Carbapenemases in non-fermenters and enteric bacteria

Laurent Poirel

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INSERM U914 « Emerging Resistance to Antibiotics »
Hospital Bicêtre
France
Enterobacteriaceae
Carbapenem-hydrolyzing β-lactamases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Penicillins</th>
<th>Cephalosporins 1st et 2nd generation *</th>
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<td>cefpirome</td>
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<td>D</td>
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* Cephamycins excluded for most class A
**The carbapenemases in Enterobacteriaceae**

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<td>D</td>
<td>Oxacillinase =OXA-48 and its derivatives</td>
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* Cephamycins excluded for most class A
ESBL of a novel type; KPC

**KPC-1:**
- plasmid encoded (50-kb)
- hydrolyses all β-lactam substrates, that means broad-spectrum cephalosporins + carbapenems

- Imipenem resistance inhibited by clavulanic acid
The worldwide dissemination of KPC

- Canada
- Puerto Rico
- Colombia
- Brazil
- Argentina
- Israel
- South Korea
- Taiwan
- India

- Single KPC-producing isolates
- Several outbreaks KPC-producing isolates
- Endemicity of KPC-producing isolates

Nordmann et al., Emerg Infect Dis 2011
• 16 KPC-2-producing *K. pneumoniae* isolates recovered from 5 countries
• All were multidrug resistant
• One major clone (9 isolates of ST258 type), and 7 minor ones
• Different plasmids (different sizes, incompatibility groups)
• One given genetic structure (transposon Tn4401)
Class B β-lactamases

- Metallo-enzymes
- Not inhibited by clavulanic acid
- Inhibited in vitro by EDTA
- Hydrolyse all β-lactam substrates, including broad-spectrum cephalosporins, but spare monobactams
- Hydrolyse carbapenems at high level
Metallo-β-lactamase; *E. coli* (NDM-1)
Metallo-β-lactamase; *E. coli* (NDM-1)

But often associated with ESBL production and permeability defects
Characterization of a New Metallo-β-Lactamase Gene, bla_{NDM-1}, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in *Klebsiella pneumoniae* Sequence Type 14 from India

Dongeun Yong,¹,² Mark A. Toleman,² Christian G. Giske,³ Hyun S. Cho,⁴ Kristina Sundman,⁵ Kyungwon Lee,¹ and Timothy R. Walsh²

Yonsei University College of Medicine, Research Institute of Antimicrobial Resistance, Seoul, Republic of Korea¹; Department of Medical Microbiology, Cardiff University, Cardiff, United Kingdom²; Clinical Microbiology, MTC—Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden³; Yonsei University College of Life Science and Biotechnology, Seoul, Republic of Korea⁴; and Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden⁵

AAC 2009; 53:5046–5054
Spread of NDM-1 from India/Pakistan to the UK

*blanDM-1*-positive bacterial clones

Courtesy Neil Woodford
K. pneumoniae NDM-1, Kenya

Poirel et al., AAC 2011;55:934-6
E. coli NDM-1, Australia

Poirel et al., AAC 2010;54:4914-6
The problem of NDM-1

- India, Pakistan, Bangladesh
- Balkan countries
  - Endemic?
- UK
  - Epidemic and recurrent importations
- Other scattered reports
  - France, Spain, Sweden, Austria, China, Japan, Canada, USA, ...
NDM producers, 2012

[Map of NDM producers in 2012 with countries such as Canada, USA, Spain, Egypt, Morocco, Iraq, and other regions marked with stars indicating different case numbers.]

STAR SIZE
- ★ 1-5 cases
- ★★ 6-50 cases
- ★★★ > 50 cases
Infections with NDM producers

E. coli, Klebsiella, Enterobacter, Serratia, Citrobacter, Pseudomonas, Acinetobacter

Asymptomatic colonisation
Wound infection / Diabetic foot
Lower urinary tract infection
Upper urinary tract infection
Nosocomial pneumonia / VAP
Intra-abdominal / pelvic infection
Bacteraemia / septicaemia
Neurosurgical meningitis

SEVERITY
Infections with NDM producers

*E. coli*, *Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter*, *Pseudomonas*, *Acinetobacter*

