Carbapenemase-producing Bacteria: The Greek Experience

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Percentage (%) of IMIPENEM-R *P. aeruginosa* from all specimens of Greek hospitals 1996-2000
The first major outbreak of metallo-β-lactamase producers in Europe

An outbreak of VIM-2-positive *P. aeruginosa* in a hospital in Thessaloniki, Greece ongoing since 1996

Tsakris A et al. JCM 2000; 38:1290

Multiclonal outbreak of multiresistant VIM-2-positive *P. aeruginosa* in several hospitals in Greece in 2001

62% of IMIPENEM-R isolates were MBL-producers!

Giakkoupi P et al. JCM 2003;41:822
VIM-producing *P. aeruginosa* endemic in tertiary-care Greek hospitals

88.7% of imipenem-R *P. aeruginosa* in Larissa, Thessaly produced VIM-2 or VIM-4

VIM-positive isolates showed clonal diversity

$bla_{\text{VIM}}$ gene cassette was carried as a single gene in a class 1 integron

Pournaras S et al. JAC 2003; 51:1409
For the history….

The first VIM-2 positive *P. aeruginosa* isolates date back to 1992 Isolated from clinical specimens of patients hospitalized in the ICU of two different tertiary-care hospitals in Athens

The first VIM-4 positive *P. aeruginosa* isolates date back to 1995 Isolated from clinical specimens of patients hospitalized in the same ICUs

I.Galani, unpublished
MBLs: More variants

In 2004 a new variant $bla_{VIM-17}$ was detected in *P. aeruginosa* clinical isolates in a hospital in Thessaloniki.

VIM-17-producing and VIM-2-producing isolates co-existed.

All isolates (irrespective of clonal lineage) carried a similar class I integron, similar to that previously described in a *P. aeruginosa* in France.

Horizontal transmission of a class I integron between different clones of *P. aeruginosa*, without evidence for plasmid carriage.

Siarkou VI et al. AAC 2009; 53:1325
Percentage (%) of IMIPENEM-R *P. aeruginosa* from all specimens of Greek hospitals 1996-2010
Figure 5.34: *Pseudomonas aeruginosa*: proportion of invasive isolates resistant to carbapenems in 2009

- **< 1%**
- **1% to < 5%**
- **5% to < 10%**
- **10% to < 25%**
- **25% to < 50%**
- **≥ 50%**
- No data reported or less than 10 isolates
- Not included

Non-visible countries:
- Luxembourg
- Malta
VIM-1 in *E. coli* isolates in Greece since 2001

Scoulica E et al. DMID 2004; 48:167
Miriagou V et al. AAC 2003; 47:395

VIM-1 in many *K. pneumoniae* isolates in various hospitals in Athens since 2002

- multiclonal outbreak
- *bla*\textsubscript{VIM-1} carried by small transferable plasmids with different restriction patterns
- one common class 1 integron structure that also included *aac6*, *dhfrI* and *aadA* (In-e541)
- many isolates exhibited an imipenem MIC in the susceptible range

Giakkoupi P et al. JCM 2003; 41:3893
VIM-1 in Enterobacteriaceae

Outbreak of MBL-producing *P. mirabilis* in a hospital in Thessaloniki from June 2004 to March 2005 [Vourli et al.] and a cluster of cases in outpatients previously hospitalized in the urology dpt of the hospital in Serres [Tsakris et al.]

MBL production sporadically in *E. cloacae* in 2003 and in *E. aerogenes* in 2004 [Galani et al.]

in *M. morganii* in 2005 [Tsakris et al.]

in *P. stuartii* in 2007 [Miriagou et al.]

in *S. liquefaciens* and *K. oxytoca* in 2006 [Miriagou et al.]

**BUT ...**
Ongoing epidemic of $\text{bla}_{\text{VIM-1}}$-positive \textit{Klebsiella pneumoniae} in Athens, Greece: a prospective survey


Outbreaks in Distinct Regions Due to a Single \textit{Klebsiella pneumoniae} Clone Carrying a $\text{bla}_{\text{VIM-1}}$ Metallo-\(\beta\)-Lactamase Gene

Alexandros Ikonomidis, Despina Tokatlidou, Ioulia Kristo, Danai Sofianou, Athanasios Tsakris, Paraskevi Mantzana, Spyros Pournaras, and Antonios N. Maniatis
From 240 patients, 23 patients either only colonised (n = 11) or infected (n = 12) by MBL-producers [9.6 patients /100 admissions]

