

JNI

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Nationales
d'Infectiologie

Poitiers

et la région Nouvelle Aquitaine

Palais des Congrès du Futuroscope

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Résistome : de quoi parle t-on?

Etienne Ruppé

Université de Paris/INSERM UMR1137 IAME

Laboratoire de Bactériologie, Hôpital Bichat-Claude Bernard, AP-HP, Paris



Déclaration d'intérêts de 2014 à 2019

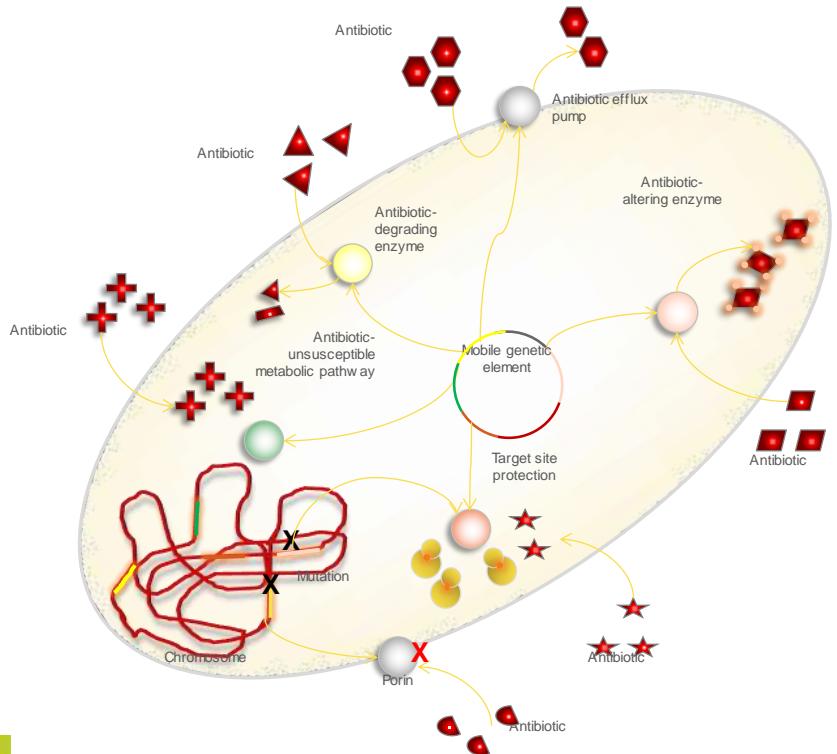
Intérêts financiers : Aucun

Liens durables ou permanents : Pathoquest, MaaT Pharma, Illumina, DaVolterra

Interventions ponctuelles : Correvio, MSD, Mobidiag

Intérêts indirects : fonds recherche bioMérieux

What is an antibiotic resistance gene (ARG)?



“From an operational perspective, an antibiotic resistance gene confers resistance to antibiotics when it is present or when it increases susceptibility to antibiotics when it is absent”.

Not considered as ARGs: gene(s), which, when mutated, cause a decrease in the susceptibility to at least one antibiotic (e.g. mutated DNA gyrases cause fluroquinolone resistance)



What does the « resistome » refer to?

NATURE REVIEWS | MICROBIOLOGY

The antibiotic resistome: the nexus of chemical and genetic diversity

Gerard D. Wright

*The antibiotic resistome is the **collection of all the antibiotic resistance genes**, including those usually associated with pathogenic bacteria isolated in the clinics, non-pathogenic antibiotic producing bacteria and all other resistance genes.*

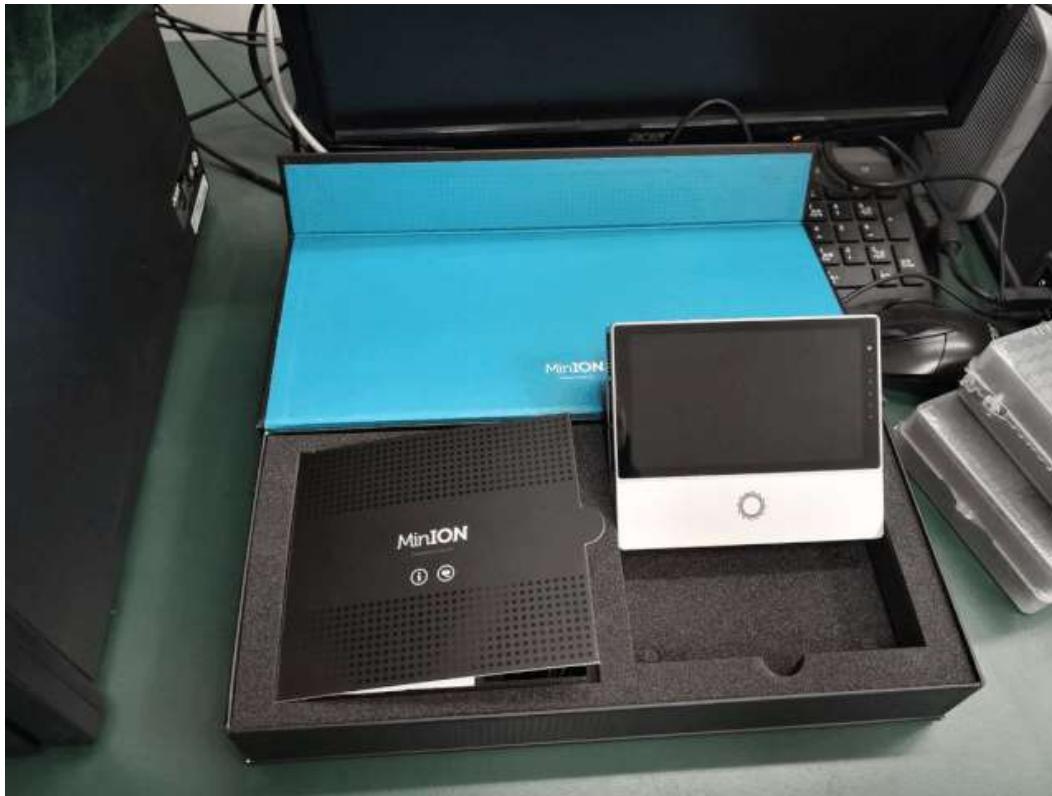


Why now?

