

Characterization of a beta-lactamase from *Bacteroidetes* bacteria found in *Escherichia coli*

Claudine Fournier^{1,2,3}, Marie Petitjean⁴, Patrice Nordmann^{1,2,3,5}, Erick Denamur⁴, Alexander Mellmann⁶, Laurent Poirel^{1,2,3}, Etienne Ruppé^{4,7}

1 Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland; 2 INSERM European Unit, IAME, Paris, France; 3 Swiss National Reference Center for Emerging Antibiotic Resistance, Fribourg, Switzerland; 4 INSERM, Université de Paris, IAME, F-75018 Paris, France. ; 5 University of Lausanne, University Hospital Center, Lausanne, Switzerland; 6 Institute of Hygiene, University Hospital Münster, Münster, Germany ; 7 AP-HP, Hôpital Bichat, Laboratoire de Bactériologie, F-75018 Paris, France.

Disclosures

Consultant for DaVolterra

Member of the Scientific Council of Pathoquest and MaaT Pharma

Research funding from bioMérieux

Interventions for Mobidia, Correvio, Eumedica and MSD.

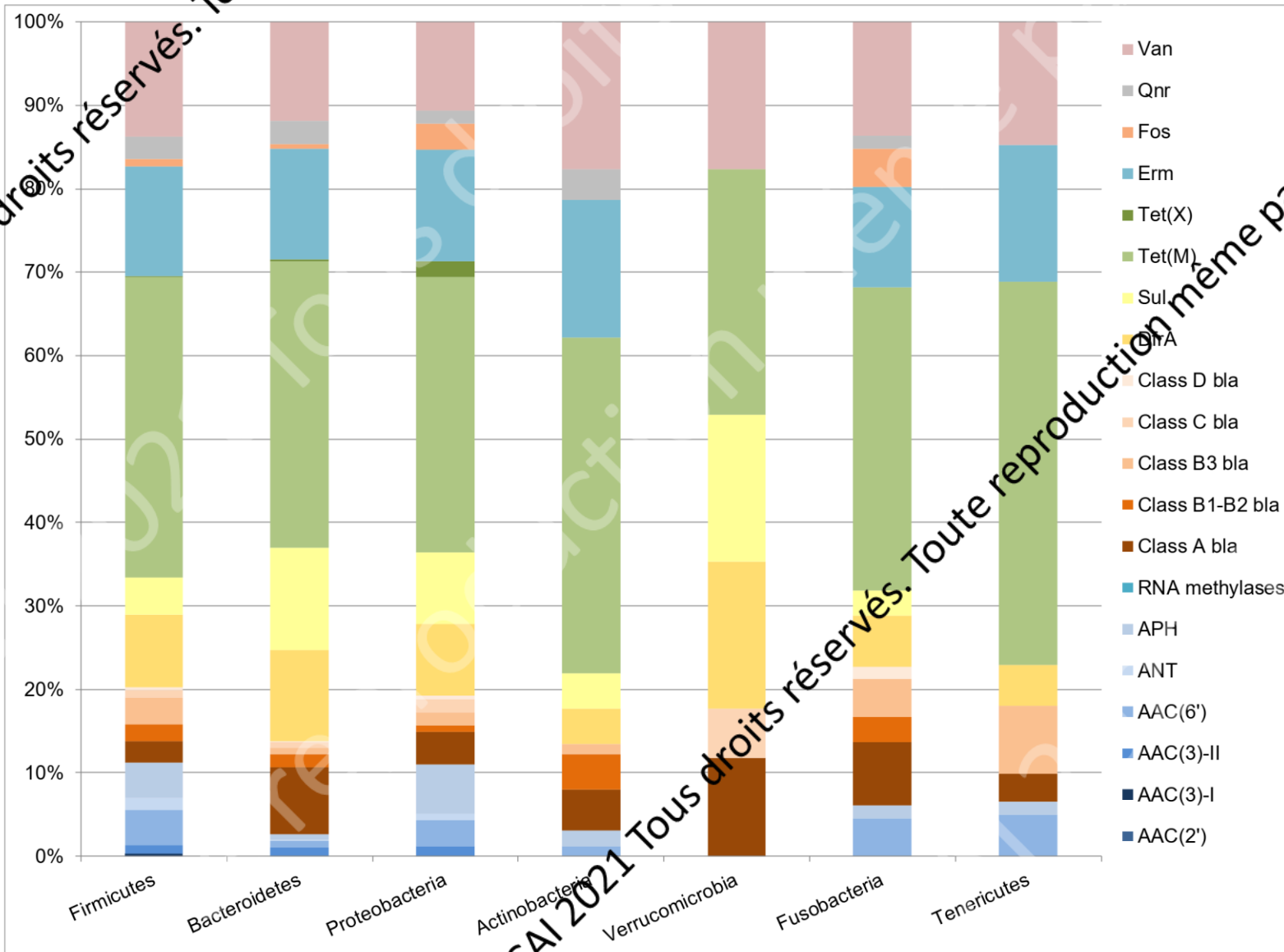
Travel expenses from Novartis, Sanofi

Is the intestinal microbiota a reservoir of antibiotic resistance genes for pathogenic bacteria?

© RICAI 2021 Tous droits réservés. Toute reproduction même partielle est interdite.

© RICAI 2021 Tous droits réservés.

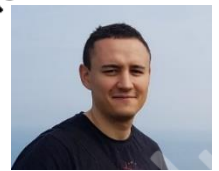
Intestinal bacteria harbor a vast diversity of antibiotic resistance genes (ARG)



- N=8995 ARG predicted (0.2% of 3.9 million gene catalogue)
- More diversity than in pathogenic bacteria
- Average 1377 ARG per subject (min. 258, max. 2367)
- **Low identity with known ARG** (mean 29.8% amino-acid identity)
- Present in the **main gut phyla**
