WHAT CAN WE LEARN FROM METAGENOMICS ABOUT ANTIBIOTIC RESISTANCE ?

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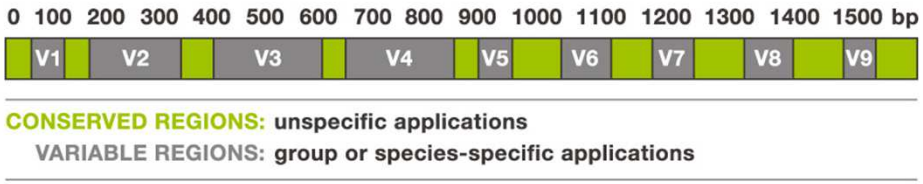
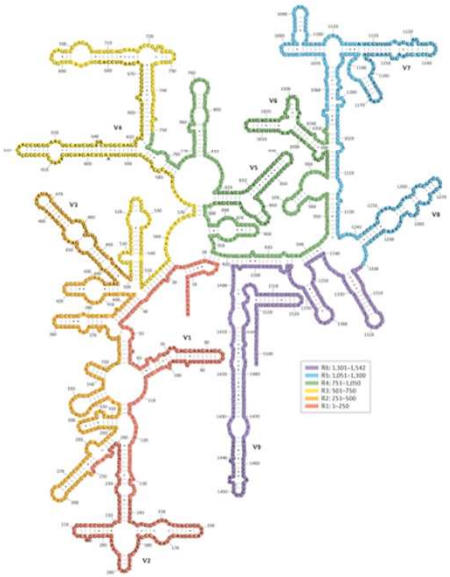
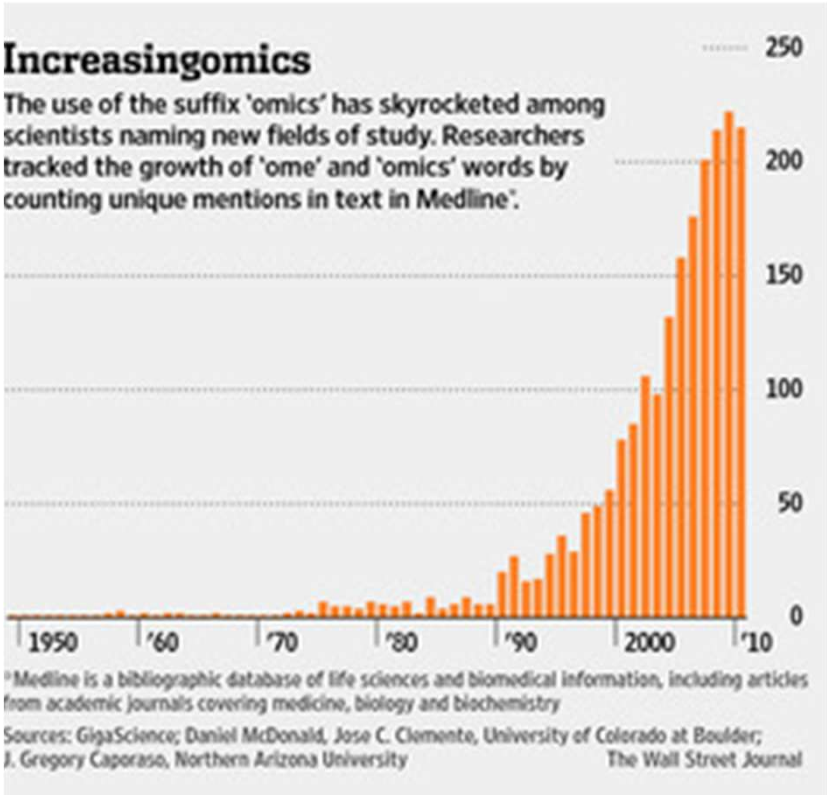
First: what is referred as “metagenomics”?

Metagenomics/metatranscriptomics: refers to the study of genetic material (DNA or RNA) recovered directly from complex samples where diverse organisms can be found.