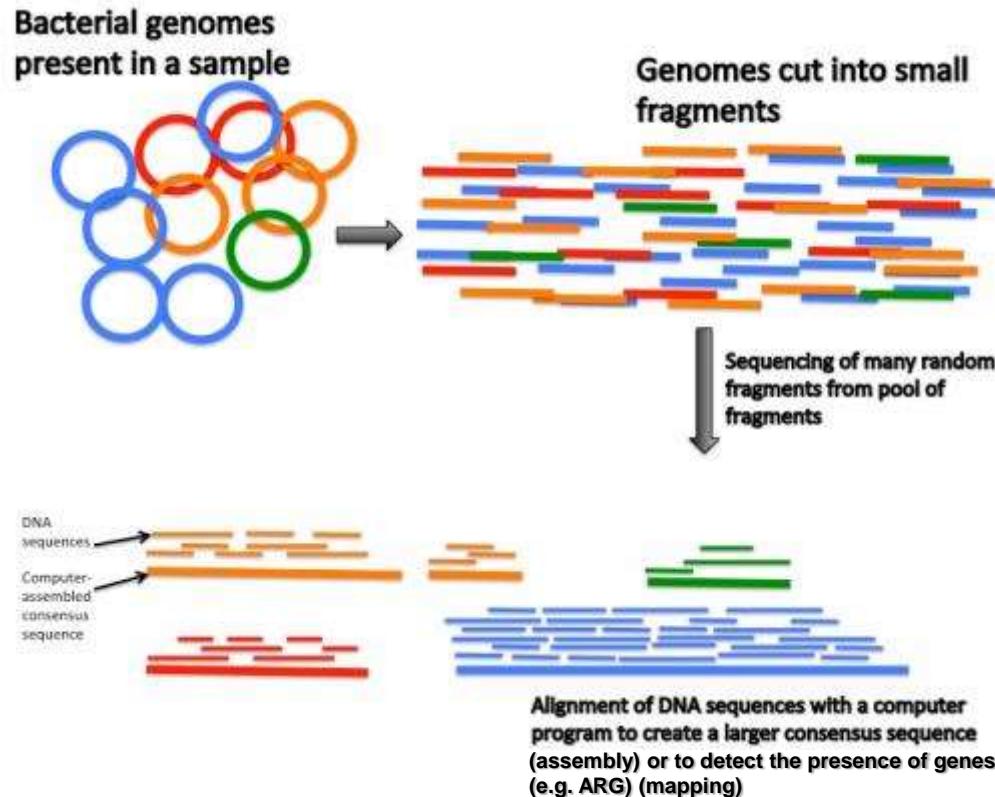


- Before 2010: ARG detection by targeted PCR
- Low throughput
- Only found what you know
- New ARGs: cloning experiments

The next-generation sequencing era (>2010)



How to deal with sequencing data?



There are plenty ARG databases to play with

No.	Database/repository	AR gene spectrum	Functionality and feature(s)	Last update ^a	Reference
1.	ARDB	All AR genes	Webtool, BLASTp, BLASTn	July 2009	²²
2.	CARD	All AR genes	Webtool, BLASTp, BLASTn, gene ontology, gene identifier V2, annotation	April 2014	²³
3.	ResFinder	All AR genes (except chromosome-specific genes)	Webtool, BLASTn	June 2015	²⁴
4.	LacED	β -Lactamases	Webtool, BLASTp, ClustalW		²⁵
5.	ResFams	All AR genes	BLASTp, Local BLAST, HMM profile	January 2015	²⁶
6.	Patric	All AR genes	Webtool link to CARD and ARDB	December 2015	²⁷
7.	HMP	Human body site-specific study resources	Webtool, BLASTp, BLASTn	November 2011	²⁸
8.	RED-DB	All AR genes	BLASTn, BLASTp		
9.	U-CARE	Organism specific (<i>E. coli</i>)	BLASTp		²⁹
10.	ARG-ANNOT	All AR genes	BLAST, BioEdit V7.2.5, annotation		³⁰
11.	BLAD	β -Lactamases	Webtool		³¹
12.	CBMAR	β -Lactamases	Webtool, BLASTn, BLASTp, ClustalW, MEME/MAST	September 2014	³²
13.	Lahey Clinic	β -Lactamases	β -lactamase classification and existing allelic no. ^b	March 2015	
14.	Institut Pasteur	OKP, LEN, OXY	MLST database with additional information on specific β -lactamases	August 2015	
15.	Tetracycline + MES nomenclature	Tetracycline and macrolide AR genes	Information on resistance mechanisms and nomenclature	June 2015	
16.	ABRES Finder	All AR genes	Links to external databases		
17.	INTEGRALL	Integron types and genetic context of AR genes	Webtool, BLASTn	August 2015	³³
18.	RAC	Genetic context of AR genes	Webtool, resistance gene cassette annotation		³⁴
19.	MvirDB	Virulence and toxin factors	BLASTn, BLASTp, link to ARGODB for AR genes	April 2014	³⁵

^a Data are based on information available on the respective websites and/or in the respective publications.

^b Data have been moved to http://www.ncbi.nlm.nih.gov/pathogens/submit_beta_lactamase/.

Culturable and/or pathogenic bacteria.

Typically thousands of alleles/hundreds of genes.

