Resistance to beta-lactams in Enterobacteriaceae: mechanisms and detection

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Classification

Extended-Spectrum \( \beta \)-Lactamases (ESBLs)

Class A
- SHV
- TEM
- CTX-M, Others

Class B
- OXA
- IMP
- VIM
- NDM-1

Class C
- AmpC
- OXY
- FOX
- MOX

mutations

Extended-Spectrum \( \beta \)-Lactamases (ESBLs)

Metallo-\( \beta \)-Lactamases (MBLs)

AmpC \( \beta \)-Lactamases

Evolution of extended-spectrum \( \beta \)-lactamases (ESBLs)

First ESBL

ESBLs

Activity vs oxyimino ceps (3rd Gen Ceph)

TEM-1
1964

Gln39→Lys

TEM-2
1970

Gln39→Lys

TEM-3
1987

Glu104→Lys

Gly238→Ser

What is an ESBL?

Serine \( \beta \)-lactamases

Group 1
AmpC

TEM/SHV

Group 2
OXA

Group 2d

CTX-M, Others

Metallo-\( \beta \)-lactamases

Group 3

IMP, VIM, GIM, SPM, SIM

ESBLs

Plasmid encoded

R to all \( \beta \)-lactams, except temocillin, (cephamycins), carbapenems inhibited by clavulanic acid

Epidemiology of ESBLs

Pre –2000
Mostly Klebsiella spp. with TEM/SHV
Nosocomial, often ICU / specialist units
1998: c. 25% of Klebs from European ICUs ESBL+
Few epidemic strains
- e.g. K. pneumoniaeK25 SHV-4 in France & Belgium
Occasional local outbreaks in Belgian hospitals
(Brussels, Gent, Leuven)
c. 5-15% of Klebsiella spp.; 40-60% E. aerogenes;
<2% E. coli

\( \beta \)-lactamases (>600)
> 50% ESBLs

Plasmid encoded

1983 SHV-type (>130)
1985 TEM-type (>180)
1988 CTX-M-type (>100)

“The oldies”

“ESBLs of growing importance”

“infrequent ESBLs”

“peculiar ESBLs”

1991 TLA-1 TLHunasca (Indian tribe)
1991 PER (7) Pseudomonas Extended-spectrum \( \beta \)-lactamase
1996 VEB (7) Vietnam Extended-spectrum \( \beta \)-lactamase
1996 BES-1 Brazilian Extended-spectrum \( \beta \)-lactamase
1998 GES (16) Guyana Extended-spectrum \( \beta \)-lactamase
2005 BEL (2) Belgium Extended-spectrum \( \beta \)-Lactamase
2005 TLA-2 ??? (Plasmid, waste water)
1998 KPC (10) K. pneumoniae Carbapenemase
1991 OXA-ESBL (18) (OXA-2, -10, -13 derivatives, OXA-18, OXA-48)
**CTX-M β-lactamases**

- >100 types, 5 clusters: evolved via escape of chromosomal β-lactamase genes from *Klebsiella* spp.
- CTX-M-1 group
  - Cefotaximases rather than cefazidimases
  - Predominant ESBLs in Argentina since 1990
  - Disseminating worldwide since 2000’s

**ESBLs in the 21st Century**

- Major shift from TEM/SHV to CTX-M
- Often in *E. coli* (variable degree of clonality)
- Often in community patients with no little HC contact
- Deaths: high attributable mortality
- CTX-M Producers often multi-resistant (quinolones, cotrimox, AGs);
  - > major driver of carbapenem use

**Epidemiology of CTX-M β-Lactamases**

- The changing epidemiology of resistance
- *The Journal of Antimicrobial chemotherapy (2009) 64 Suppl 1 T3–118*

**ESBL transmission**

- *bla* genes mobilized from chromosomes to plasmids:
  - transposases, insertion sequences, integrases involved
  - *blaCTX-M* mobilizes 10x more frequently than *blaSHV* & *blaTEM*

- Horizontal & vertical transmission of plasmids:
  - Horizontal transmission: plasmid-mediated conjugation
  - Vertical transmission: clonal transmission by normal cell division

**Diversity of Mobiles Genetic Elements (MGE) carrying ESBL genes**

- Tn3
- TEM
- SHV
- Insertion sequence
- Specific IS (IS6100), Complex integrons (ISCR1)
- Phage related elements

**ESBL transmission routes**

- Household members & pets
- Animals
- Travel
- Food
Classification of β-Lactamases

Extended-Spectrum β-Lactamases (ESBLs)

- OXA Class D
- Class A
- SHV
- TEM
- IMP
- VIM
- NDM-1
- mutations

Carbapenemases

Rising threat of multi-drug antibiotic resistance
Back to the pre-antibiotic era

PLASMID-ENCODED CLASS A KPC ENZYMES: **KLEBSIELLA PNEUMONIAE CARBAPENEMASE**

Located on large plasmids;
- conjugative and nonconjugative

*bla*KPC is usually identified within a Tn3-type transposon (Tn4001).