- Mostly **chromosomal**
- Interphyla transfers?



Julien Tap
(Danone)



Amine Ghozlane
(IP)

Transfer of antibiotic resistance genes: a phylum barrier?

- **Firmicutes**: example of glycopeptide resistance genes shared between *Clostridium* (commensal species) and *Enterococcus* (opportunistic pathogen)¹
- **Proteobacteria** (Enterobacterales, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*): resistance genes come from environmental proteobacteria².
- But exceptions exist!

CLINICAL MICROBIOLOGY REVIEWS, Jan. 2009, p. 161–182

AmpC β -Lactamases

George A. Jacoby*

Lahey Clinic, Burlington, Massachusetts

Plasmid-encoded cephalosporinases originate from Enterobacterales and *Aeromonas*

nature
microbiology

LETTERS

<https://doi.org/10.1038/s41564-019-0496-4>

Plasmid-encoded *tet(X)* genes that confer high-level tigecycline resistance in *Escherichia coli*

Jian Sun^{1,2,11}, Chong Chen^{1,2,11}, Chao-Yue Cui^{1,2}, Yan Zhang^{1,2}, Xiao Liu^{1,2}, Ze-Hua Cui^{1,2}, Xiao-Yu Ma^{1,2}, Youjun Feng^{1,2}, Liang-Xing Fang^{1,2}, Xin-Lei Lian^{1,2}, Rong-Min Zhang^{1,2}, You-Zhi Tang^{1,2,3}, Kou-Xing Zhang⁴, Han-Mian Liu⁵, Zhi-Hui Zhuang⁴, Shi-Dan Zhou⁵, Jing-Nan Lv⁶, Hong Du⁶, Bin Huang⁷, Fang-You Yu⁸, Barun Mathema⁹, Barry N. Kreiswirth¹⁰, Xiao-Ping Liao^{1,2*}, Liang Chen^{10*} and Ya-Hong Liu^{1,2,3*}

tet(X) originates from *Sphingobacterium* (Bacteroidetes)

Is the phylum barrier insurmountable for ARG?



Proteobacteria
 10^8 CFU/g feces

Bacteroidetes
 10^{11} CFU/g feces

The resistome of *Escherichia coli*:
unexpected findings

© RICAI 2021 Tous droits réservés.

© RICAI 2021 Tous droits réservés.