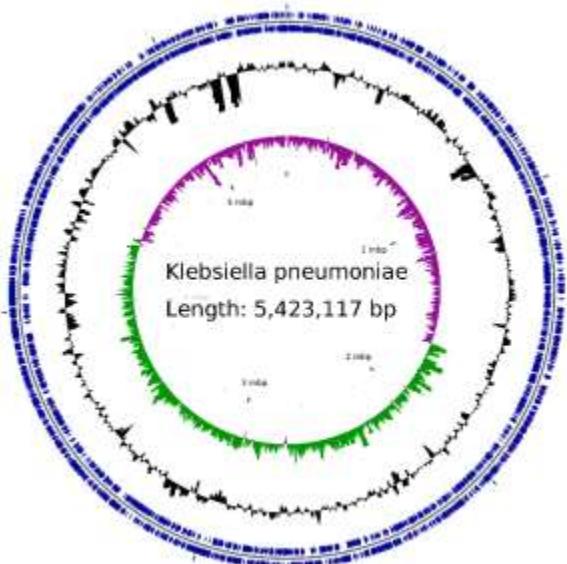
The « resistome » at the strain level

Analysis of the Resistome of a Multidrug-Resistant NDM-1-Producing *Escherichia coli* Strain by High-Throughput Genome Sequencing[▽]

Laurent Poirel, Rémy A. Bonnin, and Patrice Nordmann*

Gene	Genetic support	Protein	Function	Resistance [□]
<i>bla</i> _{NDM-1}	p271A	NDM-1	Class B β-lactamase	All β-lactams, including carbapenems, except aztreonam
<i>bla</i> _{TEM-1}	p271B and -C	TEM-1	Class A β-lactamase	Narrow-spectrum β-lactams
<i>bla</i> _{OXA-1}	p271C	OXA-1	Class D β-lactamase	Narrow-spectrum β-lactams
<i>bla</i> _{OXA-9}	p271B	OXA-9	Class D β-lactamase	Narrow-spectrum β-lactams
<i>bla</i> _{OXA-10}	p271C	OXA-10	Class D β-lactamase	Narrow-spectrum β-lactams
<i>bla</i> _{CTX-M-15}	p271B	CTX-M-15	Class A β-lactamase	Broad-spectrum β-lactams, including extended-spectrum cephalosporins
<i>armA</i>	p271B	ArmA	16S RNA methylase	All aminoglycosides
<i>rmtB</i>	p271C	RmtB	16S RNA methylase	All aminoglycosides
<i>aadA1</i>	p271B	AADA1	Adenylyltransferase	Streptomycin and spectinomycin
<i>aadA6</i>	p271D	AADA6	Adenylyltransferase	Streptomycin and spectinomycin
<i>aphA1-LAB</i>	p271C	AphA	Phosphotransferase	Kanamycin
<i>aacC2</i>	p271D	Aac(3')-II	Acetyltransferase	Gentamicin
<i>ermB</i>	p271C	ErmB	rRNA methylase	Macrolides, lincosamides, streptogramin B
<i>mph2</i>	p271B	MphB	Phosphorylase	Macrolides
<i>mel</i>	p271B	Mel	Efflux pump	Macrolides
<i>dfrA1</i>	p271D	DfrA1	Dihydrofolate reductase	Trimethoprim
<i>dfrA12</i>	p271B	DfrA-XII	Dihydrofolate reductase	Trimethoprim
<i>arr2</i>	p271B	Arr-2	ADP-ribosyl transferase	Rifampin
<i>cmlA5</i>	p271B	CmlA5	Efflux protein	Chloramphenicol
<i>catB4</i>	p271C	CatB4	Acetyltransferase	Chloramphenicol
<i>sull</i>	p271B, -C, and -D	Sull	Reductase	Sulfonamides
<i>qepA</i>	p271C	QepA	Efflux pump	Fluoroquinolones
<i>qacEΔJ</i>	p271B, -C, and -D	QacE	Efflux pump	Quaternary ammonium
<i>merRTPADE</i>	p271D	Mer	Efflux pump	Heavy metals
<i>ampC</i>	Chromosome	AmpC	Class C β-lactamase	Narrow-spectrum β-lactams (expression at low level)
<i>ompC</i>	Chromosome	OmpC	Mutated porin	Carbapenems(?), other antibiotics(?)
<i>ompF</i>	Chromosome	OmpF	Truncated porin	β-Lactams, other antibiotics(?)
<i>gyrA</i>	Chromosome	GyrA	Mutated topoisomerase	Quinolones, fluoroquinolones
<i>parC</i>	Chromosome	ParC	Modified topoisomerase	Quinolones, fluoroquinolones