- Asymptomatic colonisation
- Wound infection / Diabetic foot
- Lower urinary tract infection
- Upper urinary tract infection
- Nosocomial pneumonia / VAP
- Intra-abdominal / pelvic infection
- Bacteraemia / septicaemia
- Neurosurgical meningitis

- No difference between NDM and non-NDM producers
- No known virulence factors for NDM producers
- NDM producers will not respond to conventional antibiotics
A successful story

Hygiene

Diarrhea

Population: overcrowded

Antibiotics; misuse and overuse, over-the-counter sale

Spread of NDM-1 producers in *E. coli*, *K. pneumoniae*

Subtropical continent

...and then higher mortality rate and length of hospitalization, overuse of broad-spectrum of antibiotics....
E. coli NDM-1: community-acquired in India

The plasmid-mediated bla\textsubscript{NDM-1} gene, encoding a powerful carbapenemase, was first identified in Escherichia coli and in Klebsiella pneumoniae in Sweden from a patient who had transferred from India. It was later identified in a patient in the UK, and in patients from Pakistan in different enterobacterial species. Here we report a woman aged 60 years who was admitted to hospital in April 2013 with treatment for breast cancer.

The patient came from Darjeeling, India, where she had lived for several years and had not been hospitalised. Upon her admission in France, bacterial cultures from the surface of her breast tumour were grown. The cultures were of the E. coli isolate GUE that was resistant to most β-lactams (remaining susceptible to aztreonam) and that had reduced susceptibility to carbapenems (minimum inhibitory concentrations of imipenem 3 μg/mL, meropenem 2 μg/mL). This isolate was also resistant to gentamicin, kanamycin, tobramycin, sulfamidines, tetracycline, and fluoroquinolones, but remained susceptible to amikacin, chloramphenicol, rifampicin, and colistin. PCR and sequencing revealed that E. coli GUE harboured the \textit{bla\textsubscript{NDM-1}} gene. Mating-out assays allowed the \textit{bla\textsubscript{NDM-1}} gene to be identified on a 110 kb plasmid, with markers for kanamycin, gentamicin, tobramycin, trimethoprim, and sulfamidines resistance. Multilocus sequence typing identified E. coli GUE as an ST131 type strain, which corresponds to a genetic background that is also responsible for the worldwide diffusion of another common resistance determinant, CTX-M-15.

This case is the first identification of an NDM-1-producing E. coli isolate in France, and corresponds again to an imported case from India. This example confirms the recent data suggesting that the Indian subcontinent might represent an important reservoir, and therefore a source, of NDM-producing isolates. The patient had not been hospitalised in India; therefore, the multidrug-resistant isolate had likely been community acquired. Worryingly, this resistance gene has been identified here in an E. coli strain belonging to a genotype that has proved its ability to disseminate widely in the community.

We declare that we have no conflicts of interest. This study was mostly funded by the INSERM (U114), France, and by grants from the Ministère de l'Éducation Nationale et de la Recherche (UPR61-ECAJS3), Université Paris XI, France, and from the European Community (T5P038-QC, HEALTH-2009-241742).

Laurent Poiré, Cécile Hamtronck-Alet, Claire Frenéaux, Sandrine Bernabeu, *Patrice Nordmann

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Hôpital de Bicêtre, Department of Bacteriology, Virology, Le Kremlin-Bicêtre, Paris 94275, France.


Persistent carriage of carbapenemase producers

Persistent Carriage and Infection by Multidrug-Resistant *Escherichia coli* ST405 Producing NDM-1 Carbapenemase: Report on the First Italian Cases

Marco Maria D’Andrea, Claudia Venturelli, Tommaso Giani, Fabio Arena, Viola Conte, Paola Bresciani, Fabio Rumpianesi, Annalisa Pantosti, Franco Narni, and Gian Maria Rossolini

J Antimicrob Chemother 2011
doi:10.1093/jac/dkr236
Advance Access publication 8 June 2011

Long-term carriage of NDM-1-producing *Escherichia coli*

Laurent Poirel, Vincent Hervé, Cécile Hombrouch-Alet and Patrice Nordmann

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NDM-1 producers in the Gulf region