© RICAI 2021 Tous droits réservés.

Analysis of the resistome of *E. coli*



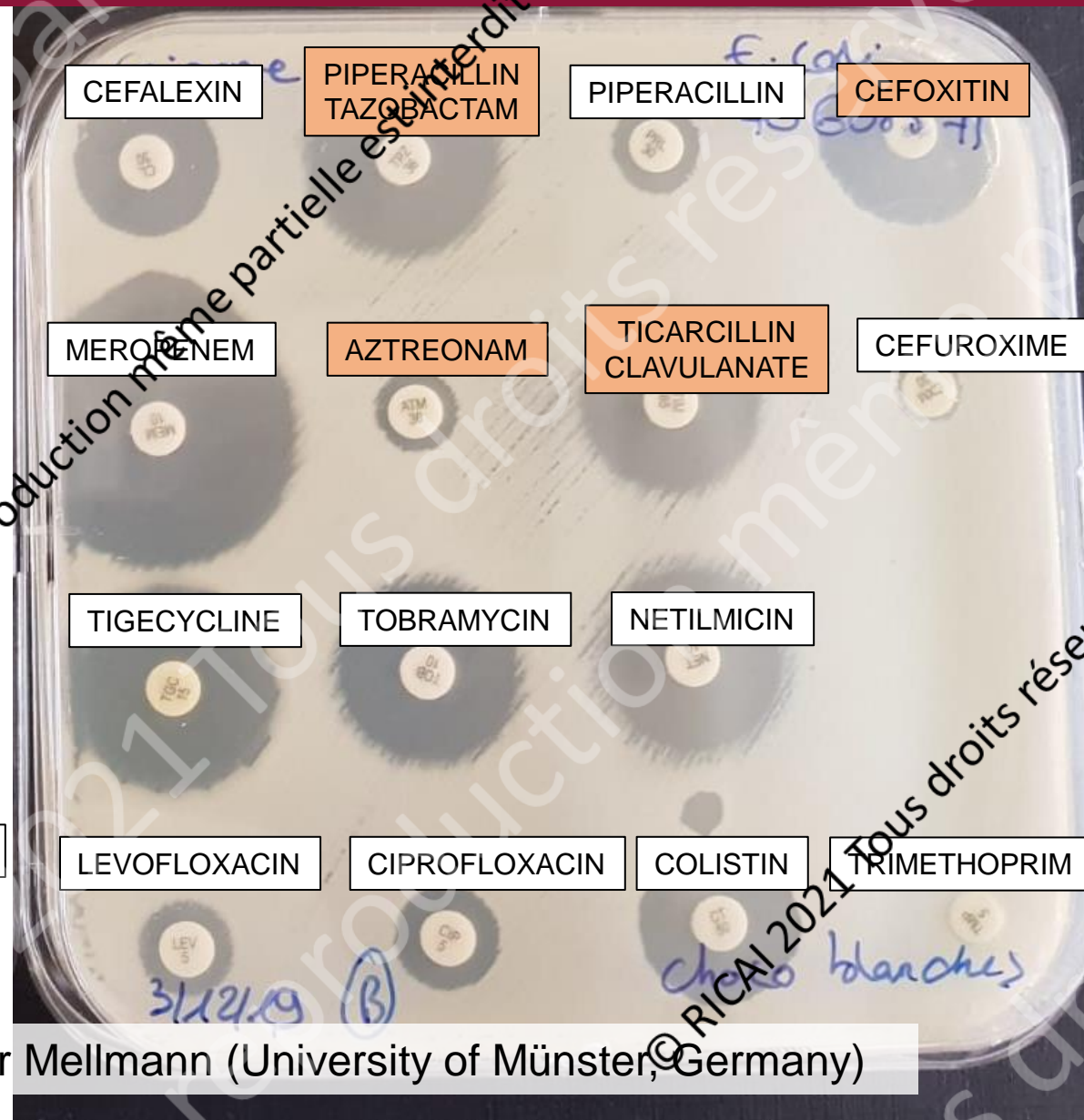
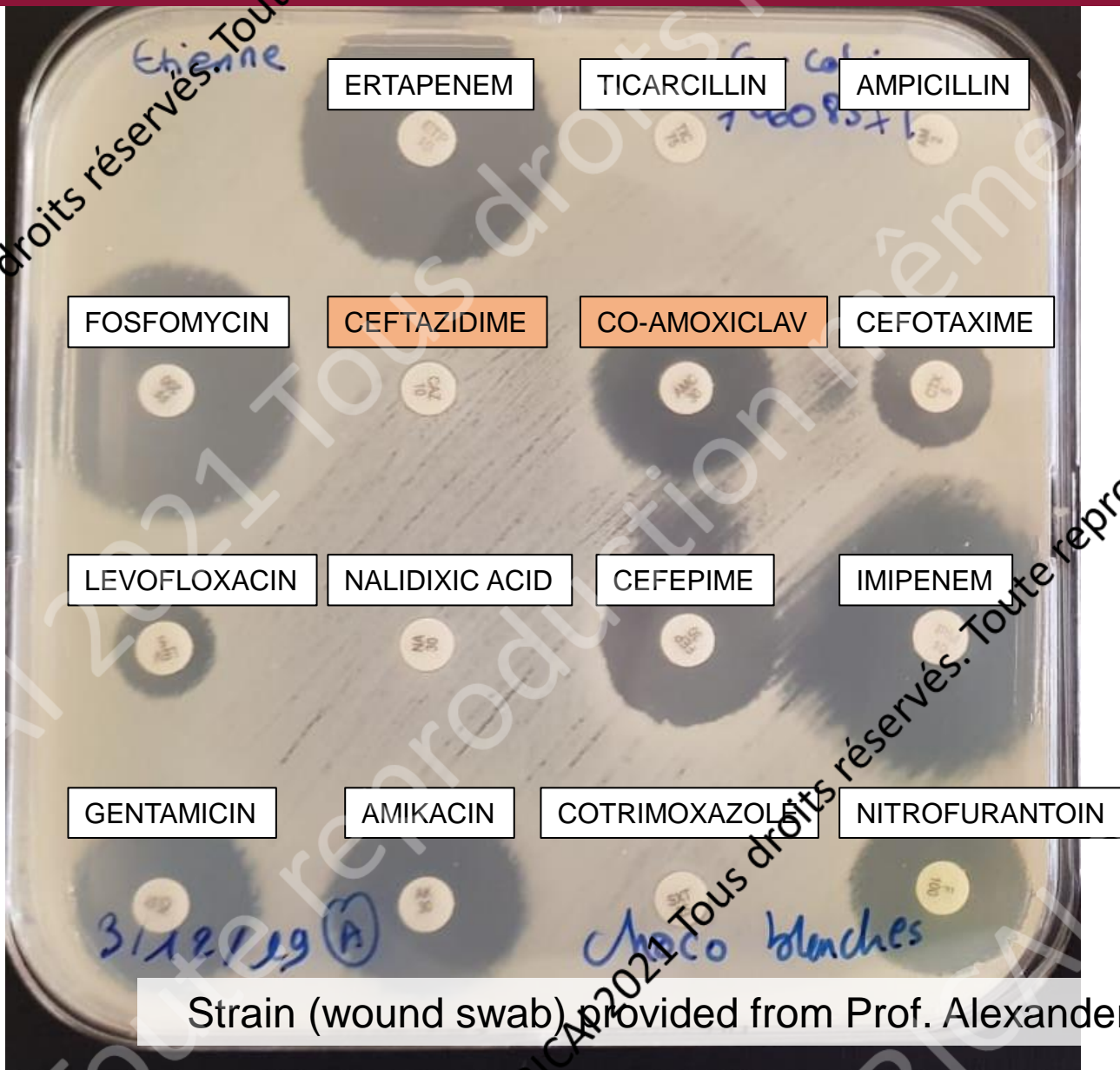
Marie Petitjean