*bla*KPC reported on plasmids with:
- Normal spectrum β-lactamases
- Extended spectrum β-lactamases
- Aminoglycoside resistance

**GENETIC APPARATUS OF TRANSFERABLE MBLs**

Plasmids, integrons and transposons

**Why are carbas in the top chart of β-lactamases?**

- Case patients were more likely than control patients to:
  - die during hospitalization (48% vs 20%; P < .001)
  - die from infection (38% vs 12%; P < .001)
- The timely administration of antibiotics with in vitro activity against CrKP was not associated with patient survival.
KPC: Klebsiella Pneumoniae Carbapenemase

VIM: Verona Integron–encoded Metallo-β-lactamase

NDM: New-Delhi Metallo-β-lactamase

OXA-48: OXacillinase

Evolution of the distribution of resistance mechanisms of carbapenemase-producing Enterobacteriaceae isolates, National Reference Centre, Belgium, January 2007–October 2011 (n=80)

Geographical origin (by country of cross-border transfer) and type of carbapenemase-encoding genes detected in patients carrying carbapenemase-producing Enterobacteriaceae, Belgium, January 2007–October 2011 (n=73)
Types of CPE isolates recovered in different hospitals (n=80)

Heterogeneous expression of MBLs in Enterobacteriaceae

Heterogeneous expression of KPC carbapenemase in Enterobacteriaceae

OXA-48 carbapenemase (Class D)

Guidelines for phenotypic screening and confirmation of carbapenemases in Enterobacteriaceae
**K. pneumoniae KPC-positive (class A carbapenemase)**

**DDST for detection of MBLs**

**Detection of asymptomatic carriage of carbapenemase in the gut flora**

- From rectal swabs (stools)
- Chromogenic selective culture media
  - Chrom ID BLSE (cefpirome)
  - CHROMagar KPC (Carbapenem)
  - Brilliance CRE (Oxoid) (Carbapenem)
  - ChromID CARBA (bioMérieux) (Carbapenem)
  - MAC+ IMI (1 mg/L)
  - MAC + 15-µg disks (ERTA/MERO/IMP)
- Limit of sensitivity of detection ($10^2$ to $10^3$)
- Enrichment broth culture not superior to direct plating

**Molecular confirmatory tests**

- **Gold standard: molecular biology +++**
  - Multiplex PCR; IMP, VIM, KPC, OXA-48, NDM...
  - Molecular confirmatory tests (Naas et al. AAC, 2010; JCM, 2011)
  - Spectrophotometric assays for Carbapenem-hydrolysis +++

**Rapid Molecular detection tools**

- 8-lactamas (>400) > 50% ESBLs
  - SHV-type (> 150), TEM-type (> 190), CTX-M-type (> 100)
  - TLA-1, PER (7), VEB (7), BES-1, GES (17), BEL (3), TLA-2, KPC (10), OXA-ESBL
- PCR and sequencing
  - The gold standard
  - Can detect all variants
  - Easy to perform but labor intensive and costly
- **PCR**
  - Multiplex-PCR, Real-time PCR (Syber green, Taqman, Hybridization probes), Real time PCR coupled with pyrosequencing, PCR ESI (Maldi)...
  - Only sequenced genes can be found
  - Only detection of DNA (not expressed genes)
  - Requires isolation of the strain for identification and susceptibility testing
  - Most of the presently used assays are confirmative tests (on cultures)
DNA extraction: 1 h  
Amplification: 3 h  
Detection: 2 h  
Results on line

Hands on time:
- 3 samples: 1 h
- 15 samples: 1 ½ h
- 72 samples: 2 ½ h
72 samples/day, cost effective run: minimum 3 samples

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**Case: 2 Patients in western Europe**

*K. pneumoniae* from wound infection  
(NDM-1; SHV-12; CTX-M-1 like)

*E. coli* from faeces  
(NDM-1; CMY-2 like; CTX-M-1 like)

Combination of resistance mechanisms detected in single experiment  
Distinction between TEM/SHV ESBLs or non ESBLs

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**Evaluate novel molecular assays**

Molecular test based on micro-array (results in < 7 h)

**Identification of ESBLs**
- of TEM and SHV type
- non-ESBL TEM and SHV type
- CTX-M type

**Identification of KPC**
- and KPC, OXA-48, NDM, VIM, IMP
- and KPC, NDM, CMY-1, CMY-2, ACT, ACC, DHA, FOX
- and KPC, OX48, NDM, VIM, IMP, CMY-1 /2, ACT, ACC, DHA, FOX

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**Result ESBL - KPC Array**

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**Multiplex end-point PCR assays**

Genotypic detection of carbapenemase-encoding genes and other important β-lactamases (ESBLs, AmpC, OXA...)