- NDM-1-positive *K. pneumoniae* isolate recovered in France from a patient transferred from Iraq (Poirel et al., 2011;55:1821-2)

- NDM-1-positive *K. pneumoniae* isolate recovered in Sultanate of Oman (Poirel et al., JAC 2011;66:304-6)

- NDM-1-positive *K. pneumoniae* isolates recovered from patients hospitalized in Kuwait (Jamal et al., IJAA 2012;39:183-4)
NDM-1 producers in the Gulf region

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All multidrug resistant and producing numerous β-lactamases
OXA-48

- OXA-48 hydrolyzes carbapenems, but spares ceftazidime and cefotaxime.
- Not sensitive to clavulanic acid.
- Responsible for carbapenem resistance in *Klebsiella pneumoniae*, *Citrobacter freundii*, and *Escherichia coli*.
**OXA-48 is an acquired Carbapenem-Hydrolyzing Class D-β-Lactamase (CHDL)**

<table>
<thead>
<tr>
<th>CHDL</th>
<th>First description in</th>
</tr>
</thead>
<tbody>
<tr>
<td>OXA-23</td>
<td><em>A. baumannii</em></td>
</tr>
<tr>
<td>OXA-24/40</td>
<td><em>A. baumannii</em></td>
</tr>
<tr>
<td>OXA-58</td>
<td><em>A. baumannii</em></td>
</tr>
<tr>
<td>OXA-143</td>
<td><em>A. baumannii</em></td>
</tr>
<tr>
<td>OXA-198</td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td>OXA-48</td>
<td><em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td>OXA-181</td>
<td><em>Enterobacteriaceae</em></td>
</tr>
</tbody>
</table>

**CHDLs**
- confer resistance to all penicillins, but not to broad-spectrum cephalosporins
- not inhibited by β-lactamase inhibitors
- confer reduced susceptibility to carbapenems
Klebsiella pneumoniae
Oxacillinase-Mediated Resistance to Imipenem

- SHV-1
- OXA-48
- OXA-47
- SHV-2a (ESBL)
- TEM-1
- Porin mutation

- OXA-48 is distantly related to all other class D β-lactamases

Poirel et al., AAC 2004
Spread of OXA-48-like enzymes

The Netherlands
Belgium
Germany
France
Morocco
Tunisia
Algeria
Egypt
Israel
Lebanon
Turkey
The Netherlands
UK
Spain
France
Morocco
Algeria
Tunisia
Senegal
India (OXA-181)

- Single OXA-48-producing isolates
- Outbreaks of OXA-48-producing isolates
- Nationwide distribution of OXA-48-producing isolates
Cross-border transmission of OXA-48-producing *Enterobacter cloacae* from Morocco to France

Laurent Poirel¹, Alain Ros², Amélie Carrèr¹, Nicolas Fortineau¹, Anne Carriço², Philippe Berthelot² and Patrice Nordmann¹*

Combating the Superbug *Klebsiella Oxa-48* Outbreak in a Dutch Hospital 15 August 2011

Over 80 patients have been infected with at Maasstad hospital, Rotterdam in the Netherlands due to the outbreak of a multiple antibiotic-resistant bacterium named *Klebsiella pneumoniae* Oxa-48. The hospital has estimated that over 2000 people were at risk of being infected.
Outbreak of *Klebsiella pneumoniae* producing OXA-48 in Rotterdam, Netherlands

98 infected patients, 27 deaths
European dissemination of a single OXA-48-producing Klebsiella pneumoniae clone

A. Potron¹, J. Kalpoe², L. Poirel¹ and P. Nordmann¹

1) Service de Bactériologie-Virologie, INSERM U914 «Emerging Resistance to Antibiotics», Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Université Paris Sud, K. Bicêtre Department of Medical Microbiology and Infection Prevention Hospital, Amsterdam, the Netherlands

Outbreak of OXA-48-Positive Carbapenem-Resistant Klebsiella pneumoniae Isolates in France

Gaelle Caution, Joceleyne Oulmass, Remy Gomaret, Thierry Naas, and Patrice Nordmann

Service de Bactériologie-Virologie, INSERM U914 «Emerging Resistance to Antibiotics», Hôpital de Bicêtre, Assistance Publique-Hôpitaux de Paris, 9475 Le Kremlin-Bicêtre, Faculté de Médecine, Université Paris Sud, France; Laboratoire de Microbiologie, Centre Hospitalier International de Villeneuve-Saint-Georges, Villeneuve-Saint-Georges, France; and Service d’Anesthésie-Rénanimation, Centre Hospitalier International de Villeneuve-Saint-Georges, Villeneuve-Saint-Georges, France.

