

# HIGH RATES OF METALLO-BETA-LACTAMASE-PRODUCING KLEBSIELLA PNEUMONIAE IN GREECE - A REVIEW OF THE CURRENT EVIDENCE

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For the last four years Greece has faced a large number of infections, mainly in the intensive care units (ICU), due to carbapenem-resistant, VIM-1-producing *Klebsiella pneumoniae*. The proportion of imipenem-resistant *K. pneumoniae* has increased from less than 1% in 2001, to 20% in isolates from hospital wards and to 50% in isolates from ICUs in 2006. Likewise, in 2002, these strains were identified in only three hospitals, whereas now they are isolated in at least 25 of the 40 hospitals participating in the Greek Surveillance System. This situation seems to be due to the spread of the blaVIM-1 cassette among the rapidly evolving multiresistant plasmids and multiresistant or even panresistant strains of mainly *K. pneumoniae* and also other *enterobacterial* species. However, the exact biological basis of this phenomenon and the risk factors that facilitate it are not yet fully understood. Moreover, the fact that most strains display minimum inhibitory concentration (MIC) values below or near the Clinical Laboratory Standard Institute (CLSI) resistance breakpoint create diagnostic and therapeutic problems, and possibly obstruct the assessment of the real incidence of these strains.

An evidence-based consensus on the therapeutic strategy for these infections is urgently needed. The problem of VIM-producing *K. pneumoniae* was timely recognized by the Greek System for the Surveillance of Antimicrobial Resistance and various guidelines, including advice on antibiotic policy and infection control, were developed by the National Centre for Disease Control and Prevention. However, these measures have yet had a relatively small impact on the situation. The best way to handle the problem of antibiotic resistance would be the development and implementation of a national integrated strategic action plan (currently under development) affirming the political commitment of the public health administration in confronting this issue.

### Introduction

Resistance to carbapenem due to the production of metallo-beta-lactamases (MBL) in Gram-negative organisms is an increasing international public health problem [1,2]. The problem of MBL-producing strains in Europe was originally confined to *Pseudomonas aeruginosa*. *P. aeruginosa* harbouring MBL of the VIM-1 type were first isolated in 1997 in Italy [3] and France [4].

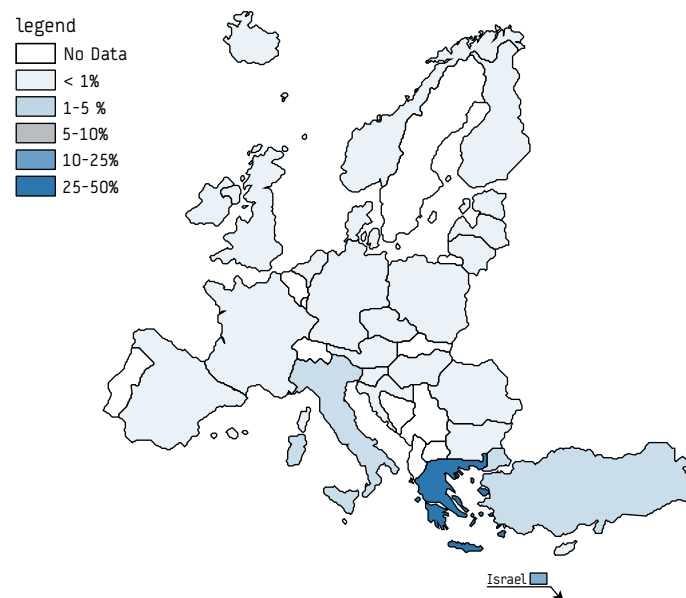
In Greece the first outbreak of *P. aeruginosa* harbouring MBL occurred in a hospital in Thessaloniki in 1996, and was reported in 2000 [5]. This enzyme was soon identified as VIM-2, an enzyme similar but not identical to VIM-1 [6]. By 2001, a multicentre study revealed that VIM-2-producing *P. aeruginosa* had already been isolated in nine out of 18 hospitals examined [7].

In the above context, the isolation of VIM-producing enteric bacteria (mainly *K. pneumoniae*, but also *Escherichia coli*, *Proteus mirabilis*, *Enterobacter* spp and other) in Greece since November 2001 seems to be an important new chapter in the epidemiology of this resistance mechanism.

It must be noted that sporadic isolates and small outbreaks of VIM-producing enteric bacteria have been reported in some European and Mediterranean countries [8-11], with the strains being traced back to Greece on some occasions [12]. However, Greece seems to be the only country where these clinical strains are isolated in high numbers (Figure 1). This constitutes a major public health problem for Greece and also a possible threat for the rest of Europe.