The « resistome » at the strain level

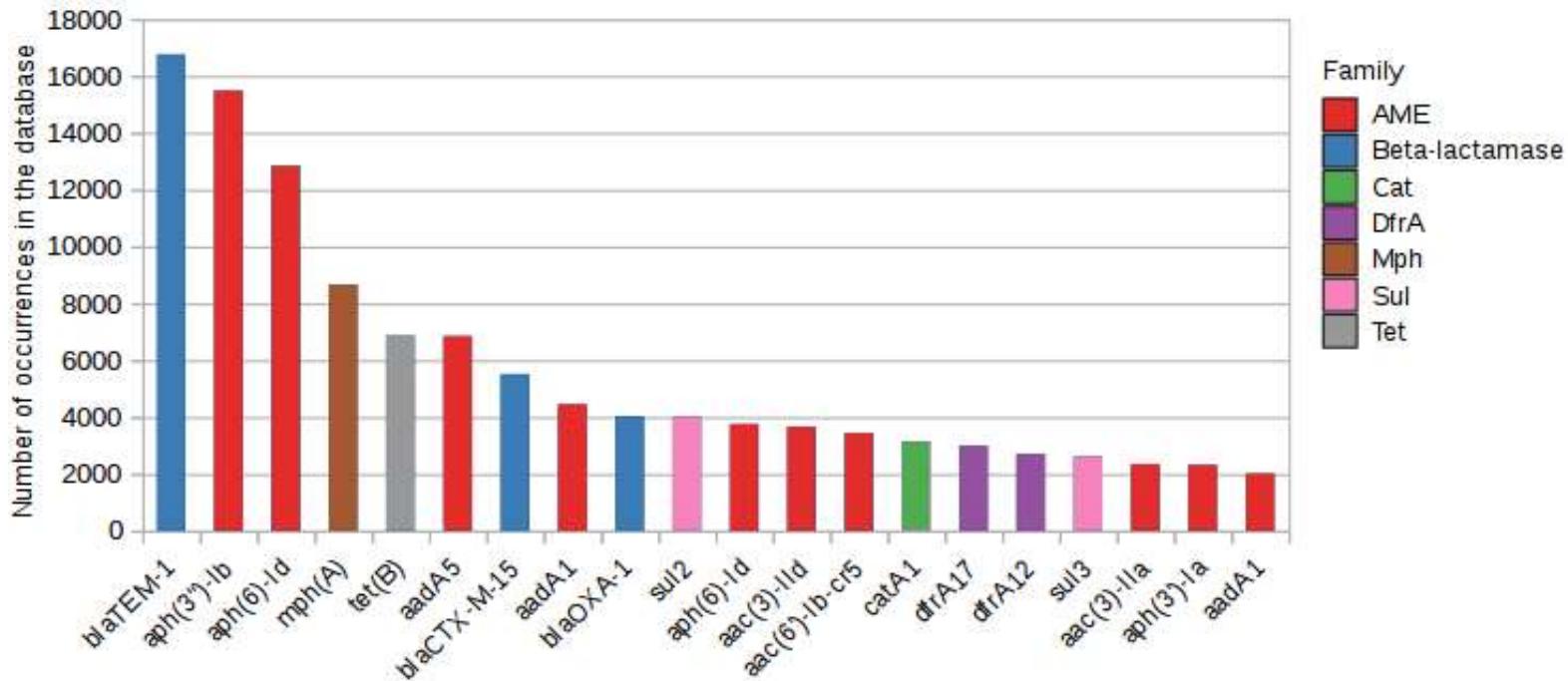


Inference of susceptibility
(next talk)
(spoiler: it works!)

Unexplained phenotype: underlying
mechanisms?



