Multiclonal outbreak of highly-resistant OXA-48-producing *Klebsiella pneumoniae* strains in a tertiary-care hospital: attack of the clones

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Disclosures:

None
OXA-48-like carbapenemases: the phantom menace

Laurent Poirel*, Anaïs Potron and Patrice Nordmann
OXA-48-type carbapenemases: global spread

Nordmann P, Poirel L. CMI 2014;20:821-30 (modified*)
van Duin D, Doi Y. Virulence. 2016 Aug 11:1-10
Albiger B et al; (EuSCAPE) working group. Euro Surveill. 2015;20(45).
OXA-48-type carbapenemases: 1st emergence in Greece, 2012

Results: All isolates harboured the \(\text{bla}_{\text{OXA-48}}\) gene along with the \(\text{bla}_{\text{CTX-M-15}}\) and \(\text{bla}_{\text{OXA-1}}\) genes. The \(\text{bla}_{\text{OXA-48}}\) gene was located on a self-transferable IncI1/M-type plasmid of \(~62\) kb, which harboured no other resistance genes. IS1999 was located upstream of the \(\text{bla}_{\text{OXA-48}}\) gene. Genetic disruptions of the \(\text{ompK35}\) and \(\text{ompK36}\) genes were not detected. The isolates belonged to a unique PFGE clone and MLST assigned them to sequence type ST11. All cases were characterized as hospital acquired and none of them was linked to immigration or history of travel in endemic areas.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>(K.) pneumoniae K1–K6 and K8–K11</th>
<th>(K.) pneumoniae K7</th>
<th>(K.) pneumoniae K12 and K13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>0.75–2</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1</td>
<td>8</td>
<td>4–8</td>
</tr>
<tr>
<td>Ertoopenem</td>
<td>4–6</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>(&gt;256)</td>
<td>(&gt;256)</td>
<td>(&gt;256)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>32 to (&gt;128)</td>
<td>(&gt;128)</td>
<td>32–64</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>(&gt;256)</td>
<td>(&gt;256)</td>
<td>(&gt;256)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>8–16</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>(&gt;256)</td>
<td>(&gt;256)</td>
<td>(&gt;256)</td>
</tr>
<tr>
<td>Amoxicillin/clavulanate</td>
<td>(&gt;256)</td>
<td>(&gt;256)</td>
<td>(&gt;256)</td>
</tr>
<tr>
<td>Piperacillin/tozobactam</td>
<td>(&gt;256)</td>
<td>(&gt;256)</td>
<td>(&gt;256)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.5–2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.5–2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Albiger B et al; (EuSCAPE) working group. Euro Surveill. 2015;20(45).
• The present study was conducted from June 2014, when OXA-48-producing *Klebsiella pneumoniae* (KP-OXA-48) re-emerged in our setting, to June 2016.
• **It outlines the establishment of KP-OXA-48**
Material/Methods

Setting: 428-bed tertiary-care hospital, 12-bed general ICU/no geographical separation between patients

Patient-to-nurse ratio in ICU: 2.5:1

Study isolates: All KP-OXA-48 isolates

Susceptibility testing
- Vitek2 Compact, Etest, BMD (colistin, tigecycline)

Screening for carbapenemase production: Modified Hodge Test
- All isolates with reduced susceptibility to carbapenems (Meropenem MIC > 0.25 mg/l)
Methods

Differentiation of carbapenemase type:
combined disc tests using meropenem ± PBA ± EDTA

Coproduction of ESBL tested using a modified CLSI ESBL Combined Disc Test

PCR: \( \text{bla}_{\text{OXA-48}} \) and other beta-lactamase genes

Molecular typing
PFGE, MLST

NGS, Resistome analysis on 5 strains (UMCG, Groningen, The Netherlands)
RESULTS

OXA-48 CRKP: evolved towards endemicity

2014, 2nd semester, 192 CRKP isolates
- OXA-48; 25%
- KPC; 44%
- MBL; 31%

2015, 534 CRKP isolates
- KPC; 61%
- OXA-48; 16%
- VIM; 16%
- KPC+MBL; 7%

2016, 434 CRKP isolates
- KPC; 34%
- OXA-48; 23%
- MBL; 40%
- KPC+MBL; 3%
Epidemic curve