<i>E. coli</i> strain ID	Phylogroup	ST (Warwick University/Pasteur Institute schemes)	Serotype	<i>fimH</i> allele	Resistance gene	Strain of origin ID
ESC_HA9845AA	A	744/2	Onovel32:H10	54	Putative β -lactamase	<i>Bacteroides uniformis</i> strain NBRC 113350
ESC_OA1280AA	E	753/920	O137:H9	124	<i>erm</i> (49)	<i>Bifidobacterium breve</i> strain CECT7263
ESC_JA0734AA	D	405/477	O102:H6	27	<i>erm</i>	<i>Clostridioides difficile</i> strain CDT4
ESC_FA9928AA	A	5943/999	O89:H11	41	<i>tetM</i>	<i>Clostridioides difficile</i> strain C161

- 70k genomes of *E. coli* (Enterobase)
- 4 putative ARG from non-Proteobacteria

© RICAI 2021 Tous droits réservés

Disk diffusion testing: a typical ESBL phenotype?



Strain (wound swab) provided from Prof. Alexander Mellmann (University of Münster, Germany)

ARG content

ARG Family	ARG name	Description	Resistance to
ANT	<i>aadA5</i>	ANT(3'')-Ia family aminoglycoside nucleotidyltransferase AadA5	Aminoglycosides
APH	<i>aph(3'')-Ib</i>	aminoglycoside O-phosphotransferase APH(3'')-Ib	Aminoglycosides
APH	<i>aph(6)-Id</i>	aminoglycoside O-phosphotransferase APH(6)-Id	Aminoglycosides
APH	<i>aph(3')-Ia</i>	aminoglycoside O-phosphotransferase APH(3')-Ia	Aminoglycosides
Cat	<i>catA1</i>	type A-1 chloramphenicol O-acetyltransferase	Chloramphenicol
Class A beta-lactamase	<i>bla_{TEM-1}</i>	class A broad-spectrum beta-lactamase TEM-1	Beta-lactams
Class C beta-lactamase	<i>bla_{EC}</i>	BlaEC family class C beta-lactamase	Beta-lactams
Dfr	<i>dfrA17</i>	trimethoprim-resistant dihydrofolate reductase DfrA17	Trimethoprim
Mph	<i>mph(A)</i>	Mph(A) family macrolide 2'-phosphotransferase	Macrolides
Sul	<i>sul1</i>	sulfonamide-resistant dihydropteroate synthase Sul1	Sulphonamides
Sul	<i>sul2</i>	Sulfonamide-resistant dihydropteroate synthase Sul2	Sulphonamides
Tet efflux	<i>tet(B)</i>	tetracycline efflux MFS transporter Tet(B)	Tetracyclines

Determination of minimal inhibitory concentrations (MIC)

Beta-lactam	MIC ($\mu\text{g/mL}$) for <i>Escherichia coli</i>		
	Clinical strain	TOP10 (pBL1-x)	TOP10
Amoxicillin	> 256	> 256	4
Amoxicillin + clavulanic acid	4	4	4
Piperacillin	> 256	> 256	2
Piperacillin + tazobactam	4	4	2
Temocillin	256	256	4
Cefuroxime	> 256	> 256	0.5
Ceftriaxone	16	16	0.12
Cefotaxime	4	2	0.12
Ceftazidime	> 256	> 256	0.25
Ceftazidime + avibactam	0.06	0.03	0.25
Cefepime	4	2	0.06
Ceftolozane + tazobactam	0.06	0.06	0.06
Aztreonam	> 256	> 256	0.03
Imipenem	0.25	0.25	0.25
Meropenem	0.03	0.03	0.03
Ertapenem	0.03	0.03	0.03

Enzymatic characterization

Beta-lactam	K_{cat} (s^{-1})	K_m (μM)	K_{cat}/K_m ($\mu M^{-1}s^{-1}$)	i_i (μM)
Penicillin G	210	95	2.2	
Ampicillin	290	280	1	
Ticarcillin	< 0.01	ND	ND	0.0036
Piperacillin	< 0.01	ND	ND	0.0072
Cephalothin	30	15	2	
Cefepime	< 0.01	ND	ND	
Cefotaxime	< 0.01	ND	ND	
Ceftazidime	< 0.01	ND	ND	0.58
Imipenem	< 0.01	ND	ND	0.0013
Meropenem	< 0.01	ND	ND	0.019
Ertapenem	< 0.01	ND	ND	0.017
Aztreonam		45	0.04	