The purpose of this report is to review the current knowledge concerning the epidemiology, microbiology, molecular biology,

**FIGURE 1**  
Proportion of carbapenem-resistant *Klebsiella pneumoniae* isolates in EARSS participating countries, 2006.



Data from European Antimicrobial Resistance Surveillance System (EARSS), <http://www.rivm.nl/earss/database/>

clinical management as well as the public health issues related to this problem.

The review is mainly based on reports published by all scientific groups working in the area of antibiotic resistance in Greece. These papers were retrieved by a systematic Medline search.

In addition, data concerning the magnitude and the development of the problem of VIM-producing enteric bacteria were derived from the Greek System for the Surveillance of Antimicrobial Resistance (GSSAR, <http://www.mednet.gr/whonet>) which has been in operation since 1996, and currently involves 40 hospitals around Greece. GSSAR participates in the European Antimicrobial Resistance Surveillance System (EARSS) and is in charge of the continuous analysis of the routine data generated in the hospital microbiology laboratories with the aid of the WHONET software. A brief description of the system can be found elsewhere [13].

### Description of the situation

The first VIM-producing enteric bacterium in Greece was an *E. coli* isolated in November 2001, and reported early in 2003, in a hospital in Piraeus [14]. Since then VIM-producing *E. coli* have been reported sporadically [15,16], and hospital outbreaks have also occurred [17].

VIM-producing *K. pneumoniae* were first reported between September and December 2002 in the intensive care units (ICUs) of three teaching hospitals located in Athens [18]. The exact origin of the index case was not revealed.

An outbreak of MBL-producing *P. mirabilis* was described in a general hospital in Thessaloniki during the period from June 2004 to March 2005 [19], as well as in outpatients believed to have been related to a general hospital in Sheres, in Northern Greece [20].

Finally MBL production was also sporadically described in *Enterobacter cloacae* in 2003 [21], in *Enterobacter aerogenes* in 2004 [15], in *Morganella morganii* in 2005 [22] and in *Providencia stuartii* in 2007 [23].

Concerning the magnitude of the problem, the GSSAR data reveal a steep increase in the proportion of imipenem-resistant *K. pneumoniae* from less than 1% in 2001 to 20% in isolates from hospital wards and to 50% in isolates from ICUs in 2006 (Figure 2). Accordingly, these resistant strains were identified in only three hospitals in 2002, and now are isolated in at least 25 of the 40 hospitals participating in the GSSAR network (Figure 2).

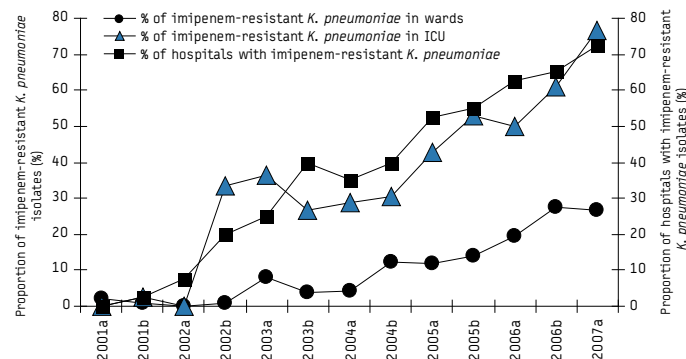
Interestingly, the proportions of imipenem-resistant enteric bacteria other than *K. pneumoniae* continue to be low (<http://www.mednet.gr/whonet>).

At this point it should be underlined that these data have to be interpreted with caution since resistance to carbapenem is monitored by the GSSAR network through the analysis of sensitivity data and not through the detection of the blaVIM gene (see next section).

Very little work has been done concerning the identification of risk factors for carbapenem-resistant infections. Fluoroquinolone and antipseudomonal penicillins have been proposed as independent risk factors in one matched case-control study [24].

FIGURE 2

### Trends in proportion of imipenem-resistant *Klebsiella pneumoniae* isolates in hospitals in Greece, 2000-2006



Data from the Greek System for the Surveillance of Antimicrobial Resistance (<http://www.mednet.gr/whonet>)

### Related clinical microbiology issues

Although the first VIM-producing *K. pneumoniae* and *E. coli* isolates were initially recognised by their *in vitro* resistance to carbapenem, i.e. displaying minimum inhibitory concentration (MIC) falling at the Clinical Laboratory Standard Institute (CLSI) resistant category in the *in vitro* sensitivity testing, it was soon documented that quite a few strains expressed low levels of resistance to carbapenem with MIC values at the CLSI intermediate resistance category (MIC 8 mg/L) or even at the sensitive category but with values near the breakpoint (MIC 2-4 mg/L). However, it must be emphasized that a strong inoculum effect has been reported – increasing the cell density by 102 CFU/mL raised carbapenem MICs by 2-6 doubling dilutions. This inoculum effect was more pronounced with imipenem [25].

