Genetics structures at the origin of acquisition of the carbapenemase genes

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Variety of carbapenemase genes

- **Class A;** KPC, GES-like
- **Class B;** IMP, VIM, NDM, GIM
- **Class D;** OXA-23, OXA-40, OXA-58, OXA-48, OXA-181
Variety of carbapenemase genes

- **Class A**: KPC, GES-like
  mostly *Enterobacteriaceae*, but also *Pseudomonas*

- **Class B**: IMP, VIM, NDM
  *Enterobacteriaceae*, *Pseudomonas*, and *Acinetobacter*

- **Class D**: OXA-23, OXA-40, OXA-58
  *Acinetobacter*
  OXA-48, OXA-181
  *Enterobacteriaceae*
Variety of genetic elements involved in mobility of β-lactamase genes

- **Transposons**
  - Class II transposons (Tn3-type)
  - Composite transposons
  - Conjugative transposons

- **Integrons**
  - Class 1 integrons

- **Insertion sequences**
Tn3-type transposons

- 2 extremities corresponding to inverted repeat sequences (IR)
- 1 transposase (TnpA) \(\Rightarrow\) recognizes IR extremities in the transposition process and cuts target DNA
- 1 resolvase (TnpR) : negative regulator of TnpA expression and recombinase mediating the resolution of the transposon integration process
Insertion sequences (IS)

- IRL
- IRR
- mARN
- Transposase

ATGCA
TACGT

Target site

ATGCA
TACGT

gene
The $bla_{KPC}$-borne transposon
Genesis of Tn4401

A

IRL  tnpR  tnpA  IRR1

KPC-2  IRR

B

IRL  tnpR  tnpA  KPC-2  IRR

ISKpn7  IRR

ISKpn6

IRR1

ATTGA

C

ATTGA  tnpR  tnpA  CCG  CCG  TA  TA  ATTGA

IRL  ISKpn7  KPC-2  ISKpn6  IRR
- **P. aeruginosa from Colombia**
  - GCGCT
  - ATTAC
  - TTGGT
  - ATTGA

- **K. pneumoniae from Colombia**
  - GCGCT
  - ATTAC
  - TTGGT
  - ATTGA

- **K. pneumoniae from Greece**
  - GCGCT
  - ATTAC
  - TTGGT
  - ATTGA

- **K. pneumoniae from NYC**
  - GCGCT
  - ATTAC
  - TTGGT
  - ATTGA
The class 1 integrons

- **Consequences**: co-resistance; co-expression; co-selection
Structure of a gene cassette

Gene cassette

---GTATAAGC---

ATG

RBS

---GCTTAAC---/---GTTRRRY---

TAA

59-be
Acquisition of resistance genes as a form of gene cassettes

- **β-lactams** => \( bla_{\text{VEB}}, \ bla_{\text{GES}}, \ bla_{\text{OXA}}, \ bla_{\text{PSE}} \ldots \)
- **Aminoglycosides** => many genes \( aac(6'), \ aadA \ldots \)
- **Chloramphenicol** => \( cmlA \)
- **Sulfonamides** => \( sul \)
- **Trimethoprim** => \( dfr \)
- **Rifampicin** => \( arr \)
Integrons are carried by transposons

The integron is not self-mobile, it is mobilized by the transposon Tn21.
bla\textsubscript{GES-like} integron structures
Integron-borne $bla_{VIM-1}$

The carbapenemase gene is usually identified in association with aminoglycosides resistance genes, and eventually with additional $\beta$-lactamase genes of narrow spectrum.

Similar features for the widespread $bla_{IMP}$ genes
Insertion sequences (IS)
Genesis of composite transposons (I)
Genesis of composite transposons (II)
Genesis of composite transposons (III)

- Composite transposon = two IS bracketing the transposed fragment

Resistance gene
Genesis of composite transposons (IV)

- Composite transposon = two IS bracketing the transposed fragment
The $\text{bla}_{\text{OXA-48}}$ gene is part of a composite transposon

- The $\text{bla}_{\text{OXA-48}}$ gene, firstly in Klebsiella pneumoniae, is widespread in Turkey

- OXA-48 hydrolyzes carbapenems, but spares ceftazidime and cefotaxime

- Responsible for carbapenem resistance in Klebsiella pneumoniae, Citrobacter freundii, and Escherichia coli
The $\text{bla}_{\text{OXA-48}}$ gene is part of a composite transposon.
$bla_{OXA-48}$ gene expression can be modulated

Prom1

Prom2

ΔIS1999

IS1R

AAGAATGTT

AAGAATGTT

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A Gram negative species isolated from lake sediments
The bla$_{OXA-23}$ gene is associated to ISAbal elements
*bla*$_{OXA-23}$ has been captured from the *A. radioresistens* chromosome
One single IS element may mobilize the resistance genes

