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Characterization of *fosD (now FosL)*, a novel plasmid-mediated fosfomycin resistance gene identified in an *Escherichia coli* isolate

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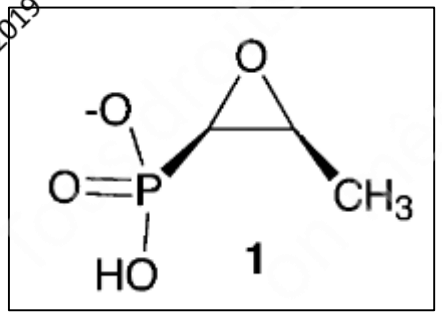
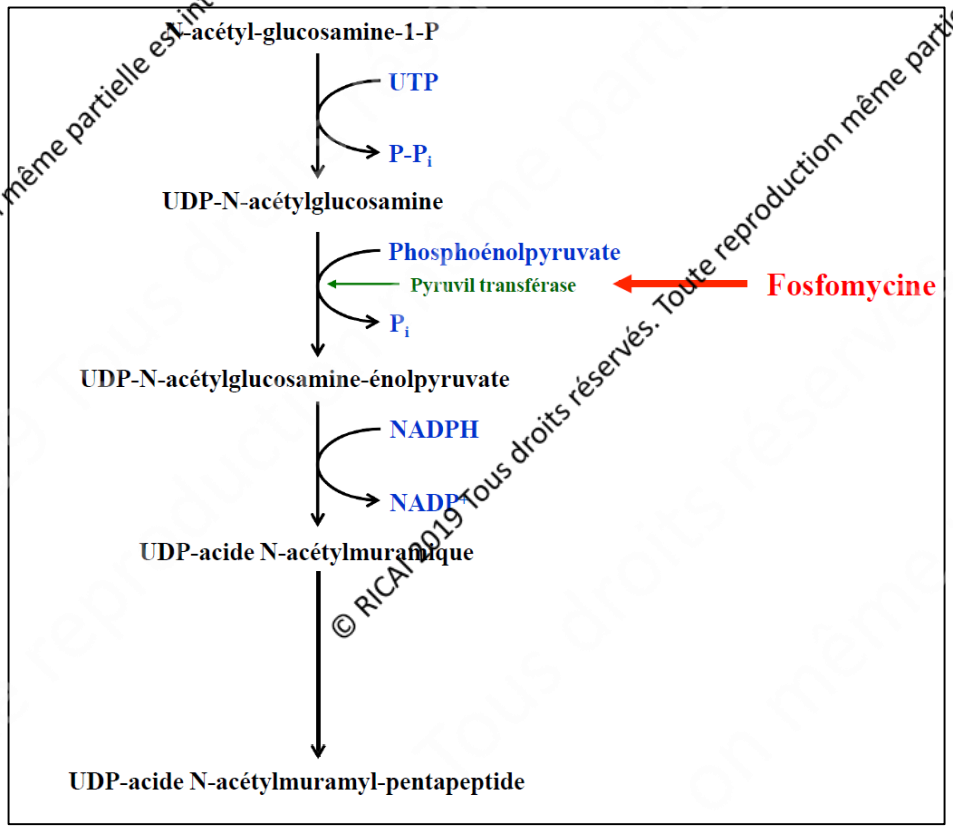
Fosfomycin

- Derivative of phosphonic acid
  - → Naturally produced by *Streptomyces viridochromogenes* and *Pseudomonas syringae/viridiflava*
- Broad spectrum antibiotic
- Inhibits the biosynthesis of peptidoglycan



Fosfomycin : mechanism of action

- Analogue of phosphoenolpyruvate
- Inhibition of the synthesis of the bacterial wall