Received 20 October 2012/Returned for modifications 2 January 2013/Accepted 1 February 2013

Seventeen Klebsiella pneumoniae isolates producing the OXA-48 carbapenemase, obtained from 18 hospitalized patients hospitalized from April to June 2012, mostly in the medical intensive care unit of the Villeneuve-Saint-Georges Hospital in a suburb of Paris, France, were analyzed. Seven patients were infected, of whom five were treated at least with a carbapenem, and five patients died. Molecular analysis showed that the isolates belonged to a single clone that harbored a 70-kb plasmid carrying the OXA-48 gene and reproduced CTX-M-15 and TEM-1 ß-lactamase. This is the first reported outbreak of OXA-48-producing K. pneumoniae isolates in France.
Letter to the Editor

Occurrence of the Carbapenem-Hydrolyzing β-Lactamase Gene blaOXA-48 in the Environment in Morocco

Anats Potron
Laurent Posiel
Florence Bussy
Patrice Nordmann*

Service de Bactériologie-Virologie
INSERM U914, Emerging Resistance to Antibiotics
Hôpital de Bicêtre

*Molecular Microbiology Unit, INSERM U914, Emergence of Resistance to Antibiotics, Hôpital de Bicêtre, Assistance Publique Hôpitaux de Paris, 94804, Créteil, France.
OXA-48 producers are often MDR but...

Table 1. MIC range of carbapenems for Enterobacteriaceae that produce several types of carbapenemases*

<table>
<thead>
<tr>
<th>Carbapenemase</th>
<th>Imipenem (mg/L)</th>
<th>Meropenem (mg/L)</th>
<th>Ertapenem (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC</td>
<td>0.5--64</td>
<td>1--64</td>
<td>0.5--64</td>
</tr>
<tr>
<td>Metallo β-lactamases†</td>
<td>0.5--64</td>
<td>0.25--64</td>
<td>0.5--64</td>
</tr>
<tr>
<td>OXA-48 type</td>
<td>1--64</td>
<td>0.5--64</td>
<td>0.25--64</td>
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Global Spread of Carbapenemase-producing Enterobacteriaceae

Patrice Nordmann, Thierry Naas, and Laurent Poirole

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 17, No. 10, October 2011
- **OXA-48-positive *K. pneumoniae* isolate recovered in France from a patient transferred from Kuwait** (Poirel et al., submitted for publication)

- **OXA-181-positive *K. pneumoniae* isolate recovered in Sultanate of Oman** (Potron et al., AAC 2011;55:4896-9)
Pseudomonas aeruginosa and Acinetobacter baumannii
The carbapenemases in *Acinetobacter baumannii*

<table>
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<tr>
<th>Enzyme Class</th>
<th>Penicillins</th>
<th>Cephalosporins 1st et 2nd generation</th>
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</tr>
<tr>
<td>D</td>
<td>Oxacillinase = OXA-23, OXA-40, OXA-58, OXA-143</td>
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* Cephamycins excluded for most class A
Acquisition of carbapenemases may lead to ...

A. baumannii
The main problem: the oxacillinases with carbapenemase properties
### Carbapenem-hydrolyzing class D β-lactamases

<table>
<thead>
<tr>
<th>Class</th>
<th>Penicillins</th>
<th>Cephalosporins 1st and 2nd generation</th>
<th>Expanded-spectrum cephalosporins</th>
<th>β-lactams/Inhibitors of β-lactamases</th>
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<tr>
<td>D</td>
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</table>
Inactivation of the $bla_{OXA-40}$ gene

$A.\ baumannii\ OXA-40(\pm)$

$A.\ baumannii\ \Delta OXA-40$
The main problem however corresponds to the acquisition of OXA-type carbapenemases.
And the bad news...