Beta-lactamase	IC_{50} (nM)		
	Clavulanic acid	Tazobactam	Avibactam
BLA-x	0.32	0.8	3.8

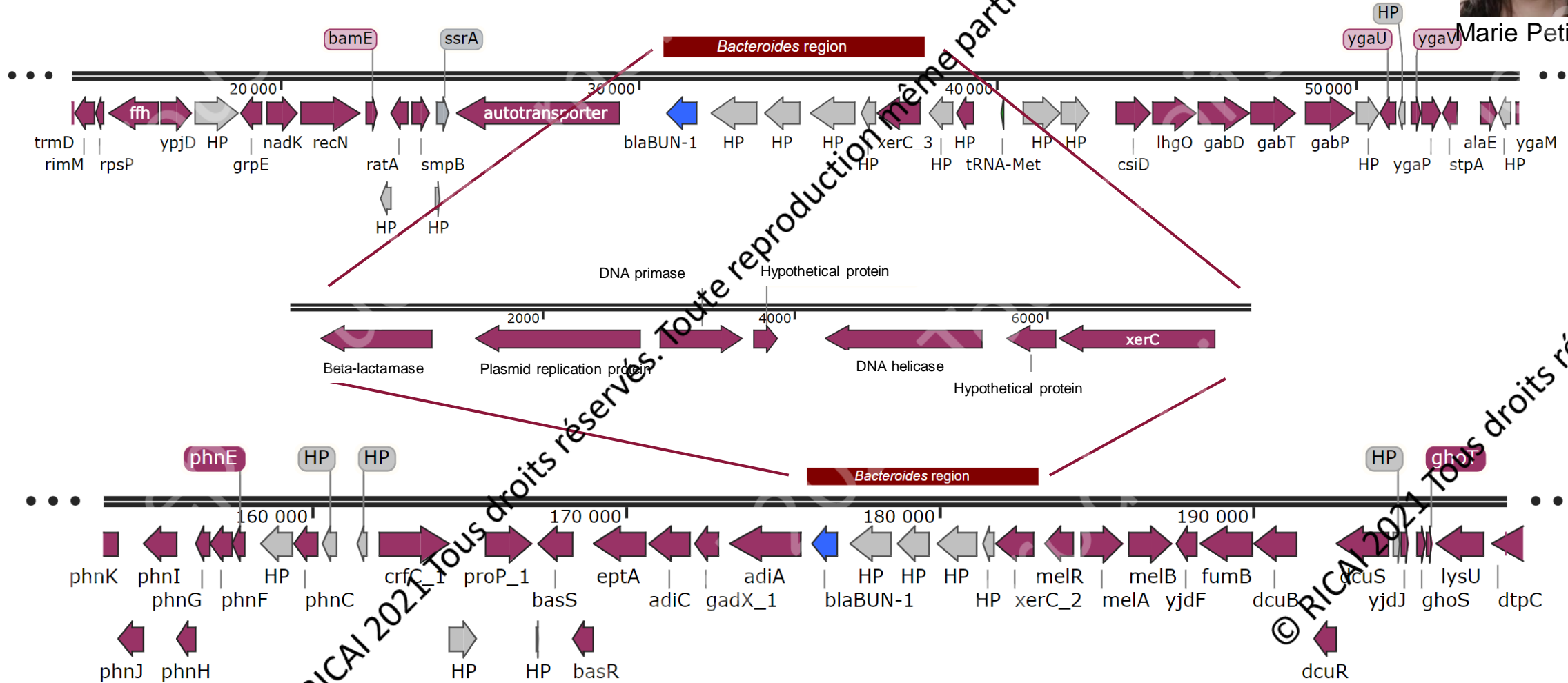
© RICAI 2021 Tous droits réservés

Genomic characterization

- Genome sequencing by Illumina MiniSeq and Nanopore MinION
- Identification of 2 copies of a beta-lactamase encoding gene from *Bacteroides* sp.
- Chromosomal location, 7600bp genomic island