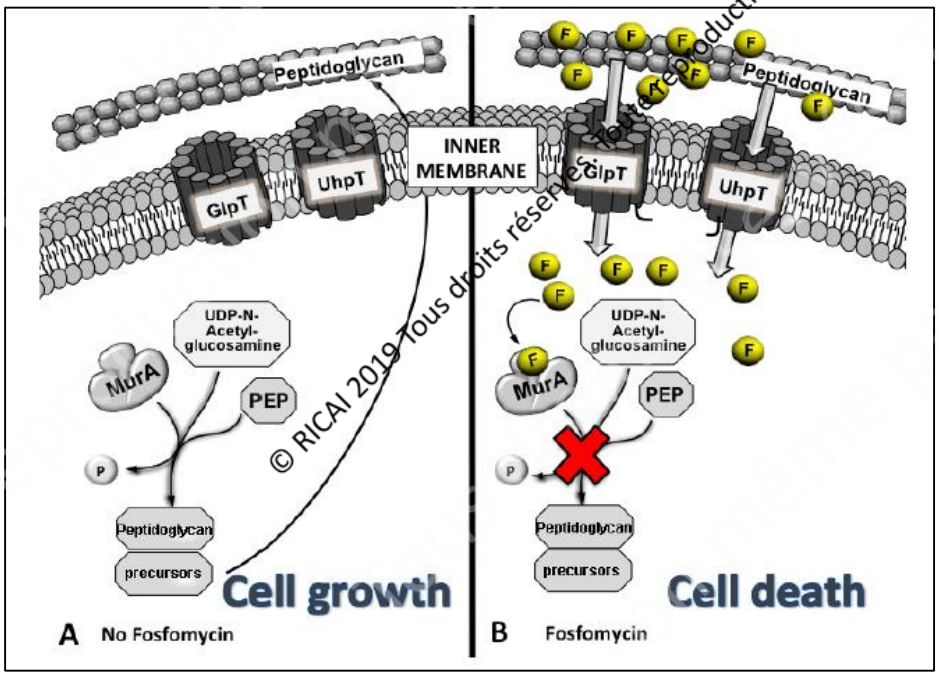


Fosfomycin : mechanism of action

Enters the bacteria through;

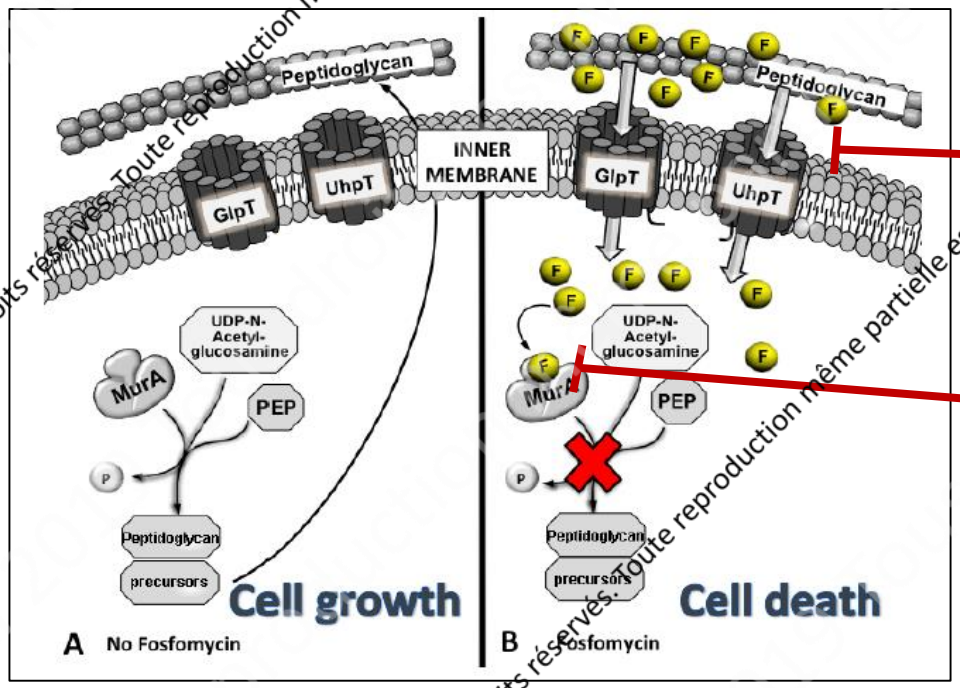
- **GlpT** (glycerol-3-phosphate transporter)
- **UhpT** (hexose phosphate transporter)

Binds to and inhibits the *MurA* activity (catalyzes the first step of peptidoglycan biosynthesis)



Alfredo Castañeda-Garcìa, Antibiotics, 2013

Fosfomycin : mechanism of resistance



Mutations in *glpT* and *uhpT* genes

Mutations in *murA*

Fosfomycin : mechanism of resistance

Modification of fosfomycin by the action of modifying enzymes:

- Kinases : FomB and FomC enzymes

- Phosphoesterases: FosC enzyme

- Bacillithiol transferases: add a thiol group to the fosfomycin molecule

- Glutathione transferases: add a GST group to the fosfomycin molecule

Fosfomycin : mechanism of resistance

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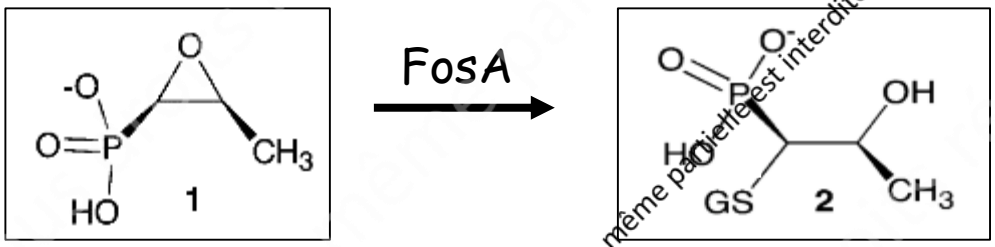
→ The most identified acquired in Enterobacteriaceae

Fosfomycin : Glutathione transferases

Largest family: FosA (in particular FosA3)

Modification of fosfomycin by the action of FosA:



Adapted from Rigsby *et al.*, Biochemistry, 2004

Fosfomycin

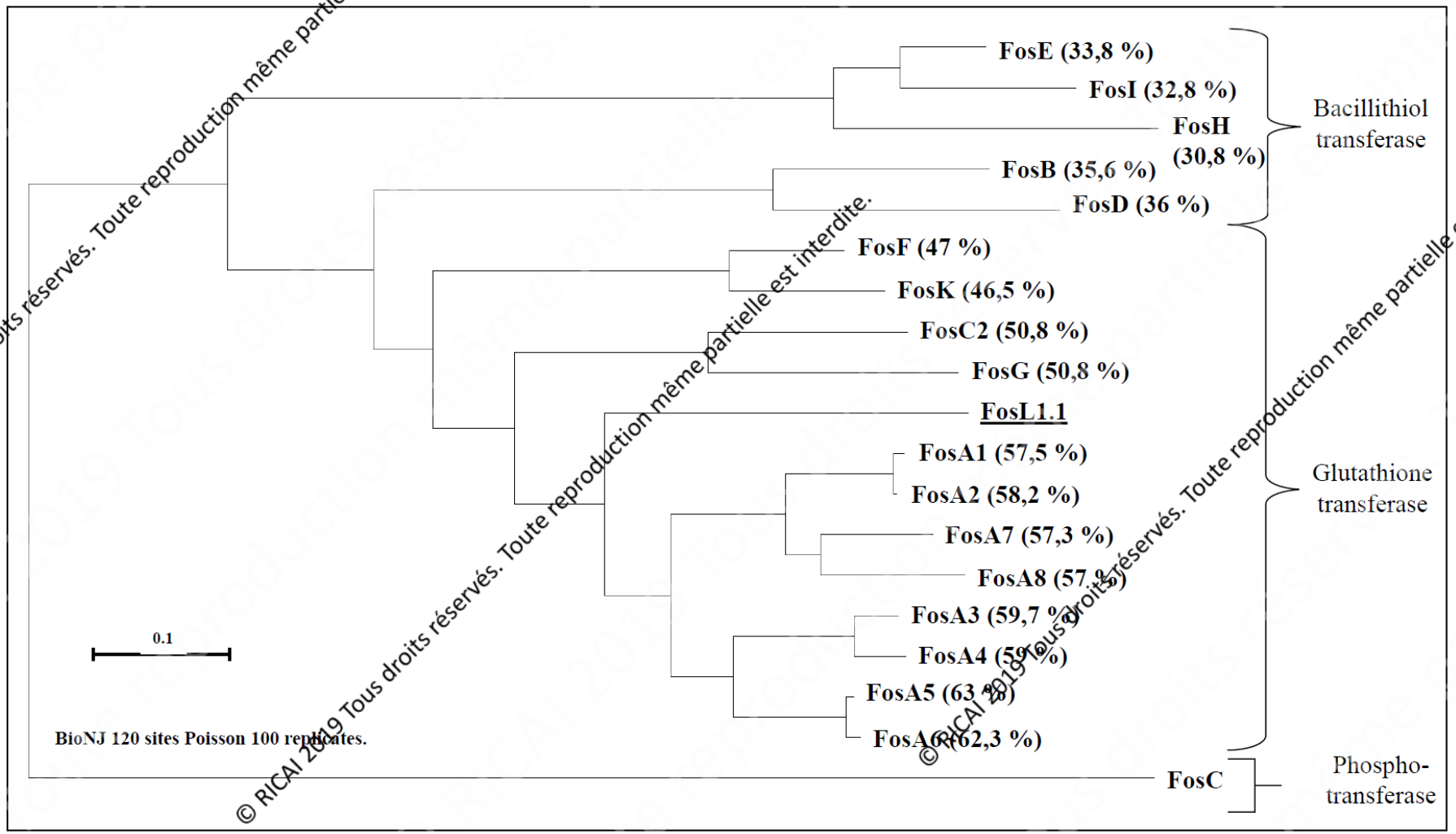
Non fonctionnal fosfomycin

FosA proteins are glutathione transferases;

→ Addition of a glutathione group to the antibiotic

→ Inactivation of the antibacterial function

Fosfomycin : *fos genes*



Identification of a new *fos* determinant

Presentation of the study:

→ Screening of a collection of 1,225 ESBL-producing *E. coli* from Switzerland

→ Detection of fosfomycin resistance using the Rapid Fosfo NP test (Nordmann et al., J Clin Microbiol 2019)

→ Detection of 17 fosfomycin-resistant *E. coli* isolates (1.4%) (Müller et al., Eur J Clin Microbiol Infect Dis 2019)

➔ Detection of two fosfomycin-resistant isolates carrying an unknown plasmid-mediated fosfomycin resistance gene

➔ FosA8 (79% AA id. avec FosA2) (Poirel et al. Antimicrob Agents Chemother 2019)

Isolate R249

- ESBL-producing *E. coli*
- Fosfomycin MIC > 512 µg/ml → Test Phosphonoformate positive (presence of a *fos* gene)
- Negative for all known *fos* genes
- Fosfomycin-resistant transconjugant was obtained : co-resistance with chloramphenicol
- Plasmid analysis identified the fosfomycin resistance determinant located onto an IncX plasmid of ca. 80 kb.

Sequencing of *E. coli* isolate R249

- Whole genome sequencing was performed
- Sequences were aligned with CLC genomic workbench and resistome was analyzed using Resfinder (cge server)

Resistome of isolate R249

Acquired antimicrobial resistance gene - Results

Aminoglycoside

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
aac(3)-IIa	99.65	861/861	249_S5_L001_R1_001_(paired)_contig_80_consensus	1919..2779	Aminoglycoside resistance	X51534
aadA5	99.87	789/789	249_S5_L001_R1_001_(paired)_contig_553_consensus	2110..2898	Aminoglycoside resistance	AF137361
aac(6')-Ib-cr	100.00	600/600	249_S5_L001_R1_001_(paired)_contig_80_consensus	410..1009	Fluoroquinolone and aminoglycoside resistance	DQ303918

Beta-lactam

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
blaOXA-534	100.00	831/279	249_S5_L001_R1_001_(paired)_contig_80_consensus	1..279		KX714285
blaOXA-1	99.81	831/526	249_S5_L001_R1_001_(paired)_contig_1096_consensus	1..526	Beta-lactam resistance	HQ170510
blaCTX-M-15	99.89	876/876	249_S5_L001_R1_001_(paired)_contig_711_consensus	244..1119	Beta-lactam resistance	AY044436

Colistin

No resistance genes found

Fluoroquinolone

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
aac(6')-Ib-cr	100.00	600/600	249_S5_L001_R1_001_(paired)_contig_80_consensus	410..1009	Fluoroquinolone and aminoglycoside resistance	DQ303918

Fosfomycin

No resistance genes found

Fusidic Acid

No resistance genes found

Glycopeptide

No resistance genes found

MLS - Macrolide, Lincosamide and Streptogramin B

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
mdf(A)	97.49	1233/1233	249_S5_L001_R1_001_(paired)_contig_63_consensus	9957..11189		Y08743

Nitroimidazole

No resistance genes found

Oxazolidinone

No resistance genes found

Resistome of isolate R249

Nitroimidazole

No resistance genes found

Oxazolidinone

No resistance genes found

Phenicol

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
catB3	100.00	633/442	249_S5_L001_R1_001_(paired)_contig_836_consensus	18..459	Phenicol resistance	U13880