The « resistome » at the species level

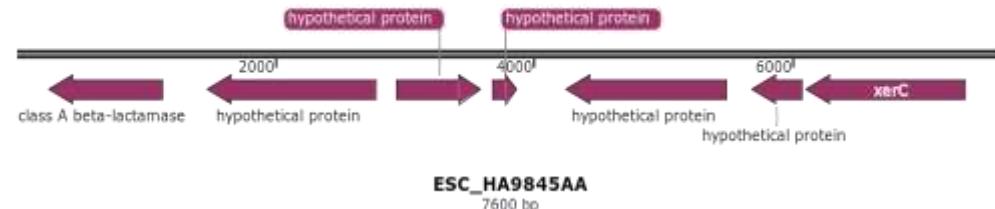


High-throughput screening brings surprises

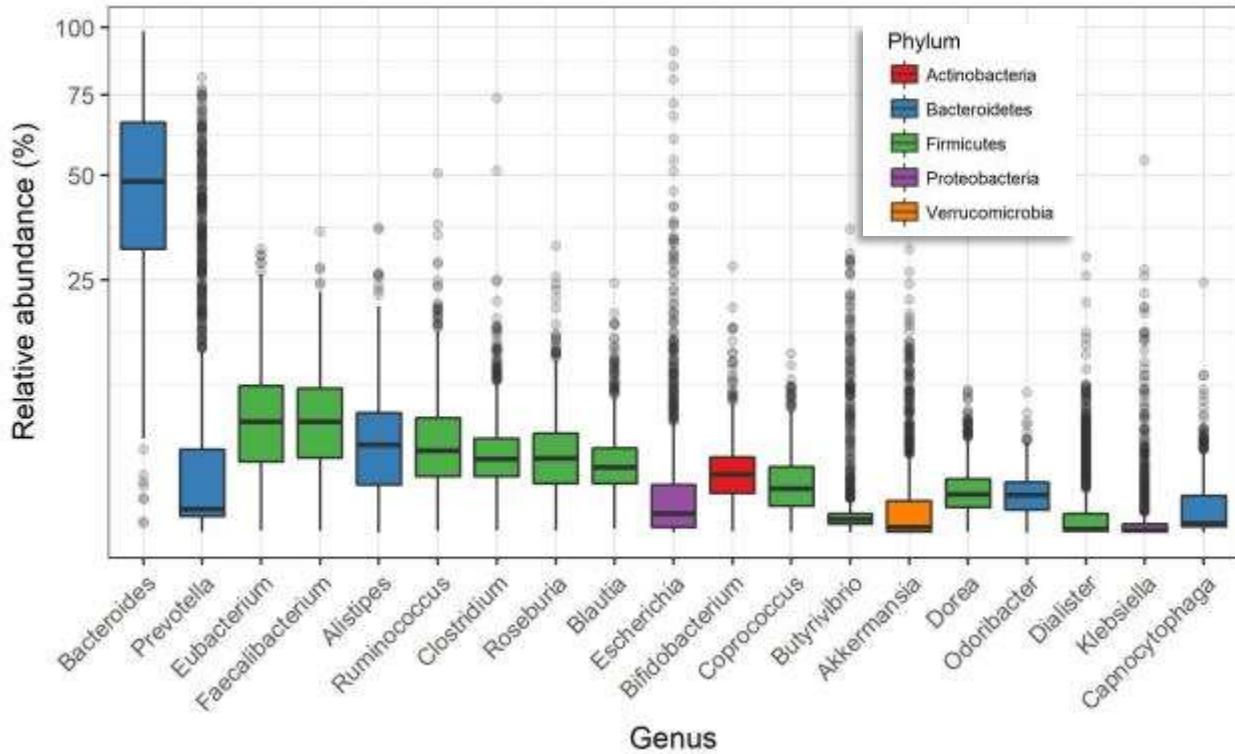


Identification of a beta-lactamase from
Bacteroides uniformis in *E. coli* o_O

Typical ESBL phenotype



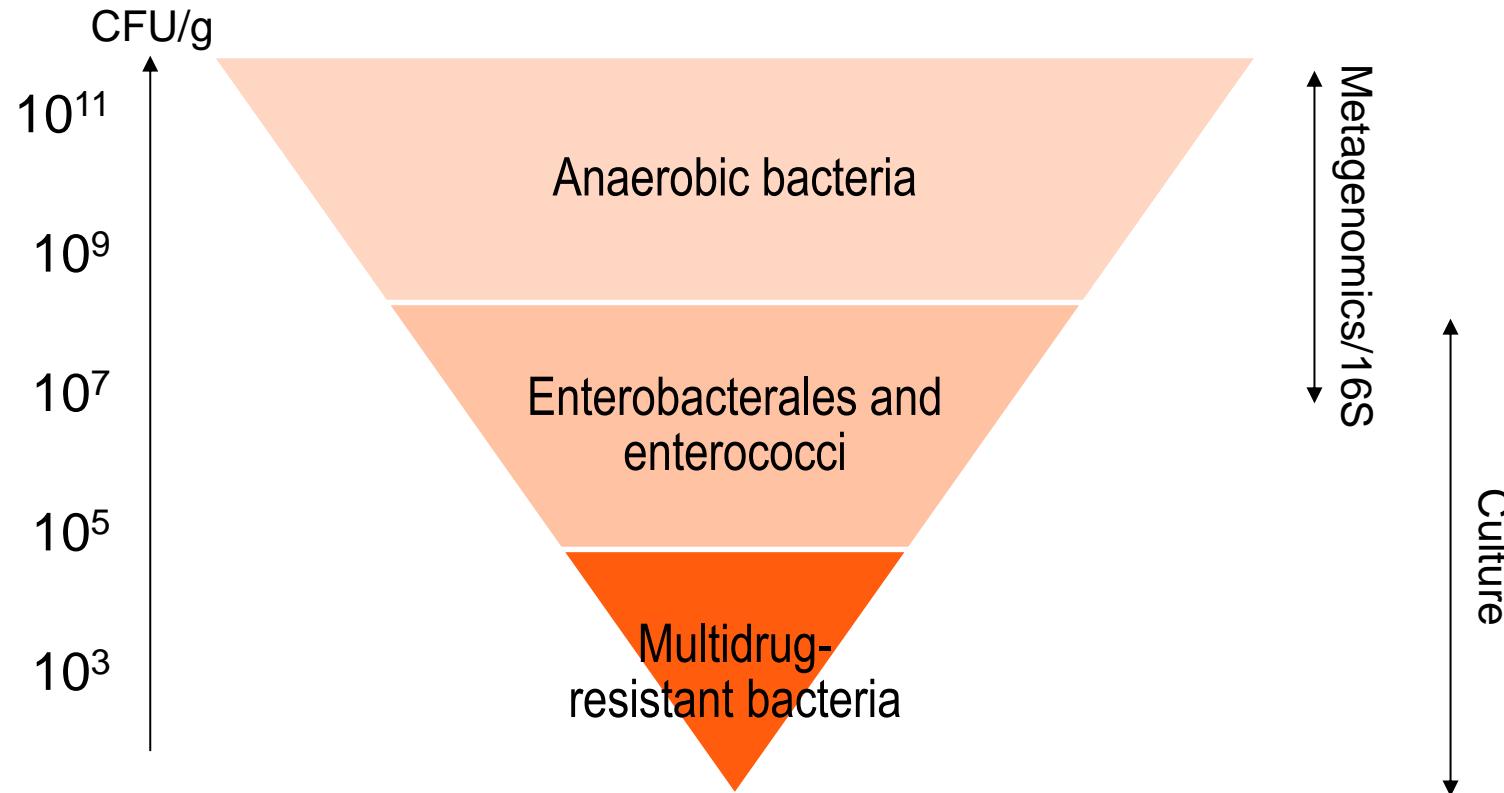
The intestinal microbiota



1. Huge number of bacterial cells (3.8×10^{13}), pathogens are subdominant
2. Great diversity (estimated hundreds of species)
3. Mostly un(hardly)culturable bacteria



Metagenomic sequencing lacks sensitivity



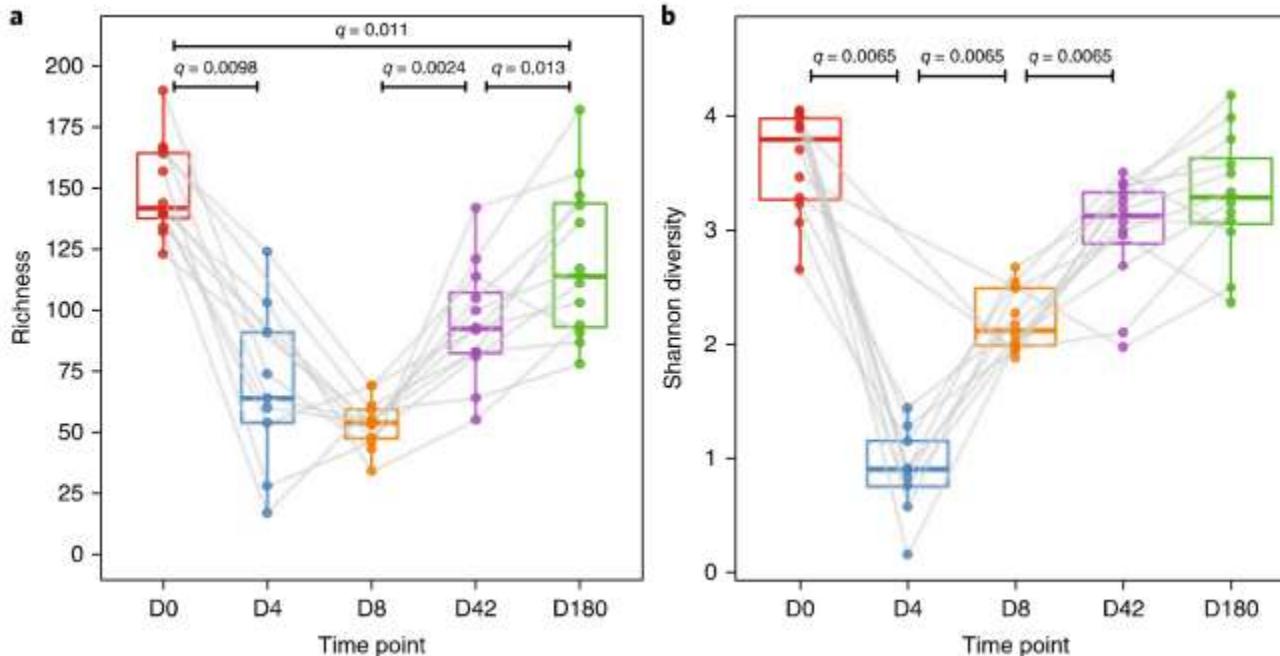
The intestinal resistome

	Forslund, K. et al	Ghosh, TS. Et al	Hu, Y. et al.
Journal	Genome Research	Plos One	Nature Communications
Published in	2013	2013	2013
N individuals	252	257	162
Origin of individuals	American, Danish, Spanish	American, Danish, French, Italian, Japanese, Spanish, Indian, Chinese	Danish, Spanish, Chinese
ARD reference database	ARDB (enriched in-house)	ARDB	ARDB
Search algorithm	Blastn	Blastx	Blastp
N unique ARDs (>95%)	100	157	156
Beta-lactamases	TEM, SHV, AmpC_E. coli, CCRA, CBLA, CFXA, CEPA	TEM, LEN, SHV, OXY,CTX-M, CFXA, CBLA, CEPA, AmpC_E. coli, CMY-2	KPC, ROB, TEM, CTX-M, OXY, PER, SHV, CARB, PSE, LCR, OXA-1, OXA, SME, L1, IMP