227 KP-OXA-48 isolates, 79 patients

Index patient ST147
## Patients’ characteristics

<table>
<thead>
<tr>
<th>Demographic and epidemiological characteristics of the 79 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, male</strong></td>
</tr>
<tr>
<td><strong>Age, y (median)</strong></td>
</tr>
<tr>
<td><strong>Department of hospitalization</strong></td>
</tr>
<tr>
<td><strong>Prior hospitalization</strong></td>
</tr>
<tr>
<td><strong>Hospital stay, days from admission to OXA-48 KP isolation (median, range)</strong></td>
</tr>
<tr>
<td><strong>Isolation of KP-OXA-48 during the first 48 hours of hospitalization</strong></td>
</tr>
<tr>
<td><strong>Rectal screening at admission</strong></td>
</tr>
<tr>
<td><strong>BSI</strong></td>
</tr>
<tr>
<td><strong>Overall mortality</strong></td>
</tr>
</tbody>
</table>
Bacterial isolates

No of KP-OXA-48 strains per patient: 1-17
Most isolates were colonizers

Source of isolation

ASC protocol for CPKP
- ICU patients
  - at admission
  - weekly (unless acquiring CPKP)
- Patients with risk factors
- Contacts of colonized/infected patients

Pournaras S et al. JCM 2013; 51:2986-90
Bacterial isolates, resistances %

- FOSF: 16.4%
- CHLORA: 94.7%
- FEP: 90.0%
- CTX: 95.1%
- CAZ: 82.3%
- ATM: 82.3%
- CIP: 100.0%
- SXT: 74.9%
- GENTA: 94.7%
- AMI: 62.2%
- TETRA: 99.0%
- TIGE: 54.1%
- COL: 81.9%
- ERT: 100.0%
- MER: 99.6%
- IMP: 96.9%

36.3% I (MIC=2 mg/l)
PCR for ESBLs, carbapenemases among the 227 KP-OXA-48 isolates

80.6% carried \( \text{bla}_{\text{CTX-M}} \)

<table>
<thead>
<tr>
<th>Additional carbapenemase gene</th>
<th>No of strains</th>
<th>Other ( \beta )-lactamases genes</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{bla}_{\text{KPC}} )</td>
<td>3</td>
<td>( \text{bla}<em>{\text{CTX-M}} ) ( \text{bla}</em>{\text{CTX-M}} ) ( \text{bla}_{\text{CTX-M}} )</td>
<td>ST147</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ST101</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ST101</td>
</tr>
<tr>
<td>( \text{bla}_{\text{VIM}} )</td>
<td>4</td>
<td>( \text{bla}<em>{\text{CTX-M}} ) ( \text{bla}</em>{\text{CTX-M}} ) ( \text{bla}<em>{\text{CMY-4}} ) ( \text{bla}</em>{\text{CMY-13}} ) ( \text{bla}<em>{\text{OXA-10}} ) ( \text{bla}</em>{\text{SHV-129}} ) ( \text{bla}_{\text{VEB-1}} )</td>
<td>ST101</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ST101</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ST383</td>
</tr>
<tr>
<td>( \text{bla}_{\text{NDM}} )</td>
<td>1</td>
<td>( \text{bla}<em>{\text{CTX-M}} ) ( \text{bla}</em>{\text{TEM}} )</td>
<td>ST383</td>
</tr>
</tbody>
</table>