- NDM-1 identified in *A. baumannii* in India (Karthikeyan et al., J Antimicrob Chemother 2010)

- Those isolates co-produced the OXA-23 carbapenemase and the ArmA 16S RNA methylsase conferring high level resistance to ALL aminoglycosides
NDM-2-producing *A. baumannii* in Germany

- *A. baumannii* recovered in 2010
- Transfer from a hospital in Cairo, Egypt
The same transposon at the origin of $bla_{NDM-1/-2}$ acquisition in *A. baumannii*

Tn125-Related Acquisition of $bla_{NDM}$-Like Genes in *Acinetobacter baumannii*

Laurent Poirel, Rémy A. Bonnin, Anne Boulanger, Jacques Schrenzel, Martin Kaase, and Patrice Nordmann

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Carbapenemase producers in the Gulf region

- NDM-2-positive *A. baumannii* isolate recovered in the United Arab Emirates
  (Ghazawi, Sonnevend, Bonnin, Poirel, Nordmann, Hashmey, Rizvi, Hamadeh & Pal, CMI 2012;18:E34-6)

- OXA-23, OXA-58, and OXA-72-positive *A. baumannii* isolates in the Kingdom of Bahrein
  (Mugnier, Bindayna, Poirel & Nordmann, JAC 2009;63:1071-3)

- OXA-23-positive *A. baumannii* isolates in United Arab Emirates
  (Mugnier, Poirel, Pitout & Nordmann, CMI 2008;14:879-82)
# The carbapenemases in *Pseudomonas aeruginosa*

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<tr>
<td><strong>D</strong></td>
<td>Oxacillinase = OXA-198</td>
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</tr>
</tbody>
</table>

* Cephamycins excluded for most class As

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**Notes:**
- Penicillins and cephalosporins are divided into generations based on their antimicrobial activity.
- Carbapenems are considered the most effective ß-lactams.
- Metallo-enzymes are a distinct class of ß-lactamases that act on a wide range of ß-lactams.
- Oxacillinases are enzymes that hydrolyze ß-lactams.
Metallo-β-lactamases: carbapenemases

- Confer resistance to all β-lactams, except aztreonam
- Genetic support: plasmids, transposons, integrons...
- Numerous co-associated resistances, especially aminoglycosides
And also.... KPC in *P. aeruginosa*......

Colombia, but also Trinidad and Tobago, Puerto Rico, and USA
Warning !!!

ESBL can possess some carbapenemase activity

GES-1/GES-2: one mutation

« classical ESBL »

« carbapenem-hydrolyzing ESBL »
Take-home message: some ESBLs may compromise the carbapenem activity

A consequence of the antibiotic selective pressure

Wild-type *P. aeruginosa*

*P. aeruginosa* expressing GES-2
Carbapenemases in *P. aeruginosa*

- Metallo-β-lactamases of VIM-types: Europe, Canada, Korea
- Metallo-β-lactamases of IMP-types: Japan, Turkey
- Metallo-β-lactamase SPM: Brazil
- Metallo-β-lactamase GIM: Germany

- Class A carbapenemase KPC: North and Central America, Columbia
- Class A of the GES-type (ESBL derivatives): South Africa, Brazil
- A new class D: OXA-198 in Belgium
Location of Mobile MBL-Containing Organisms

- IMP metallo-\(\beta\)-lactamase
- VIM metallo-\(\beta\)-lactamase
- SPM metallo-\(\beta\)-lactamase
- GIM metallo-\(\beta\)-lactamase
- NDM-1
- SIM-1
Conclusion (1)

- Increased prevalence of multidrug-resistant isolates in nosocomial isolates of developing/developed countries

- Extended development of resistance in the community

Why?

- Selective effect of old and novel antibiotics in the community
- Human-to-human transmission: migrations of populations
- Lack of detection of antibiotic resistance; efforts of surveillance urgently required
Conclusion (2)

- **Main threats:**
  - KPC, NDM and OXA-48 in *K. pneumoniae*
  - NDM and OXA-48 in *E. coli*
  - OXA-23, OXA-58, and NDM in *A. baumannii*
  - VIM in *P. aeruginosa*