Only a few hundreds of ARGs among millions of genes?
> Issue of the ARG database (ARGB not curated)
> Issue of the searching method

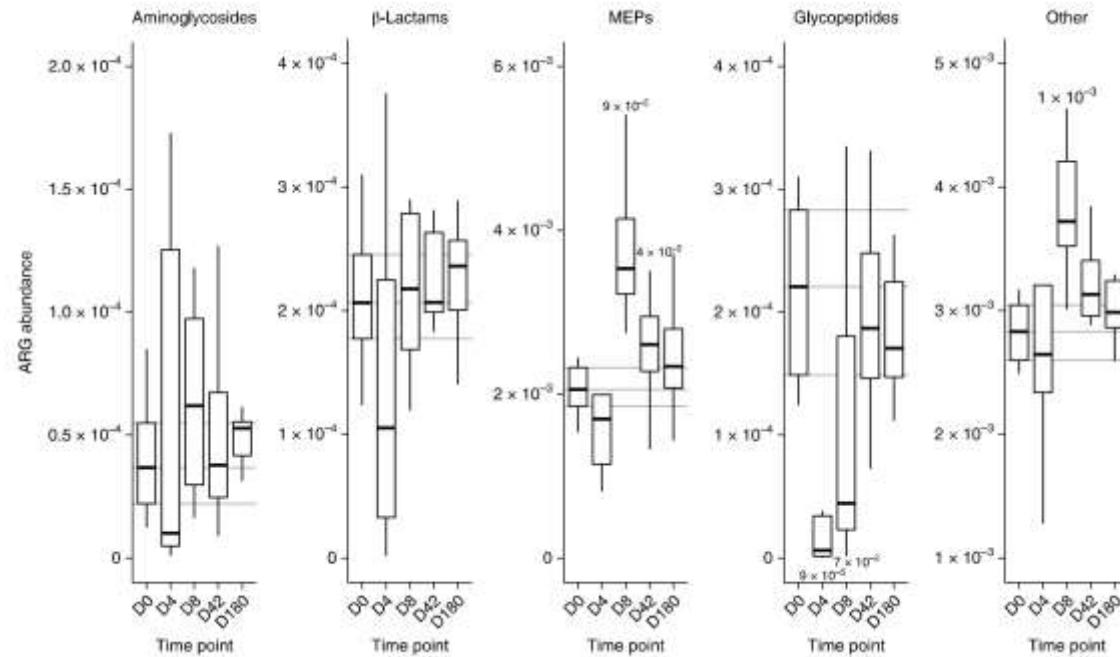
Effects of a short high exposure to antibiotics on the intestinal microbiota

12 healthy volunteers, 4 day-course of meropenem, vancomycin and colistin.

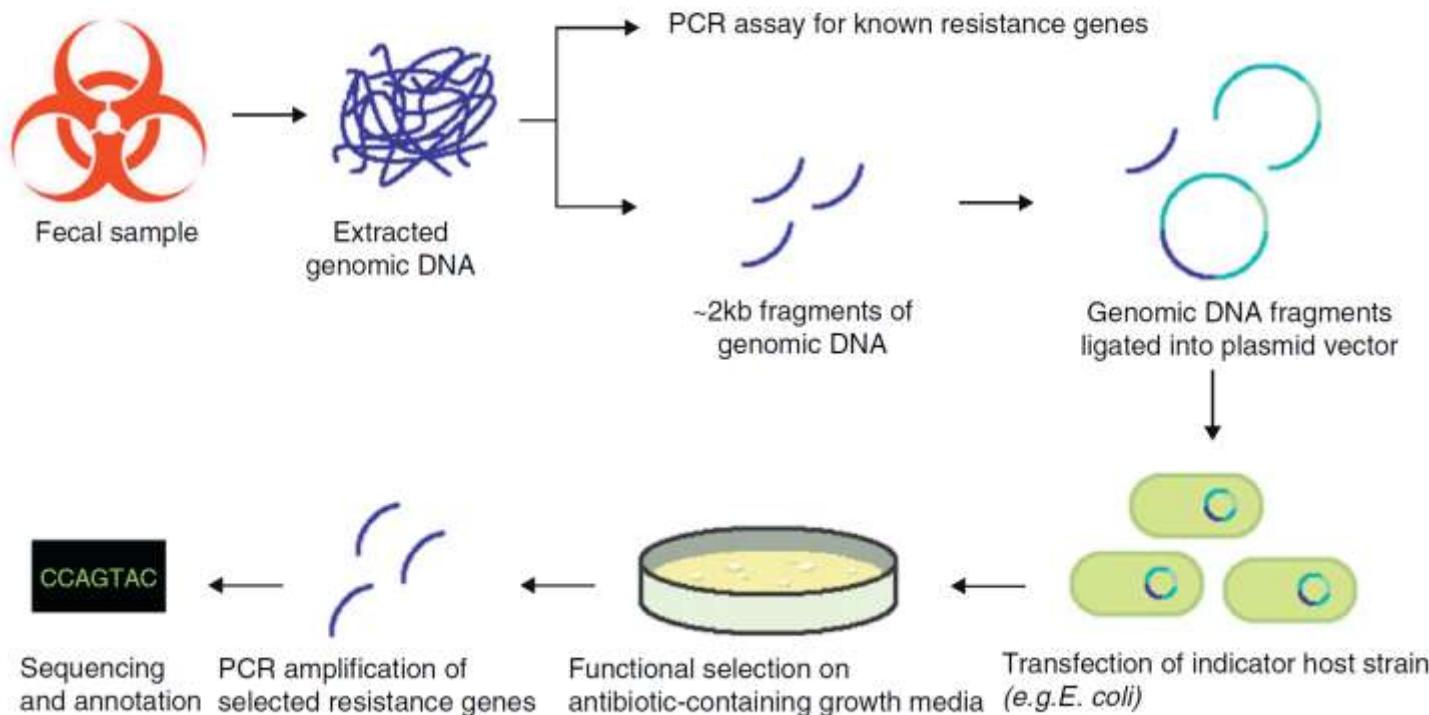


Effects of a short high exposure to antibiotics on the resistome

12 healthy volunteers, 4 day-course of meropenem, vancomycin and colistin.

