



Theme 5 – Surveillance – 5 June 2024

Plasmid outbreak

Case studies


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Intended Learning Objectives (ILOs)

 **Bioinformatician:** develop expertise in plasmid identification, characterization, and typing



 **Microbiologist:** apply clinical knowledge to study medically relevant plasmids biology and impact



 **Epidemiologist:** analyze spread and prevalence of plasmid-borne genes



Outline



This session will consist of three applied case studies characterized by varying levels of difficulty (i.e., easy, intermediate and difficult)

This session encourages networking and collaboration within “country teams”.

Within each team every participant (bioinformatician, epidemiologist and microbiologist) will bring his experience in solving the case



Outline

This session will consist of three applied case studies

In addition to ILOs, this session encourages networking and collaboration within “country teams”.

Furthermore, if you don't understand something, ask for help!

Outline

 Case study 1 (easy)

 Case study 2 (intermediate)

 Case study 3 (difficult)

Case study 1

Framework

The institution you work at receives a notice

During screening analyses intended to identify 3rd generation cephalosporin and beta-lactam/beta-lactamase inhibitor resistant microorganisms in a pig factory, two carbapenem resistant isolates were identified from the same fecal sample

What do you do?



Case study 1

Identification, antimicrobial susceptibility testing and PCR screening

Antimicrobial	MIC	Interpretation
Amikacin	>8	R
Amoxicillin/Clavulanic Acid	>8	R
Ampicillin	>8	R
Aztreonam	>4	R
Cefepime	>8	R
Cefotaxime	>2	R
Ceftazidime	>4	R
Ceftazidime/avibactam	>8	R
Ceftolozane/tazobactam	>4	R
Ciprofloxacin	>1	R
Colistin	<=2	S
Ertapenem	>2	R
Gentamicin	>4	R
Imipenem	>4	R
Levofloxacin	>1	R
Meropenem	>8	R
Piperacillin	>16	R
Piperacillina/tazobactam	>16	R
Tigecyclin	<= 0.5	S
Trimetoprim/sulfamethoxazole	>4/76	R

<i>bla</i> _{KPC}	-
<i>bla</i> _{NDM}	-
<i>bla</i> _{VIM}	+
<i>bla</i> _{IMP}	-
<i>bla</i> _{OXA-48}	-

Antimicrobial	MIC	Interpretation
Amikacin	>8	R
Amoxicillin/Clavulanic Acid	>8	R
Ampicillin	>8	R
Aztreonam	>4	R
Cefepime	>8	R
Cefotaxime	>2	R
Ceftazidime	>4	R
Ceftazidime/avibactam	>8	R
Ceftolozane/tazobactam	>4	R
Ciprofloxacin	>1	R
Colistin	>8	R
Ertapenem	>2	R
Gentamicin	>4	R
Imipenem	>4	R
Levofloxacin	>1	R
Meropenem	>8	R
Piperacillin	>16	R
Piperacillina/tazobactam	>16	R
Tigecyclin	2	S
Trimetoprim/sulfamethoxazole	>4/76	R

Strain 1

Citrobacter koseri

Strain 2

Morganella morganii



Case study 1

Short-reads only assembly

Strain 1 (*Citrobacter koseri*)

MLST: ST1175

Acquired resistance genes: *aph(6)-Id*,
aph(3'')-Ib, *aph(3')-XV*, *aadA1*, *aac(6')-Ib3*,
bla_{SHV-12}, *bla_{VIM-1}*, *catB2*, *qnrS1*, *sul1*, *sul2*,
dfrA14

Replicon content: IncA

<https://pubmlst.org/organisms/plasmid-mlst>

IncA pMLST: ST12

Strain 2 (*Morganella morganii*)

MLST: Not developed

O-AGC (O-antigen synthesis cluster): Type 2

Acquired resistance genes: *aph(6)-Id*,
aph(3'')-Ib, *aph(3')-XV*, *aadA1*, *aac(6')-Ib3*,
bla_{SHV-12}, *bla_{VIM-1}*, *catB2*, *qnrS1*, *sul1*, *sul2*,
dfrA14

Replicon content: IncA

Case study 1

Long-reads first hybrid assembly

Strain 1 (*Citrobacter koseri*)

Number of contigs: 2

Chromosome length: \approx 4,9 Mbp

IncA plasmid length: 158,944 bp

Strain 2 (*Morganella morganii*)