The behaviour of these strains in the various automatic sensitivity testing systems was also studied quite early, and discrepancies were reported [26]. Moreover, due to inadequate scaling, the MBL-detecting Etest strips containing imipenem plus EDTA produced a synergy image between imipenem and EDTA, occurring as a “phantom zone”, and making the interpretation of the result difficult.

On the contrary, the fact that *Proteus* spp, displaying intrinsically high MICs to imipenem in the wild type population (see wild-type distributions published by EUCAST at: <http://www.srga.org/eucastwt/MICTAB/index.html>), has resulted in many false positive reports of imipenem-resistant *Proteus*, mainly in laboratories that use automatic susceptibility testing methods.

All these characteristics hamper the detection of the VIM-producing strains, pose therapeutic questions and obstruct the assessment of the real incidence of these strains, due to a possible iceberg phenomenon created by the presence of the *in vitro* “sensitive” strains harbouring the blaVIM gene.

Consequently, it was soon recognised that a special phenotypic test for the detection of these strains should be adopted. The double-disk imipenem – EDTA synergy test already in use for the detection of MBL-producing *P. aeruginosa* [2,7] was suggested for the identification of MBL production in all enteric bacteria isolates with an MIC to imipenem  $\geq 1$  mg/ml [27]. However, this problem

has not been studied further and official recommendations have not been issued yet.

The diversity of carbapenem resistance levels in the *K. pneumoniae* carrying blaVIM-1 gene was associated in one study [28] either with multiple copies of the gene on the plasmid backbone – a procedure generated by IS26 activity – or due to porin loss – a fact indicating that the clinical use of carbapenem and, to a lesser extent, cefepime and aztreonam, against the phenotypically susceptible isolates of this group may have possibly contributed to the selection of the high-level resistance isolates.

### Related molecular epidemiology issues

#### Genes

The spread of MBL-producing enteric bacteria in Greece is generally found to be due to VIM-1 type genes in the form of gene cassette [14,16-19,21,22] which are genetically different to the VIM-2 type genes isolated in *P. aeruginosa* in this country [6,7].

Interestingly, the blaVIM-1 cassette (including the 81 nucleotides of the 59-base element) was found identical to that originally described in *P. aeruginosa* in Italy and other European countries [14,18,21].

A different blaVIM gene termed blaVIM-12 was isolated in one *Klebsiella pneumoniae* and one *E. coli* isolate. This gene could be viewed as a blaVIM-1/blaVIM-2 hybrid being identical to blaVIM-1 from the 5' end up to nucleotide 663, and to blaVIM-2 from nucleotide 614 up to its 3' end [29,30]. Furthermore, the 59-base element of the blaVIM-12 gene cassette (72 bp in length) was identical to the element commonly found in blaVIM-2 cassettes and differed significantly from the 59 bp of the blaVIM-1 gene cassettes [29,30].

#### Integrations

The VIM gene was generally found to be part of related type I integrations. The cassette region of these integrations typically contains (from 5' to 3') the blaVIM-1, and the aacA4, dhfrI, and aadA genes [14,18,19].

However, a type I integron carrying the blaVIM-1 gene and a 6'-N-aminoglycoside acetyltransferase (aac(6)-Ib) gene cassette was described in an *E. cloacae* clinical isolate [21]. Moreover, a different integron structure suggesting a different evolution process rather than a transfer, and the spread of the mobile element among the Greek hospitals was described in a cluster of four *E. coli* isolates in Crete [17].

Similarly, a novel class 1 integron carrying a carbapenemase gene (blaVIM-1) associated with a trimethoprim (dfrA1), a streptothricin (sat1) and two aminoglycoside resistance genes (aacA7 and aadA1) was detected in a *Morganella morganii* clinical isolate [22]. Moreover, a class I integron carrying only the blaVIM-1, and the dhfrI and aadA genes was found in a plasmid isolated from three different bacterial genera [15]. Lastly, an integron solely carrying the blaVIM-1 gene was described in an *E. coli* isolate [16].