- Multiclonal outbreak [10 PFGE types]
- Unrelated clones of K.pn and both clones of E.cl shared the same integron structure (In-e541)
- C.fr contained a class I integron (bla\text{VIM-1}, aac(6)-IIc) previously described in an E.cl with no apparent epidemiological link and in K.pn from Genoa, Italy
- A single patient could harbour up to 4 unrelated clones of MBL (+) K.pn
- Different patients shared some common clones of K.pn even without apparent time link between them
- Most clonal types were responsible for both colonisation and infection
*bla*<sub>VIM-1</sub> did not originate from *P.aeruginosa*

**BUT...**

*bla*<sub>VIM-12</sub>, a *bla*<sub>VIM-1</sub>*/bla*<sub>VIM-2</sub> hybrid was detected in *K.pneumoniae* and *E.coli* clinical isolates from a hospital in Thessaloniki

A wide dissemination of that gene is documented locally causing an outbreak in that hospital

*Pourmaras et al. AAC 2005; 49:5153*
*Iconomidou et al. AAC 2007; 51:3038*
*Tokatlidou et al. JCM 2008; 46:1005*
Carb-R Kpn (VIM-1-positive) was identified in
- 3 hospitals in Greece in 2002
- 25 of 40 hospitals participating in GSSAR in 2008

Low level of imipenem resistance is common among VIM-1 producing Kpn

Higher MICs are due to either multiple copies of the gene on the plasmid – a procedure generated by IS26 activity – or to porin loss

Loli et al. JAC 2006
Percentage (%) of IMIPENEM-R *K. pneumoniae* from all specimens of Greek hospitals 1998-2007

www.mednet.gr/whonet
Figure 5.25: *Klebsiella pneumoniae*: proportion of invasive isolates resistant to carbapenems in 2009

- Green: < 1%
- Light green: 1% to < 5%
- Yellow: 5% to < 10%
- Orange: 10% to < 25%
- Red: 25% to < 50%
- Dark red: ≥ 50%
- Gray: No data reported or less than 10 isolates
- Light gray: Not Included

Non-visible countries:
- Green: Luxembourg
- Green: Malta
Risk factors for VIM-positive *K. pneumoniae* infections and outcome

- Independent predictors of VIM-positive *K. pn* bloodstream infections (*Daikos GL et al. 2010*)
  
  ICU stay
  
  prior use of carbapenems
  
  prior exposure to >3 different classes of antibiotics
  
- Prospective observational study on the outcome of patients with *K. pn* bloodstream infections (*Daikos GL et al. 2009*)
  
  14-day mortality:
  
  15.8% for patients infected with VIM-negative *K. pn*
  
  18.9% for those infected with VIM-positive Carb-S *K. pn*
  
  42.9% for those infected with VIM-positive Carb-R *K. pn*
  
  mediated by the failure to provide effective therapy
Recurrent healthcare-associated community-onset infections due to *Klebsiella pneumoniae* producing VIM-1 metallo-β-lactamase

Aggeliki Poulou\(^1,2\), Nicholas Spanakis\(^2\), Spyros Pournaras\(^3\), Vassiliki Pitiriga\(^2\), Kyriaki Ranellou\(^2\), Fani Markou\(^1\) and Athanassios Tsakris\(^2*\)  

*JAC* 2010; 65: 2538–2542

Large Dissemination of VIM-2–Metallo-β-Lactamase–Producing *Pseudomonas aeruginosa* Strains Causing Health Care-Associated Community-Onset Infections


12 patients with UTI caused by VIM-1 *K.pn*, 2-4 months after discharge  
Only 2 patients with known colonization during recent hospitalization

45 patients with UTI or bacteremia caused by VIM-2 *P.aer*, 40 reported previous hospitalization 1mo-1y before, 5 without link to health-care facilities
In 2007…

Outbreak of infections due to KPC-2-producing *Klebsiella pneumoniae* in a hospital in Crete (Greece)

H.C. Maltezou a,*, P. Giakkoupi b, A. Maragos a, M. Bolikas c, V. Raftopoulos a, H. Papahatzaki c, G. Vrouhos d, V. Liakou c, A.C. Vatopoulos b

Interestingly, the first KPC-2-producing *K. pneumoniae* strains detected in France and in Sweden concerned two patients previously hospitalized in this hospital in Crete


Tegmark Wissel K et al. Eurosurveil 2007

Tn4401 was identified at the origin of acquisition of *bla*KPC-2 in the Greek *K. pneumoniae* as well as in all international strains studied

