 2017.

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Alterations of the *mgrB* gene		N	Percentage (%)
94 sequence insertions	ISK*pn*26 - IS5	42	19.7
	IS*Ec*68 - IS5	19	9
	ISK*pn*14 - IS1	13	6.1
	IS1R - IS1	10	4.6
	ISK*pn*25 - ISL3	6	2.8
	IS903 - IS5	2	1
	IS5 - IS5	2	1
Amino acid substitutions		24	11.3
Premature stop codon		4	1.9
Intact		65	30.5
Not detected	Total deletion	21	9.8
	Partial deletion	5	2.3
Total		213	100

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