Integrations are not self-transferred elements, and are commonly associated with various transposons. An IS26 insertion into the 5' conserved segment of an In4-type integron and an IS26-mediated recruitment of resistance genes of diverse origin have been suggested as a mechanism for the evolution of various

multiresistant integrations, including those that harbour the blaVIM-1 genes [31]. However, further work on the exact mechanism of their development and dissemination is needed.

The coexistence of the blaVIM gene with various other, newer beta-lactamases, including SHV-5 [18], the IBC-1 [32], the GES7 [16] the CMY-4 [33] and the CTX-M [17] genes have also been reported.

#### Plasmids

The blaVIM containing integrations are mainly found to be harboured by transferable plasmids in most enteric bacteria species including *K. pneumoniae* [18], *E. coli* [14,17], *P. mirabilis* [15], *Enterobacter aerogenes* [15] and *Providencia stuartii* [23].

Interestingly, the chromosomal location of the VIM containing integrations was also documented on several occasions, including an epidemic clone of *P. mirabilis* in Thessaloniki [19], and sporadic *E. coli* [16], *Enterobacter cloacae* [21] and *Morganella morganii* [22] isolates.

The epidemiology of the blaVIM harbouring plasmids is an important prerequisite for understanding the dynamics of the growing proportion of VIM-producing strains. These plasmids were generally found to display different restriction patterns [18], although the spread of plasmids with identical patterns in isolates of the same species [17,18], or even among isolates of different species [15] has also been described. Most importantly, in at least one study, plasmids harbouring the blaVIM-1 gene were found to belong to the incompatibility group N [34], a fact consistent with the possible spread of evolving plasmids. However, these issues must be further elucidated. Plasmids of other than N incompatibility groups have also been sporadically isolated [33].

#### Bacterial strains

Another important condition for understanding the situation is the study of the possible clonal spread of the VIM-producing strains. Although much work needs to be done on this issue, the epidemics seem to be generally multiclonal, with clones differing between hospitals and sometimes even different clones present within a single hospital [18], with no particular clone prevailing (unpublished data from our department). A few exceptions to this rule have been reported: an outbreak in distinct regions of Greece due to a single *K. pneumoniae* clone carrying a blaVIM-1 gene [35], a small nosocomial outbreak due to a VIM-producing *E. coli* clone [17], and one caused by a VIM-producing *P. mirabilis* clone [19].

A recently published study on blood isolates from three hospitals in Athens revealed that 37.6% of all *K. pneumoniae* blood isolates were blaVIM-1-positive. 77.8% of these were taken from ICUs. PFGE identified eight clusters (A-H) with related (>80%) patterns, as well as four unique types. Microorganisms producing both VIM-1 and SHV-5 constitute the prevalent multidrug-resistant population of *K. pneumoniae* in this setting [36].

In conclusion, the large and still increasing proportion of VIM-producing *K. pneumoniae* seems to be due to the spread of the blaVIM-1 cassette among rapidly evolving multiresistant plasmids and multiresistant or even panresistant strains mainly of *K. pneumoniae* but also, of other enteric bacteria species. However, further work is needed to elucidate the possible contribution of plasmid or bacterial clone spread.

### Related clinical issues

Imipenem-resistant isolates are generally found to be multidrug-resistant, the majority displaying resistance to at least one aminoglycoside, quinolones and trimethoprim [37, unpublished data from the GSSAR]. Interestingly, most isolates were found to be resistant to aztreonam, indicating the simultaneous presence of other extended-spectrum beta-lactamases (ESBL) as well [37].

The multidrug-resistant nature of these isolates dramatically limits the therapeutic options, leaving colistin, a toxic and difficult-to-use drug, as the only antibiotic with *in vitro* activity against VIM-producing enteric bacteria. However, VIM-producing *K. pneumoniae* displaying resistance to colistin, with an MIC up to 64 mg/L have sporadically been isolated [unpublished data from the GSSAR], and at least one outbreak has been described [38].

Taking this into account, and given the *in vitro* low levels of resistance displayed by most isolates, the question of the possible treatment of these patients with high levels of carbapenem has so far been addressed by two published reports.

The *in vivo* activity of imipenem against VIM-producing *K. pneumoniae* was assessed in a thigh infection model in neutropenic mice by Daikos et al. [39]. The authors concluded that while their results cannot provide firm conclusions regarding the treatment of infections caused by VIM-producing *K. pneumoniae* strains with MIC of imipenem in the susceptible range, they suggest that the administration of imipenem at higher doses may prove to be of some benefit.