• Wide intrahospital spread of KPC-producing *K. pneumoniae* clones
• Apart from *bla*KPC-2 most isolates carried also *bla*SHV-12 and *bla*TEM-1
• The predominant clone was closely related to the hyperepidemic clone from the USA and Israel
• The crude mortality rate of infected patients was 27.7%
Surveillance in 21 Greek hospitals from 2/2008 until 12/2008

All K.pn with imipenem MIC ≥1mg/L

77% of 225 isolates from 18 hospitals in 3 different areas of the country were KPC-2 producers

One major genetic clone which has emerged in Crete in spring 2007 and then spread in the rest of the country

The clone was indistinguishable from the KPC-2 Israeli clone!
Intercontinental spread of KPCs
KPCs are frequent flyers
Clusters of linked and unlinked STs in the entire *K. pneumoniae* MLST database

The globally distributed ST of *K. pneumoniae* associated with KPC enzyme production is ST258

The rapidly, multifocal emergence of clonally related isolates with *blaKPC* is highly suggestive of direct, human-mediated international spread, rather than the repeated acquisition of similar plasmids by a prevalent clone.

*Woodford N et al. FEMS Microbiol Rev 2011*
Percentage (%) of IMIPENEM-R *K. pneumoniae* from all specimens of Greek hospitals 1998-2010
An Outbreak of Infection due to β-Lactamase
*Klebsiella pneumoniae* Carbapenemase 2–Producing*K. pneumoniae* in a Greek University Hospital:
Molecular Characterization, Epidemiology, and Outcomes

M. Souli et al. *Clinical Infectious Diseases* 2010; 50:364
Prevalence rate: isolates/100 admissions

<table>
<thead>
<tr>
<th>K. p positive for</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>$bla_{VIM-1}$</td>
<td>32.9</td>
<td>13.5</td>
</tr>
<tr>
<td>$bla_{KPC-2}$</td>
<td>1.8</td>
<td>9.9</td>
</tr>
</tbody>
</table>

50 patients: 36% infection and 64% colonization

82% of the isolates belong to a common clonal type

Source of acquisition: cross-transmission 58.8%
imported 29.4%
undetermined 11.8%

46% GI colonization

Bacteremia the most common source of infection

Crude mortality: 61%
Attributable mortality: 27.8%
Comparing our experience with clinical infections by VIM producing Enterobacteriaceae in the same hospital, although differences were not significant we noticed that:

In the cohort of KPC (+) Kpn infections

• Patients were less seriously ill
• Colonization was detected sooner after admission
• The duration of previous colonization before infection was shorter
• Mortality was 27.8% vs 18.8%
• Fewer strains with low-level Carb-resistance
1-15 September 2008

WARD C

WARD A

WARD B
9 MARCH 2009

WARD C

WARD A

WARD B
All-Cause in Hospital Mortality of 353 Patients with *Klebsiella pneumoniae* BSIs

Daikos GL, unpublished
The ongoing challenge of acquired carbapenemases

Emerging *Klebsiella pneumoniae* Isolates Co-producing KPC-2 and VIM-1 Carbapenemases

In 2009 *K. pneumoniae* co-producing VIM-1 and KPC-2 were simultaneously identified in diverse locations in Greece.

These strains evolved through acquisition of a $\text{bla}_{\text{KPC-2}}$-carrying plasmid by an established VIM-1-producing *K. pneumoniae* strain.

*Giakkoupi P et al. AAC 2009; 53:4048*
During 2009, a third of all carbapenem non-susceptible *K. pneumoniae* from a single center were VIM-1 and KPC-2 co-producers.
Continuing evolution of MBLs under carbapenem pressure in Greek hospitals

Detection of the new metallo-β-lactamase VIM-19 along with KPC-2, CMY-2 and CTX-M-15 in Klebsiella pneumoniae

Spyros Pournaras¹*, Aggeliki Poulou²,³, Evangelia Voulgaris³, Georgia Vrioni³, Ioulia Kristo¹ and Athanassios Tsakris³

JAC 2010; 65:1604

Emergence of carbapenem-resistant Enterobacter cloacae carrying VIM-4 metallo-beta-lactamase and SHV-2a extended-spectrum beta-lactamase in a conjugative plasmid.