Multiple protocols (3-7 sets, 11-25 target genes)

Design of specific group of primers
- Definition of different reaction mixtures
- Optimization of PCR conditions (one single amplification protocol)
- Distinct amplicon sizes for the different genes

Rapid (<4 h), reliable (specific)

Screening of clinical isolates, epidemiological surveys

Customizable to local needs (endemic/non endemic settings)

Lower costs (Vs multiple PCR assays)
**Rapid (<4 h), reliable (specific)
Screening of clinical isolates, epidemiological surveys**
Customizable to local needs (endemic/non endemic settings)

**Lower costs (Vs multiple PCR assays)**

**Real-time Multiplex PCR**
Design of specific primers and fluorescent probes (TaqMan)
Rapid (<2 h), one amplification reaction in single tube
Less prone to contamination (closed system, no tube opening)
Different ways for identification of amplicons
- Real-time probe-based PCR
- Capillary electrophoresis of amplicons (QIAxcel –QIAGEN)
- DNA sequencing of amplicons (confirmation, identification of specific alleles – e.g. GES 170 Gly-Ser with carbapenemase activity)
Would allow more rapid guidance of antibiotic treatment and infection control measures

**NDM-1-qPCR**
A: Serially diluted Kp NDM-1 in 10⁸ E. coli WT
B: Serially diluted Kp NDM-1 in stool

**Real-Time PCR for Detection of NDM-1-carbapenemase genes from Stools**

<table>
<thead>
<tr>
<th>No</th>
<th>Bacteria</th>
<th>MIC (µg/ml)</th>
<th>Lowest limit of detection in water (E. coli)</th>
<th>Lowest limit of detection in stool (E. coli)</th>
<th>Lowest detection limit of NDM-1-qPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>E. cloacae</td>
<td>8</td>
<td>2 x 10⁴</td>
<td>2 x 10⁵</td>
<td>2 x 10⁶</td>
</tr>
<tr>
<td>25</td>
<td>E. coli</td>
<td>8</td>
<td>2 x 10⁴</td>
<td>2 x 10⁵</td>
<td>2 x 10⁶</td>
</tr>
</tbody>
</table>

**Real-Time PCR for Detection of NDM-1-carbapenemase genes from Stools**

Highly specific and sensitive qPCR (limit of detection +/-10⁴ bacteria / 100 mg of stool)
=> use of enrichment culture (increased detection?)
Useful tool for outbreak management (rapid cohorting of patients, infection control measure evaluation, …)
At least as sensitive as culture on stool samples, but 4h turn around time vs 48h-72h for culture
Needs to be evaluated on rectal swabs &/in outbreak situation
Extraction can be automated (less hands on time)
**Multiplex PCR-ELISA**

1. DNA extraction
2. Multiplex PCR
3. Reverse hybridization of PCR products to specific capture oligonucleotide probes
4. Detection of hybridization complex by peroxidase conjugated antibody

Results in less than 2 hours!

**Evaluation of hyplex® MBL ID Multiplex PCR-ELISA**

(AmpLEXDiagnostics)

Identification of MBL (VIM/IMP) genes directly from clinical specimens

<table>
<thead>
<tr>
<th>Clinical specimens</th>
<th>MBL (producers (+))</th>
<th>True Positive (TP)</th>
<th>True Negative (TN)</th>
<th>False Positive (FP)</th>
<th>False Negative (FN)</th>
<th>Sens (% spec)</th>
<th>Spec (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (n=90)</td>
<td>P. aeruginosa (8)</td>
<td>74</td>
<td>16</td>
<td>71</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>other specimens (n=236)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine (60)</td>
<td>P. aeruginosa (2)</td>
<td>46</td>
<td>1</td>
<td>45</td>
<td>0</td>
<td>0</td>
<td>98</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>Bronchial/sputum (n=85)</td>
<td>P. aeruginosa (26)</td>
<td>58</td>
<td>7</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td>98</td>
<td>98</td>
<td>98</td>
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<tr>
<td>P.aeruginosa (2)</td>
<td>62</td>
<td>3</td>
<td>59</td>
<td>0</td>
<td>0</td>
<td>98</td>
<td>98</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>K. pneumoniae (6)</td>
<td>34</td>
<td>2</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>98</td>
<td>98</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>P. stuartii (2)</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>P. stuartii (2)</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Total (n=326)</td>
<td></td>
<td>77</td>
<td>73</td>
<td>300</td>
<td>6</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Conclusions**

The epidemiology of carbapenemase (sources of acquisition, risk factors, real prevalence/incidence) is poorly known and most probably largely underestimated because of difficulties in their detection. It is obvious that carbapenemase-producing organisms are distributed worldwide and their prevalence is increasing.

"This rapid spread of carbapenemases will be a major healthcare issue for the next decades - The "fight" against MDR carbapenemase-producing bugs, will require:
- Fast detection and identification of the reservoirs
- Reduced (appropriate) consumption of antimicrobials
- Strict adherence to standard hygiene practice

**Acknowledgments**

CHU Mont Godinne (UCL)
National Reference Laboratory
- Daniel Huang
- Alexia Vermanen
- Pierre Bogaerts
- Caroline Baunang
- Catherine Berfyn
- Roberta Rosende de Castro
- Warda Bouchourouf
- Amélie Guisset
- Farid El Garch

Hôpital ULB-Erasme
Associated Ref. laboratory
- Ariane Deplano
- Sandrine Rosin
- Claire Nonhoff
- Ricardo De Mendonca
- Marie Hallin
- Olivier Denis

Institute of Public Health
- Bea Jans
- Boudewijn Catry
- Sophie Quoilin