Not found
Clonality analysis

**ST147 index patient**, directly admitted from another hospital Screened positive upon admission

**ST101 (and ST383!) index patient**, admitted from another hospital (ICU); yielded the ST101 isolate on day 19

**ST383** was yielded on day 31

<table>
<thead>
<tr>
<th>PFGE type</th>
<th>ST type</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>ST147</td>
<td>26</td>
<td>40.9</td>
</tr>
<tr>
<td>A1</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>ST101</td>
<td>37</td>
<td>56.1</td>
</tr>
<tr>
<td>C</td>
<td>ST383</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>C1</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Patient/Strain ID</td>
<td>PFGE/ST type</td>
<td>Carbapenemases genes</td>
<td>Other β-lactamases genes</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>----------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>PT1.4 O0149 13/10/14 Rectal</td>
<td>A/147</td>
<td>$\text{bla}_{\text{OXA-48}}$</td>
<td>$\text{bla}<em>{\text{TEM-1B}}$, $\text{bla}</em>{\text{CTX-M-15}}$, $\text{bla}_{\text{SHV-11}}$</td>
</tr>
<tr>
<td>PT2.2 O0147 13/10/14 Rectal</td>
<td>C/383</td>
<td>$\text{bla}<em>{\text{OXA-48}}$, $\text{bla}</em>{\text{VIM-1}}$</td>
<td>$\text{bla}<em>{\text{TEM-1B}}$, $\text{bla}</em>{\text{CMY-4}}$, $\text{bla}<em>{\text{CMY-13}}$, $\text{bla}</em>{\text{OXA-10}}$, $\text{bla}<em>{\text{SHV-129}}$, $\text{bla}</em>{\text{VGB-1}}$</td>
</tr>
<tr>
<td>PT2.3 O0151 13/10/14 Rectal</td>
<td>B/101</td>
<td>$\text{bla}_{\text{OXA-48}}$</td>
<td>$\text{bla}<em>{\text{TEM-1A}}$, $\text{bla}</em>{\text{OXA-1}}$, $\text{bla}<em>{\text{OXA-9}}$, $\text{bla}</em>{\text{CTX-M-15}}$, $\text{bla}_{\text{SHV-1}}$</td>
</tr>
<tr>
<td>P3.2 O0133 7/10/14 Blood</td>
<td>A/147</td>
<td>$\text{bla}_{\text{OXA-48}}$</td>
<td>$\text{bla}<em>{\text{TEM-1B}}$, $\text{bla}</em>{\text{CTX-M-15}}$</td>
</tr>
<tr>
<td>PT3.3 O0144 13/10/14 Rectal</td>
<td>A/147</td>
<td>$\text{bla}_{\text{OXA-48}}$</td>
<td>$\text{bla}<em>{\text{TEM-1B}}$, $\text{bla}</em>{\text{CTX-M-15}}$, $\text{bla}_{\text{SHV-11}}$</td>
</tr>
</tbody>
</table>
Discussion: Clone ST383

Circulating in our hospital since 2010:
- Associated with KP VIM-19 and KPC-2 & VIM-19 producers
- First reported from Greece in 2010 (ST383 with VIM-4 & KPC-2 & CMY-4)
- Probably endemic clone in Greek hospitals since 2008 (VIM-19 KP in a Swedish patient hospitalized in Greece)
- Most KPC & VIM-producing KP in Greek hospitals during 2009-2010 were ST383
- ST-383 not frequently associated with OXA-48 producers
- ST383 was predominant in a single KP-OXA-48 outbreak recently reported in China

Sabirova JS et al. JAC 2016; 71:1501-9
Papagiannitsis CC et al. IJAA 2010; 36:573-4
Samuelsen Ø et al. CMI 2011; 17:1811-6
Discussion: The main clones ST147, ST101

**ST147**: circulating in our hospital since 2009, associated with KPC-2 and KPC-2 & VIM-1 KP isolates
- Probably endemic clone in Greece since 2005, associated with epidemics of VIM-1 and later KPC producers in Greece
- Linked to the global spread of different carbapenemases (OXA-48, NDM-1, VIM-1 and KPC-2) or CTX-M-15
  - **ST147 OXA-48 KP have been reported from remote countries worldwide**

**ST101**: first detected in our hospital during the current outbreak and established thereafter
- First correlation with carbapenemase-producing *K. pneumoniae* dissemination in Greece
- Linked to OXA-48 KP spread all over Europe and in North Africa
Discussion: multiclonal outbreak of OXA-48 KP, Spain

4-month period (Sep - Dec 2014)
68 isolates from 41 patients
Conclusions

- The multiclonality of KP-OXA-48 circulating in our hospital implies an endemic situation, which adds to the established KPC and VIM epidemic.
- The XDR/PDR susceptibility profile of the isolates, is alarming, leading to therapeutic dead-ends.
- Our data may indicate wide dissemination of KP-OXA-48 in Greece.

It seems that... controlling the overall spread of CPKP in Greece:
- is a major challenge
- necessitates hospital and countrywide implementation of multifaceted interventions.
Appreciation

- Gian Maria Rossolini
- Tommaso Giani
- Alexander W. Friedrich
- John Rossen