Number of contigs: 2

Chromosome length: \approx 4,2 Mbp

IncA plasmid length: 158,944 bp

[IncA comparison](#)

Case study 1

Visually compare and analyze plasmids (Clinker)

Command line approach

Install clinker via pip or conda

Annotate the single plasmid .fasta files using prokka/bakta

```
clinker *.gbk -p output.html
```

GUI approach (CAGECAT webserver)

Upload all plasmid .gbk files to be analyzed on the CAGECAT webserver (input files)

Choose parameters

Launch clinker and explore the results



Case study 1

Geographical distribution of IncA plasmids

PLSDB <https://ccb-microbe.cs.uni-saarland.de/plsdb>



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51 IncA plasmids from:

 *Enterobacterales* (47/51)

 *Aeromonadales* (4/51)

(both Gammaproteobacteria)

→ Broad host range plasmid ←

Global spread

Case study 1

Geographical distribution of *bla*_{VIM} carrying plasmids

PLSDB <https://ccb-microbe.cs.uni-saarland.de/plsdb>

295 *bla*_{VIM} carrying plasmids

Global spread

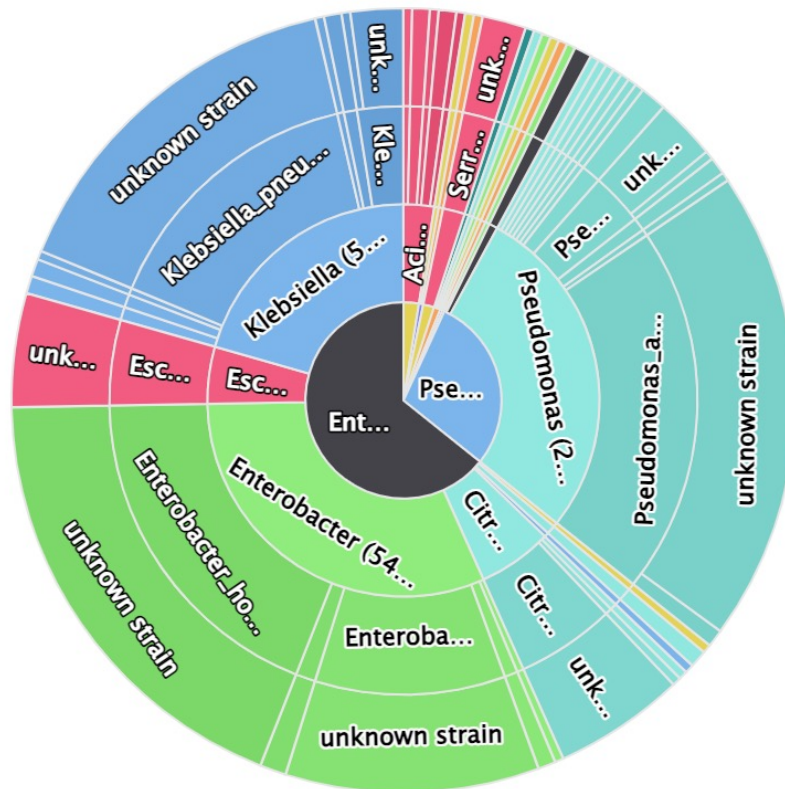


Highcharts.com © Natural Earth

Case study 1

Species distribution of *bla*_{VIM} carrying plasmids

PLSDB <https://ccb-microbe.cs.uni-saarland.de/plsdb>



295 *bla*_{VIM} carrying plasmids

> 20 hosting species, mostly:

 *Enterobacteriales* (187)

 *Pseudomonadales* (88)

Case study 1

Global epidemiology of IncA plasmids *bla*_{VIM-1}

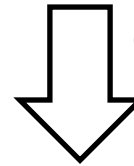
PLSDB <https://ccb-microbe.cs.uni-saarland.de/plsdb>

		<i>bla</i> _{VIM-1}		Row total
		+	-	
IncA	+	25	26	51
	-	272	36438	36710
Column total		297	36464	36761

Case study 1

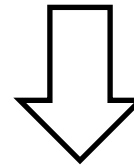
Proposal for a swift "damage containing" surveillance protocol