ISEcp1
• Belongs to the IS1380 family

• Possesses two imperfect 18-bp inverted repeats

• One transposase of 420 amino acid long, sharing 41% with that of IS1380

• Generates 5-bp duplication of the target site upon transposition
ISEcp1 recognizes variable IRR sequences during its transposition process.
ISEcp1 and other β-lactamase genes

Involved in the acquisition of plasmid-borne CTX-M and AmpC-type β-lactamase genes

\[ \text{bla}_{\text{CTX-M}} \] (originating from Kluyvera spp.)

\[ \text{bla}_{\text{CMY}} \] (originating from Citrobacter freundii)
\[ \text{bla}_{\text{DHA}} \] (originating from Morganella morganii)
\[ \text{bla}_{\text{ACC}} \] (originating from Hafnia alvei)
Different mobilization events for closely-related $bla_{CTX-M}$ genes
Role of $ISEcp1$ in expression of β-lactamase genes

A so-called «strong» promoter
Genetic environment of $\text{bla}_{\text{OXA-181}}$

IRL

\[\text{CCTAGATTCTACGT}\]

IRR1

\[\text{ACGTGGAATTTAGG}\]

IRR2

\[\text{ACATGAAATCCTCCG}\]

$\text{ISEcp1}$

\[\text{ATATA} \quad \text{ATATA}\]

$\Delta\text{lysR}$

$\Delta\text{ere}$

Tn2013
Genetic environment of \( \text{bla}_{\text{OXA-181}} \) compared to that of \( \text{bla}_{\text{OXA-48}} \)

\[
\text{Tn2013} \\
\text{Kp} \ 481814 \\
\text{ISEcp1} \\
\text{Kp} \ 11978 \\
\text{IS1999} \\
\text{blaoxa-181} \\
\Delta \text{lysR} \\
\Delta \text{ere} \\
\text{Tn1999} \\
\text{blaoxa-48} \\
\text{lysR} \\
\text{IS1999} \\
\]

94% identité
The transposition of Tn2013 was demonstrated *in-vitro*.

*Potron et al., AAC 2011*
Plasmid pKP3-A carrying bla\textsubscript{OXA-181}

A ColE-type mobilizable and broad-host range plasmid

7,605 bp
The $\text{bla}_{\text{OXA-181}}$ originates from *Shewanella xiamenensis*.

- Hyp. protein *Shewanella sp.* 92%
- Desoxyribonuclease *Shewanella sp.* 96%
- lysR *Shewanella sp.* 99%
- Carbamoyl-phosphate synthase *Shewanella sp.* 99%

Kp 481814

100% identity

### Diagram:

- **bla$_{\text{OXA-181}}$**
- **Hyp. protein** *Shewanella sp.* 96%
- **Desoxyribonuclease** *Shewanella sp.* 96%
- **lysR** *Shewanella sp.* 99%
- **Carbamoyl-phosphate synthase** *Shewanella sp.* 99%
- **Δere**
- **ΔlysR**
And what about the famous $bla_{NDM-1}$ gene?
The $\text{bla}_{\text{NDM-1}}$ gene is part of composite transposon Tn125 in *A. baumannii*

- $\text{ISA}ba125$ belongs to the IS30 family, and encodes a 322 amino-acid long transposase

Poirel et al., AAC in press
ISAba125 provides the -35 box constituting the $\text{bla}_{\text{NDM-1}}$ promoter.
Analysis of a $\text{bla}_{\text{NDM-1}}$-carrying plasmid in an $\text{E. coli}$ isolate

A plasmid possessing an IncN-type scaffold, but with a new replicase

Poirel et al., AAC 2011
Close genetic environment of \( \text{bla}_{\text{NDM-1}} \)

- Two novel insertion sequences are bracketing the \( \text{bla}_{\text{NDM-1}} \) gene
- \( \text{ISEc33} \) has interrupted insertion sequence \( \text{ISAba125} \)
- No target site duplication, two different IS \( \Rightarrow \) likely not a composite transposon
**ISAb125** provides part of the promoter sequences for \(bla_{NDM-1}\) expression.
A bit further...

△ISAb125  IRR  ISEc33  IRL  IRL  bla  NDM-1  ble  IRR  ISSen4  IRL  tnpR  tnpA

CAGGGGTC

△Tn501

⇒ A Tn501-made transposon


**bla<sub>NDM-1</sub> gene environment among different Enterobacteriaceae**

A. 

B. 

C. 

D. 

E.
The same promoter is always present

A. bla<sub>NDM-1</sub> IR L IS

B. bla<sub>NDM-1</sub> IRR ISSen4 IR L

C. bla<sub>NDM-1</sub> IR L IS

D. efflux pump ∆ldh ∆bla<sub>DHA-1</sub> ∆PAI

Prom
Conclusions

- A diversity of genetic tools for carbapenemase genes acquisition and dissemination is identified.
- Genetic plasticity at the origin of certain carbapenemase gene diffusion => increasing spread.
- Searching for the reservoirs:
  - Reservoir of the resistance genes themselves?
  - Reservoir of the mobilizing elements?
  - Which are the factors enhancing the mobilization processes?