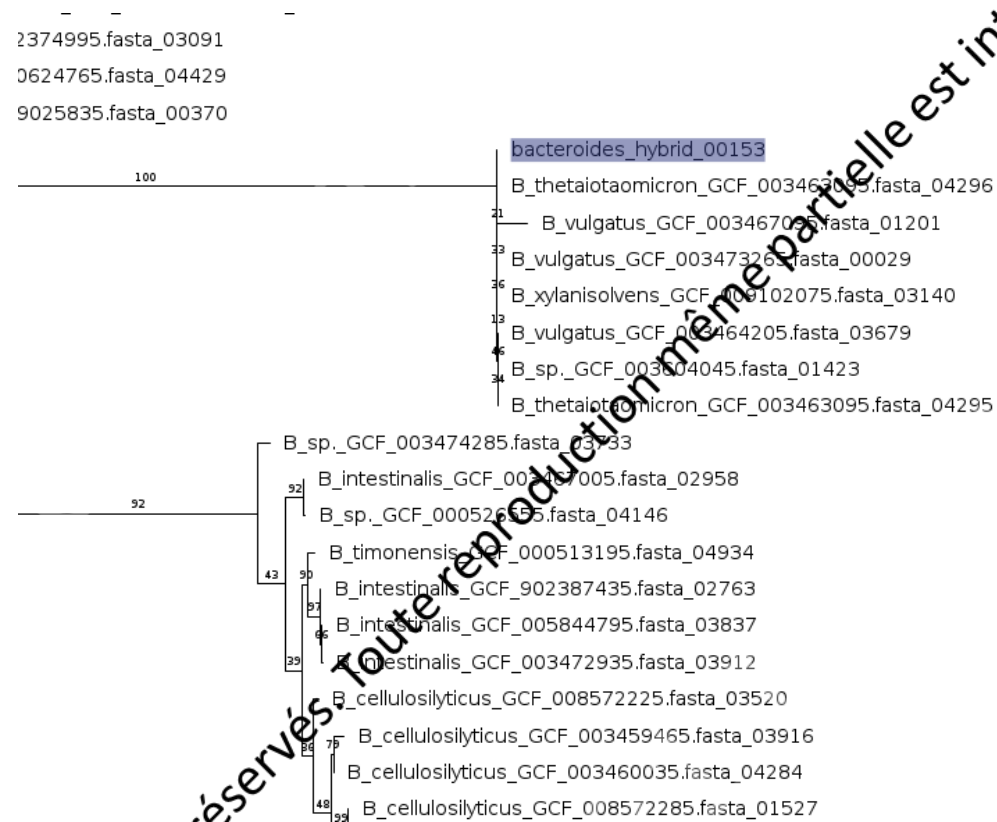
Marie Petitjean



Tous droits réservés. Toute reproduction même partielle est interdite.

© RICAI 2021 Tous droits réservés.

Origin of the gene



Search for 7600 bp contig in RefSeq database:

- Hits in *Bacteroides* (*B. uniformis* (n=4), *B. vulgatus* (n=6), *B. caccae* (n=3), *B. eggerthii* (n=3), *B. salyersiae* (n=3), *B. stercoris* (n=3) and *B. fragilis* (n=2)
- But also in *Parabacteroides distasonis* (n=2) and *Prevotellamassilia timonensis* (n=1)

In conclusion

© RICAI 2021 Tous droits réservés. Toute reproduction même partielle est interdite.

Discussion

- 1st (?) identification of *bla* from intestinal Bacteroidetes to Proteobacteria (*E. coli*)
- Transfer from Bacteroidetes to *E. coli* most likely, or shared reservoir?
- Atypical phenotype (resistance to aztreonam and temocillin)
- Mechanism of transfer?
- Limited success, **impact on fitness?**

Tous droits réservés. Toute reproduction partielle est interdite



Thank you for your attention

Etienne Ruppé

etienne.ruppe@inserm.fr

@RuppeEtienne

© RICAI 2021 Tous droits réservés

© RICAI 2021 Tous droits réservés