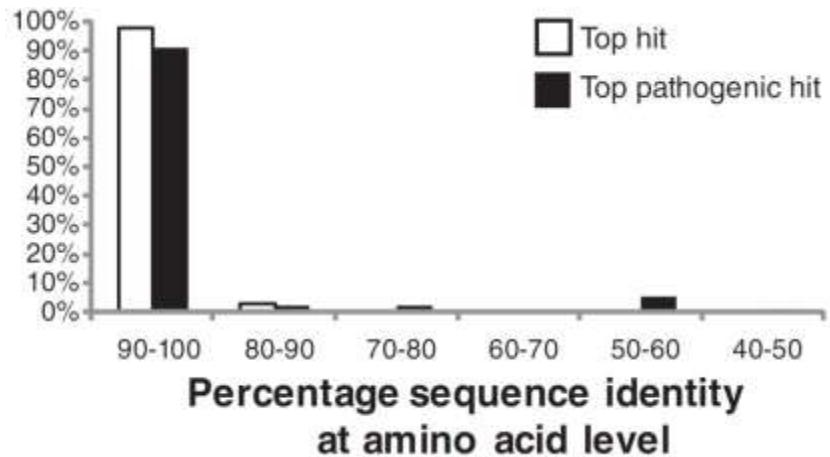


Functional metagenomics

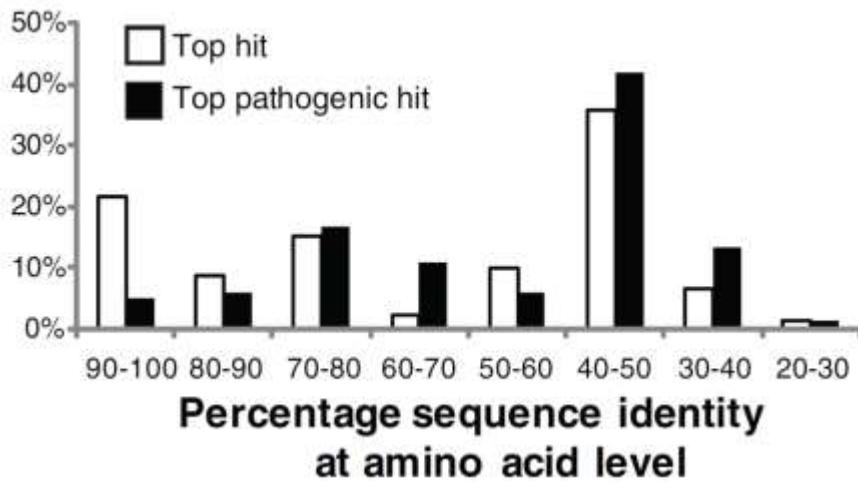


Functional metagenomics

Culturable → ARG databases



Non-culturable → under the radar

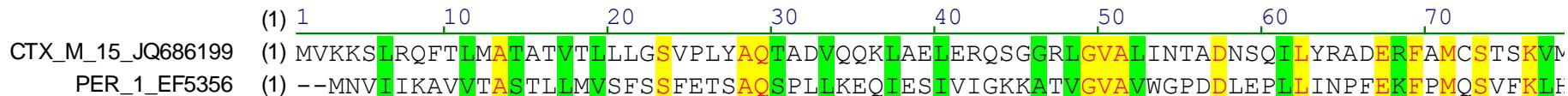


The expected iceberg thing

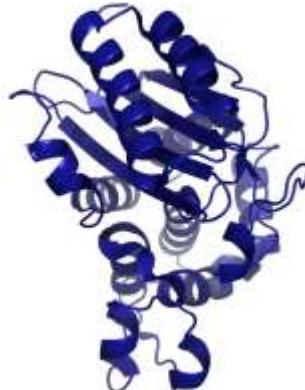


Leveraging the protein structure to find ARG

PER-1 and CTX-M-15 do not share more than 21% identity in aminoacid (1-dimension)



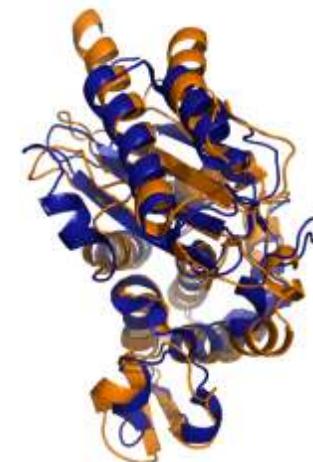
But they do share a high homology in 3-dimensions!