Moreover, a retrospective analysis of 28 cases of VIM-producing *K. pneumoniae* bloodstream infections [40] revealed a striking difference in mortality between patients infected with VIM-producing *K. pneumoniae* with MIC of imipenem >4 g/mL and control group patients infected with non-VIM-producing *K. pneumoniae*. In contrast, patients infected with VIM-producing *K. pneumoniae* but with MIC of carbapenems in the susceptible range displayed no difference in mortality compared to the control group.

In addition to these studies, Galani et al. have reported both successful [15] and non-successful [21] outcomes of patients infected with low-level-resistant VIM-producing enteric bacteria and treated with imipenem.

However, all these reports must be regarded as preliminary, and well designed prospective studies are urgently needed to tackle the therapeutic issues set by VIM-producing *K. pneumoniae*, as well as the possible need to modify the clinical breakpoints to carbapenems for the blaVIM harbouring strains.

### Related public health issues

It is well recognized that the main tools for confronting antibiotic resistance are antibiotic policy and infection control strategies [41].

The problem of VIM-producing *K. pneumoniae* was timely recognized by the GSSAR, and its significance adequately assessed and publicized by the Infectious Disease and Clinical Microbiology community in Greece. Moreover, the National Early Warning System for the Recognition of New and Emerging Resistance Mechanisms, which has been in operation in Greece for the last two years, was successfully used for the early tracing and reporting of VIM-

producing enteric bacteria. Additionally, the National Centre for Disease Control and Prevention at the Greek Ministry of Health (KEELPNO) issued guidelines which were distributed to the hospitals as soon as a VIM-producing strain had been isolated there. These guidelines were mainly addressed to the "Infection Control Committee" of the respective hospitals and included issues on antibiotic policy and infection control.

To date, however, these measures have made a relatively small impact on the still increasing proportion of VIM-producing strains.

It is well accepted that antibiotic resistance is a difficult-to-manage public health problem, especially when it is established. This is particularly true in the case of the complex molecular epidemiology of the VIM-producing *K. pneumoniae* problem in Greece.

Furthermore, Greece is among the countries which for decades have been reporting the highest levels of resistance to most antibiotics [42,43] and therefore physicians may not always recognize the possible significance of a new mechanism of resistance.

Antibiotics are the most important risk factors in the development of resistance, and therefore an effective antibiotic policy, in addition to being an important element of good medical practice, is an important public health measure in confronting the problem of antimicrobial resistance [44,45]. Especially since Greece is among the European countries with the highest rates of antibiotic use in both hospital and community settings [46,47].

It must be emphasized, however, that for the antibiotic policy to be effective, it must be based on a good understanding of the molecular basis of the resistance mechanisms [48]. Moreover, in an area such as Greece, with high resistance rates and very few effective antibiotics left at the physician's disposal, antibiotic policy has very narrow limits. What is more, antibiotic policy must always be combined with infection control.

In addition to the above difficulties, certain characteristics of the public health system in Greece, especially the fact that public health is relatively undersized within the national health system, hinders the effort to confront antibiotic resistance. The hospital epidemiologist is not a recognized specialist in Greece and hospital epidemiology is not part of the everyday practice in Greek hospitals. Although there is expertise available in many hospitals and university laboratories, the strains isolated from cases of healthcare-associated infections are not routinely typed. Hospital outbreaks are not routinely studied and the possible role of the spread of drug-resistant clones in these outbreaks is not routinely assessed. The "Infection Control Committees" in hospitals do not have administrative authority, infection control measures are not always implemented in practice, while infectious diseases specialists, with no official training in epidemiology, are mainly focused on antibiotic policy [49,50].

In summary, a national Strategic Action Plan is a necessary public health instrument to coordinate efforts, prioritize activities, set goals and audit actions, and thus to answer all important issues related to the spread of drug-resistant enteric bacteria discussed in this paper. Such Strategic Action Plan is currently

under development and hopefully will be available in the next few months. The plan will affirm the political commitment of the Greek health administration in confronting the issue of antimicrobial resistance. It will put emphasis on this public health problem and its risk factors in a way to be understood by the wider medical community, the health policymakers and the wider community. It will allocate specific tasks to the responsible bodies and coordinate and prioritize the necessary scientific research. The Action Plan will be based on the collaboration, coordination and consensus of opinions of all parties involved.

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The hospitals, members of the Greek System for Surveillance of Antimicrobial Resistance are listed at: <http://www.mednet.gr/whonet>

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