Emergence of Klebsiella pneumoniae of a novel sequence type (ST383) producing VIM-4, KPC-2 and CMY-4 β-lactamases

Papagiannitsis et al.
IJAA 2010; 36:573
An update of the evolving epidemic of $bla_{KPC-2}$-carrying Klebsiella pneumoniae in Greece (2009–10)

Giakoupi P et al. JAC 2011; 66:1510

378 K. pn isolates with IMP MIC $\geq 1 \mu g/ml$ and boronic test (+) were collected from 40 Greek hospitals from Jan 2009 to Apr 2010

• All KPC-2 (+) and ~5% KPC-2 and VIM-1 or VIM-4 (+)

• A wide range of carbapenem MICs was observed; 1 to $\geq 64$ mg/L

• ~ 40% (up to 65%) of all K.pn isolated during the study period were KPC (+)

• Compared to data from the 2008 surveillance, an abrupt increase in isolation rates was noted in almost all participating hospitals

• 13 PFGE types were detected assigned to 11 STs

Table 1. Regional distribution of 378 KPC-2-producing K. pneumoniae isolates and their classification by molecular typing

<table>
<thead>
<tr>
<th>Geographical distribution (no. of hospitals)</th>
<th>PFGE types (STs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Athens (26)</td>
<td>216</td>
</tr>
<tr>
<td>Other mainland cities (6)</td>
<td>43</td>
</tr>
<tr>
<td>Crete and other islands (8)</td>
<td>63</td>
</tr>
<tr>
<td>Total no. of hospitals (40)</td>
<td>322</td>
</tr>
</tbody>
</table>
Almost all STs harbored identical plasmids carrying a Tn4401-type transposon, either isoform a or b.

Isolates carrying a Tn4401b transposon have lower MICs probably due to a lower level of transcription of blaKPC-2.

Double carbapenemase producers were assigned to STs 147, 323 and 383.

ST 147 was common in Greek hospitals up to 2007 and harbored only blaVIM. It can be supported that some of these isolates acquired blaKPC-2 after the introduction of the respective plasmids in Greece.

The epidemic is at present essentially clonal but the recent spread of KPC-2-encoding plasmids in a wide variety of STs is a development that will probably further aggravate the situation.

Giakkoupi P et al. JAC 2011; 66:1510
Geographic distribution of KPC worldwide

Acquired Carbapenemases among A. baumannii in Greece

$bla_{OXA-58}$ is frequently identified in Carbapenem–R A. baumannii

$bla_{OXA-58}$ was identified in 94.4% of carbapenem–non-susceptible and in 37.5% of carbapenem–susceptible isolates collected over a 10-year period from 8 Greek hospitals.

$ISAba3$ adjacent to $bla_{OXA-58}$ upregulates gene expression.

$bla_{VIM-1}$ is only rarely found and sometimes weekly expressed and for that reason cannot be detected by phenotypic tests.

Occasional isolates with $bla_{VIM-4}$

Ikonomidis A et al. JCM 2008; 46:346
Tsakris A. et al. EID 2006; 12:981
Gogou V et al. JAC 2011
Tsakris A. et al CMI 2008; 14:588
Figure 1. Proportion of A. baumannii strains resistant to carbapenems. Souli et al. Eurosurveillance 2008;13(47):1-11
In November 2010 a National Action Plan for Monitoring and Containment of MDR pathogens was launched by the Greek Center for Disease Control and Prevention (KEELPNO) and the Greek Ministry of Health under the name "Procrustes"
Prevalence of bacteremias by *Kl. pneumoniae* in Laiko General Hospital

Daikos GL, unpublished
Preliminary Results from the first level of this plan concerning the **Early Detection** of cases of MDR pathogens from a nationwide surveillance network of ...cooperating hospitals

<table>
<thead>
<tr>
<th>Species</th>
<th>%</th>
<th>Bacteremia %</th>
<th>UTI %</th>
<th>URTI %</th>
<th>SSI %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. pneumoniae</em></td>
<td>43</td>
<td>35</td>
<td>30</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>35</td>
<td>24</td>
<td>29</td>
<td>35</td>
<td>12</td>
</tr>
<tr>
<td><em>A. baumannii</em></td>
<td>22</td>
<td>31</td>
<td>8</td>
<td>46</td>
<td>15</td>
</tr>
</tbody>
</table>

Carbapenem-R *K. pneumoniae*: 45% ICUs, 55% regular wards

Estimated crude mortality rate: 10/100.000 population
Carbapenemase-Producing Bacteria: The Greek Experience

VIM-2 and VIM-4 MBLs are the most frequent carbapenemases in *P. aeruginosa*

VIM-2 MBL and KPC-2 are mainly responsible for the extremely high rates of carbapenem-resistance among *K. pneumoniae*

OXA-58 is the most frequent carbapenemase among Acinetobacter

Most of these MDR Gram-negatives are already endemic in major Greek hospitals
Thank you for your attention