Rifampicin

No resistance genes found

Sulphonamides

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
sul1	99.77	867/867	249_S5_L001_R1_001_(paired)_contig_553_consensus	3418..4284	Sulphonamide resistance	EU780013

Tetracycline

Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
tet(34)	84.75	465/118	249_S5_L001_R1_001_(paired)_contig_110_consensus	7166..7285	Tetracycline resistance	AB061440

Trimethoprim

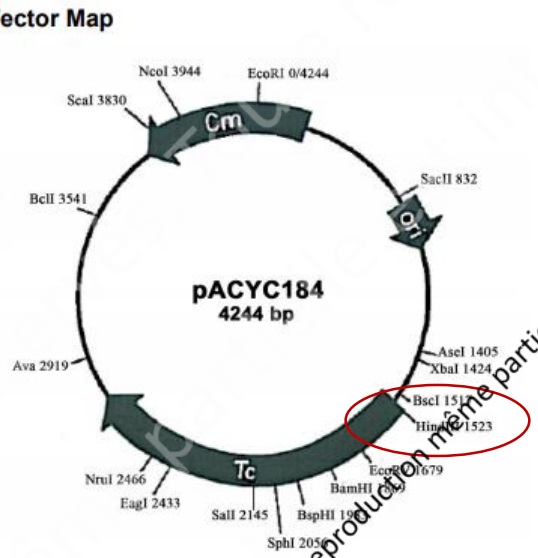
Resistance gene	Identity	Query/HSP	Contig	Position in contig	Phenotype	Accession no.
dfrA17	99.58	474/474	249_S5_L001_R1_001_(paired)_contig_553_consensus	1506..1979	Trimethoprim resistance	FJ460238

No fosfomycin resistance gene detected ?

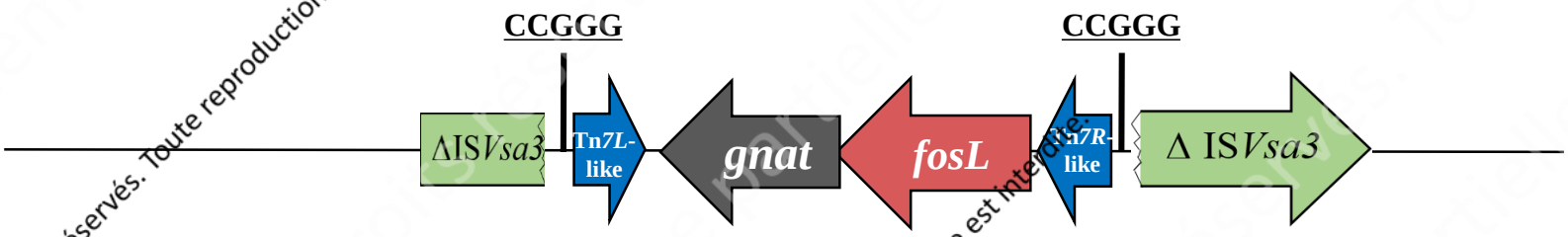
→ Shotgun cloning in order to detect the contig containing the new *fos* gene

Cloning of the gene encoding fosfomycin resistance

- Experiment done with DNA from the transconjugant
- The pACYC184 recombinant plasmid was used
- A single colony grew on fosfomycin supplemented LB-agar
- 10-kb insert
- Detection of the contig containing the new *fos*