Phenotypic screening



Carbapenem resistant isolates

PCR identification for
carbapenemase genes



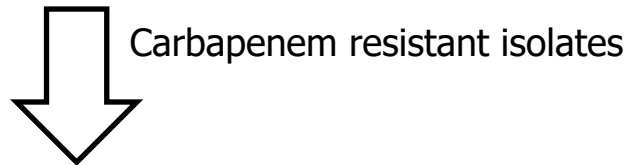
*bla*_{VIM} positive isolates

PCR identification for
IncA replication gene

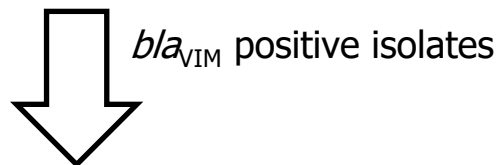
Case study 1

Proposal for a swift "damage containing" surveillance protocol

Phenotypic screening



PCR identification for
carbapenemase genes



PCR identification for
IncA replication gene

repA-F AAGAGAACCAAAGACAAAGAC
repA-R GCTGCTTACGCTTGTTGGA
repA amplicon 982 bp



<https://pubmlst.org/organisms/plasmid-mlst>



Case study 1

Conclusions

Both isolates (*C. koseri* and *M. morganii*) carried identical IncA plasmid belonging to IncA/C ST12 and harbored the *bla*_{VIM} metallo-beta lactamase gene.

The detection of the same plasmid in two different species from the same sample suggests high conjugation capabilities.

Global epidemiological data indicates that the IncA plasmid has a broad host range, posing potential future challenges in controlling the spread of the associated antibiotic resistance determinants.

Case study 2

Framework

The institution you work at receives a notice

A case of deadly enteritis in a pre-weaning piglet has been identified in a local swine farm. Preliminary testing results report the presence of multidrug-resistant *Escherichia coli*

What do you do?

Case study 2

Antimicrobial susceptibility testing and short-reads only assembly

Antimicrobial	MIC	Interpretation
Amikacin	<=2	S
Amoxicillin/Clavulanic Acid	>32	R
Ampicillin	>32	R
Aztreonam	>4	R
Cefepime	>8	R
Cefotaxime	>2	R
Ceftazidime	>4	R
Ciprofloxacin	<=0.12	S
Cloramphenicol	<=1	S
Colistin	<=2	S
Ertapenem	<=0.12	S
Gentamicin	<=1	S
Levofloxacin	<=0.12	S
Meropenem	<=0.12	S
Piperacillin/tazobactam	>16	R

Escherichia coli

MLST: ST2165

Acquired resistance genes: *aph(3')-Ib*,

*bla*_{CTX-M-15}, *bla*_{TEM-1B}

Replicon content: IncI1, IncFIB

Case study 2

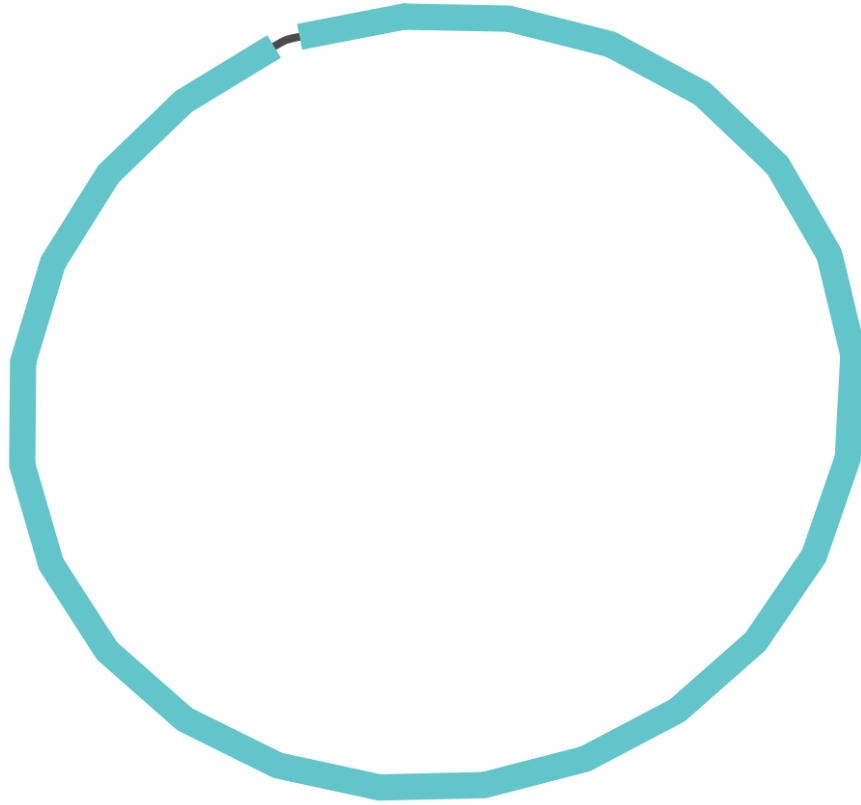
Long-reads first hybrid assembly

Analyze the **E_coli.fasta** file from the
“Case 2 data” folder.