CTX-M-15

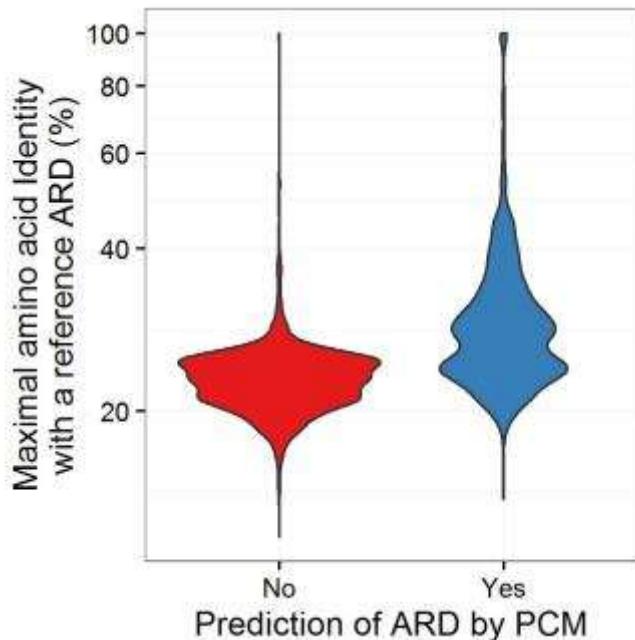


PER-1



Alignment of CTX-M-15 and PER-1
(TMscore > 0.9)

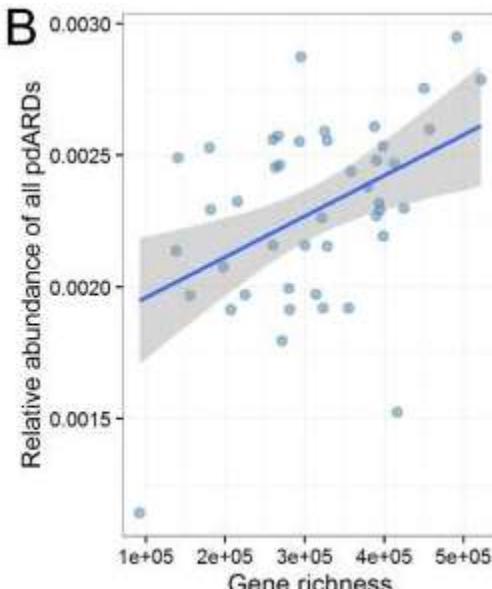
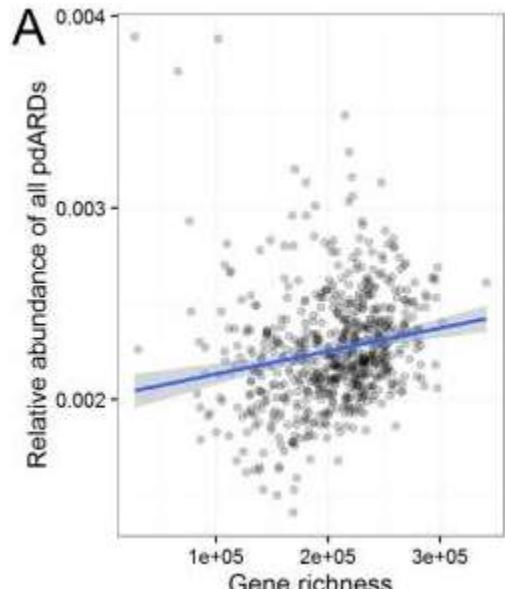
The intestinal resistome



6,095 ARGs in a 3,9M protein catalogue
Mostly distant from known ARG
Mostly chromosomal

Beneficial??

Could ARG be good to us?



Positive link between gene richness and the abundance of ARG

Could ARG be good to us?

Table 1. Ceftriaxone concentration, β -lactamase activity, and bacterial counts in feces of six volunteers before and after one daily intravenous injection of 1 g of ceftriaxone for 5 d.

	Volunteer					
	1	2	3	4	5	6
Ceftriaxone concentration (μ g/g of feces)						
Before	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
After*	<0.1	1,800	<0.1	<0.1	2,000	<0.1
β -lactamase activity†						
Before	3+	1+	1+	3+	0	0
After	4+	0	4+	4+	0	4+

The more intestinal beta-lactamases, the more the gut is protected from beta-lactams



KEEP
MICROBIOTA
 **GREAT**

The word "MICROBIOTA" is written in white capital letters. The letter "I" is replaced by a small blue map of the state of Minnesota. Below the word "GREAT" are six stars arranged in two rows of three. The first and fourth stars from the left are red, the second and fifth are white, and the third and sixth are blue.

2020

Oral beta-lactamase prevents from *C. difficile* infection



P1A beta-lactamase from *Bacillus subtilis* and its derivative P3A manufactured in *E. coli* (Asp276Asn): Ribaxamase

	Placebo (N=206)	Ribaxamase (N=206)
Local laboratory-confirmed <i>C difficile</i> infections		
Number of patients (%)	7 (3.4%)	2 (1.0%)
Risk reduction (95% CI)	..	2.4% (-0.6 to 5.9)*
One-sided p value†	..	0.045
Central laboratory-confirmed <i>C difficile</i> infections		
Number of patients (%)	8 (3.9%)	2 (1.0%)
Risk reduction (95% CI)	..	2.9% (-0.2 to 6.6)
p value	..	0.027
Patients receiving treatment for <i>C difficile</i> infections‡		
Number of patients (%)	6 (2.9%)	1 (0.5%)
Risk reduction (95% CI)	..	2.4% (-0.3 to 5.8)
p value	..	0.028

	Placebo	Ribaxamase	p value
<i>Clostridium difficile</i>			
Screening	5 (2%)	3 (1%)	0.239
End of treatment period 2	14 (8%)	7 (4%)	0.059
4-week follow-up visit	18 (9%)	11 (6%)	0.088
Vancomycin-resistant enterococci			
Screening	8 (4%)	5 (2%)	0.198
End of treatment period 2	69 (37%)	36 (19%)	0.0001
4-week follow-up visit	71 (36%)	40 (20%)	0.0002
Extended-spectrum, β-lactamase-producing Gram-negative bacilli			
Screening	46 (22%)	37 (18%)	0.134
End of treatment period 2	30 (16%)	31 (17%)	0.565
4-week follow-up visit	44 (22%)	49 (25%)	0.714

Take-home messages

The resistome is the census of ARGs in a given sample (strain/environment/microbiota)

The resistome of culturable/pathogenic bacteria is well-characterized and can be used in diagnostic

But knowledge on the other resistomes is lacking

We need to identify and phenotypically characterize ARGs outside culturable bacteria

Not all ARGs are bad!

Exciting times!!!



International Conference on Clinical Metagenomics

C O V I D - 1 9

A VIRTUAL CONFERENCE OCTOBER 15-16 2020

www.clinicalmetagenomics.org



Happy to take questions!



etienne.ruppe@inserm.fr