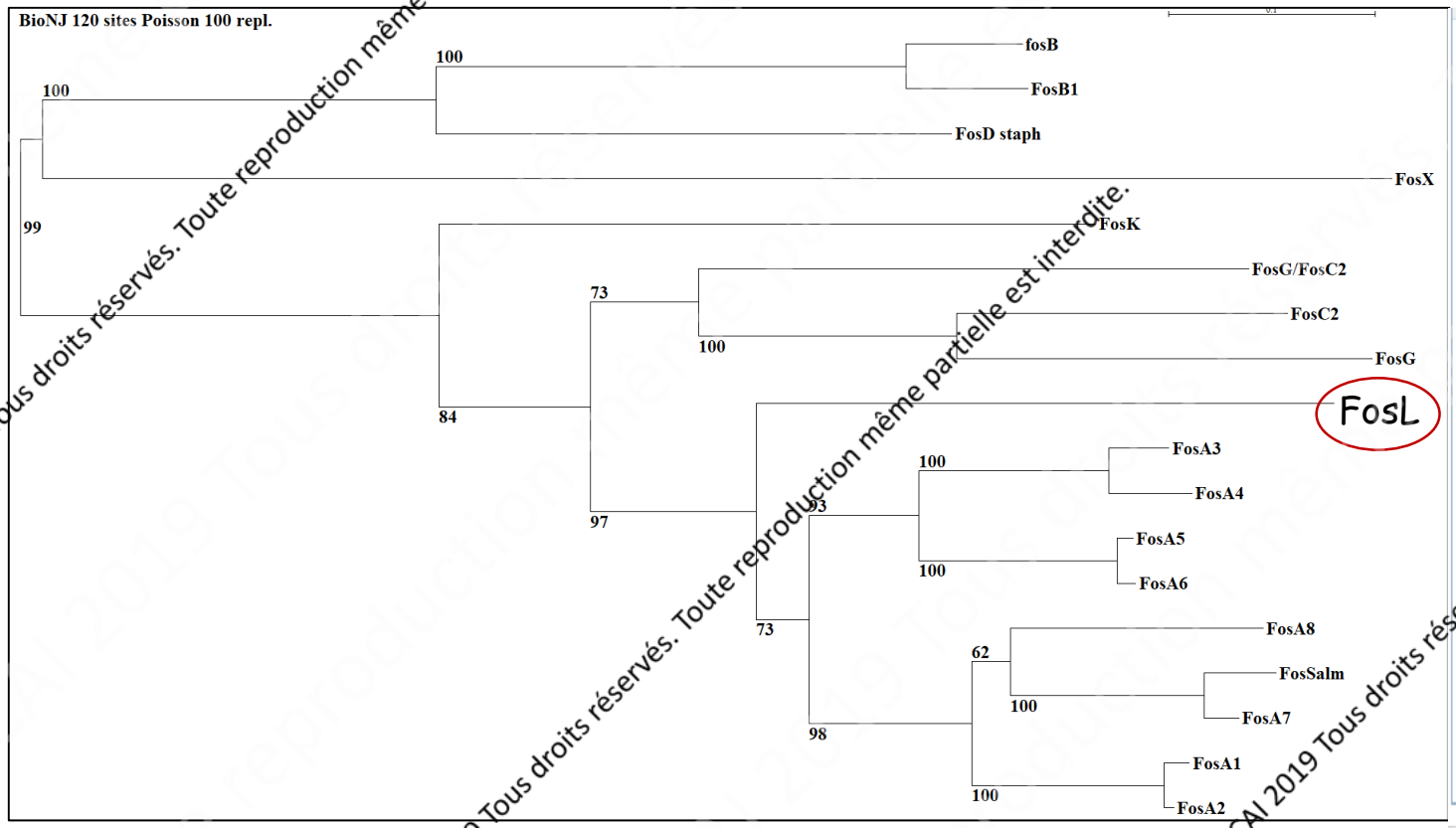


A new *fos* gene



- *fosL*: new *fos* gene detected by WGS and by shotgun cloning
- Tn*7L* and Tn*7R*-like: Sequences showing high nucleotide identity with the Tn*7* extremities recognized by the transposases of the transposon Tn*7*
- *gnat*: gene encoding a putative N-acetyltransferase from *Achromobacter denitrificans* (95%)
- IS*Vsa3*: insertion sequence truncated by the insertion of the *fosL* cassette

But where is FosL ?



FosA5	63.04%
FosA6	62.32%
FosA3	59.71%
FosA4	58.99%
FosA2	58.21%
FosA1	57.46%
FosA8	57.04%
FosA7	57.35%
FosSalm	58.11%
FosG/FosC2	48.48%
FosC2	50.00%
FosG	50.00%
FosK	46.51%
FosB1	33.33%
FosD staph	36.00%
fosB	32.56%
FosX	29.41%

Conclusion

- We identified a totally novel fosfomycin resistance determinant gene acquired by an ESBL-producing *E. coli* isolate
- The Phosphonoformate positive test performs very well for all FosA producers
- Whole genome sequencing does not always give teh answer…
- Further epidemiological work is needed to evaluate whether this gene has already spread.