What can we say about its plasmids?

Case study 2


Long-reads first hybrid assembly



IncI1 plasmid: 91,072 bp, ST31

 *bla*_{CTX-M-15}, *bla*_{TEM-1B}

FIB plasmid: 128,277 bp, closest match FIB67

 Multiple virulence determinants (*clbA*, *papI*, *papB*, *papa_12*, *papH*, *papC*, *papD*, *papJ*, *papK*, *papE*, *papF*, *iutA*, *iucD*, *iucC*, *iucB*, *iucA*, *astA*)

UT plasmid: 19,634 bp

 *aph(3')-Ib*

Case study 2




Geographical distribution of IncI1 plasmids

PLSDB <https://ccb-microbe.cs.uni-saarland.de/plsdb>



Highcharts.com © Natural Earth

1,036 IncI1 plasmids, mainly hosted by:

-  *Escherichia coli/fergusoni* (729/1,036)
-  *Salmonella* (222/1,036)
-  *Shigella* (42/1,036)

→ Narrow host range plasmid ←

Global spread

Case study 2

IncI1 plasmids carrying *bla*_{CTX-M-15}

PLSDB <https://ccb-microbe.cs.uni-saarland.de/plsdb>

26 IncI1/*bla*_{CTX-M-15} plasmids:

 *Escherichia coli* (18/26)

 *Salmonella* (1/26)

 *Shigella* (7/26)

pMLST <https://pubmlst.org/organisms/plasmid-mlst>

Case study 2

FIB virulence plasmids & untypeable plasmids carrying *aph(3')-Ib*

PLSDB <https://ccb-microbe.cs.uni-saarland.de/plsdb>



Highcharts.com © Natural Earth



Highcharts.com © Natural Earth

Case study 2

Question time

Is it already possible to:

- 🦠 say which plasmid is more worrisome?
- 🦠 deduce the evolutionary origins of this isolate?

Case study 2

Predict plasmid mobility (MOB-typer)

Command line approach

Install the MOB-suite via pip, source or conda

```
mob_typer --infile assembly.fasta --out_file mobtyper_results.txt
```

GUI approach (Galaxy servers)

Upload all plasmid .fasta files the Galaxy platform

Launch mob-typer and explore the results

Case study 2

Mobility classification of the three plasmids

IncI1

Conjugative

IncFIB

Non-mobilizable




UT

Mobilizable

Case study 2

Conclusions

The *Escherichia coli* causing this pre-weaning enteritis case hosts three plasmids:

-  A conjugative IncI1 carrying *bla*_{CTX-M-15}
-  A non-mobilizable FIB carrying multiple virulence genes
-  A mobilizable untypable plasmid carrying *aph(3')-Ib*

Given that more stringent controls are needed to prevent future cases of virulent and resistant *E. coli* enteritis, it is safe to state that in this case the virulence determinants will not spread horizontally.

Case study 3

Framework



During a prevalence study of antimicrobial resistance in *Campylobacter coli* isolates from the same poultry factory, it was observed that most isolates fell into one of four distinct antimicrobial susceptibility profiles



Case study 3

Antimicrobial susceptibility testing profiles

Antimicrobial	MIC	Interpretation
Ciprofloxacin	≤ 0.12	I
Erythromycin	≤ 2	S
Tetracycline	≤ 0.5	S

1 (62,5%)

Antimicrobial	MIC	Interpretation
Ciprofloxacin	≤ 0.12	I
Erythromycin	≤ 2	S
Tetracycline	> 4	R

3 (22%)

Antimicrobial	MIC	Interpretation
Ciprofloxacin	> 2	R
Erythromycin	≤ 2	S
Tetracycline	> 4	R

2 (14%)

Antimicrobial	MIC	Interpretation
Ciprofloxacin	> 2	R
Erythromycin	> 8	R
Tetracycline	$\leq 0,5$	S

4 (1.5%)

Case study 3

Antimicrobial susceptibility testing profiles

Antimicrobial	MIC	Interpretation
Ciprofloxacin	≤ 0.12	I
Erythromycin	≤ 2	S
Tetracycline	≤ 0.5	S

Profile 1 (82,5%)

Antimicrobial	MIC	Interpretation
Ciprofloxacin	≤ 0.12	I
Erythromycin	≤ 2	S
Tetracycline	> 4	R

Profile 3 (2%)

Antimicrobial	MIC	Interpretation
Ciprofloxacin	> 2	R
Erythromycin	≤ 2	S
Tetracycline	> 4	R

Profile 2 (4%)

Antimicrobial	MIC	Interpretation
Ciprofloxacin	> 2	R
Erythromycin	> 8	R
Tetracycline	$\leq 0,5$	S



Profile 4 (1.5%)

Which antimicrobial susceptibility profile(s) is/are likely to be the most problematic from the perspective of horizontal gene transfer? Why?

Case study 3

Antimicrobial susceptibility testing profiles

Typically, in *Campylobacter coli*

-  quinolone resistance is a consequence of single/double mutational alterations of the chromosomal *gyrA* gene, hence cannot be horizontally transferred
-  macrolide resistance is mediated by either 23S rRNA mutations or by the 23S rRNA methyltransferase *erm(B)* gene (primarily integrated in the chromosome, see <https://card.mcmaster.ca/ontology/36514>) which cannot be or are unlikely to be, respectively, horizontally transferred

therefore

Case study 3

Antimicrobial susceptibility testing profiles

under a horizontal gene transfer perspective, the most relevant determinant is *tet*(O)

→ this case will focus only on plasmids from tetracycline resistant *Campylobacter coli* (Profiles 1 and 3)←

Case study 3

tet(O) carrying *Campylobacter coli* plasmids

In the "Case_3_data/fasta" folder you will find all the
plasmids from the PLSDB database carryin *tet(O)*

WITHOUT the corresponding chromosomal sequence

What can we say about these plasmids?

Case study 3

Long-reads first hybrid assembly - Profiles 1 and 3

Mean length: 71,355 bp (σ 50,541 bp)

Replicon content: All untypeable

Mobility classification: 25 predicted conjugative, 1 predicted non-mobilizable

Case study 3

tet(O) carrying *Campylobacter coli* plasmids clustering (*mge-cluster*)

Command line approach

Install *mge-typer* via pip, source or conda

Since there is no *Campylobacter* model, we will have to use the *--create* flag

Since we are working with a small dataset, we will have to lower the *--min_cluster* parameter

Generate an input file with the absolute .fasta path (*ls -d -1 \$PWD/*.fa > input.txt*)

```
mge_cluster --create --input input.txt --outdir mge-results --perplexity 30 --min_cluster 5 --threads 8
```

Case study 3

tet(O) carrying *Campylobacter coli* plasmids



Analyze the **MOB-typer.tsv** and the
mge-cluster.csv files from the
"Case_3_data/results" folder.

Case study 3

Conclusions - I

During this monocentric study on prevalence of antimicrobial resistance in *Campylobacter coli*, most isolates fell into one of four distinct antimicrobial susceptibility profiles.

Typically, in *Campylobacter*

-  quinolone resistance is mediated single/double mutational alterations of the chromosomal *gyrA* gene, hence cannot be horizontally transferred
-  macrolide resistance is mediated by either 23S rRNA mutations or by the 23S rRNA methyltransferase *erm(B)* gene, primarily integrated in the chromosome, which cannot be or are unlikely to be, respectively, horizontally transferred

but

Case study 3

Conclusions - II

tet(O)-mediated tetracycline resistance is associated with horizontal gene transfer

Most *tet(O)*-carrying plasmids are predicted to be conjugative (25/26), and group into two main clusters (0, 5 plasmids and 1, 16 plasmids)

This hints to the existence of horizontal gene transfer within the population

These findings highlight the need for monitoring the spread of horizontal gene transfer to control tetracycline resistance in *Campylobacter coli*.

Thank you for your attention!

Questions?

Acknowledgements

The creation of this training material was commissioned by ECDC to Institut Pasteur with the direct involvement of <name of speaker(s)>