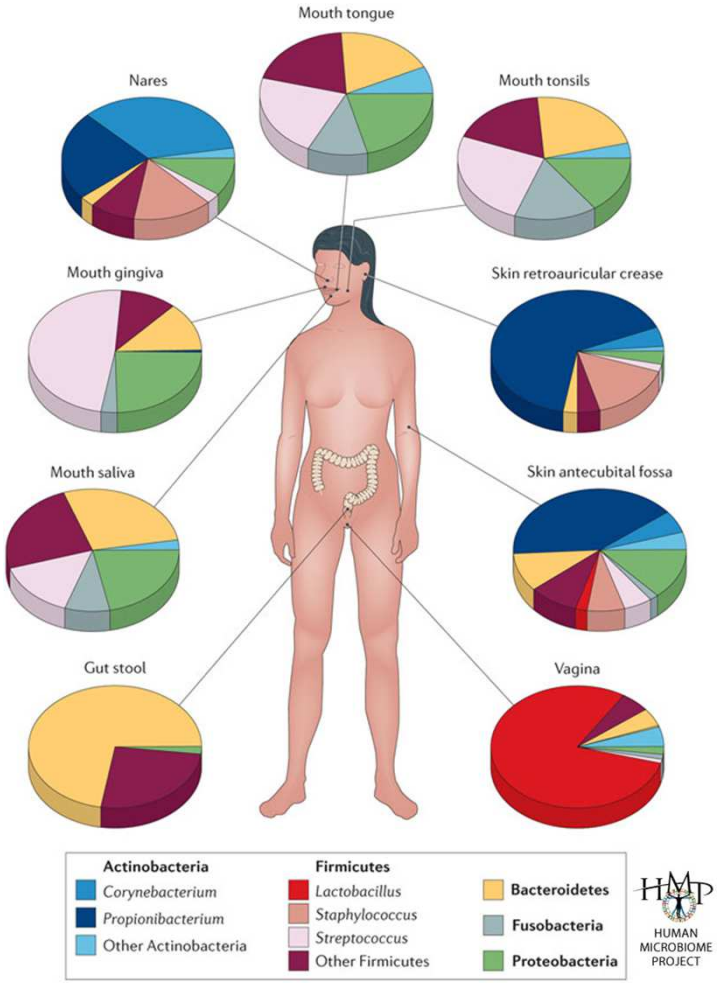
16S profiling: amplification and sequencing of a part or the entire 16SrRNA gene. Does not allow the detection of functions (including antibiotic resistances genes)



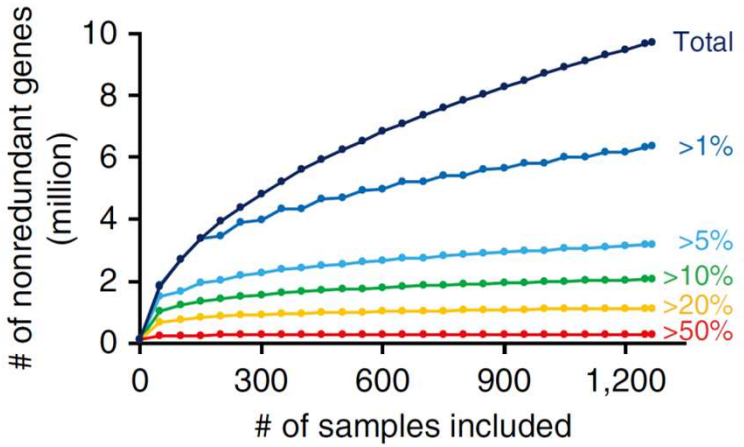


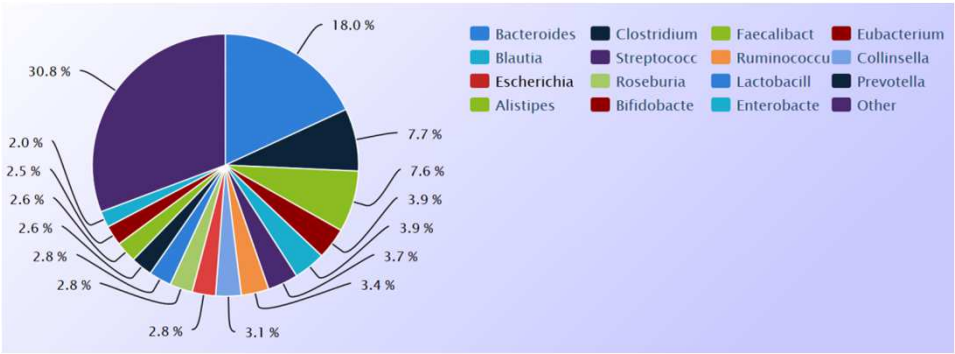


16S profiling and metagenomics allowed a more in-depth vision of our microbiota



A 10M genes catalogue!



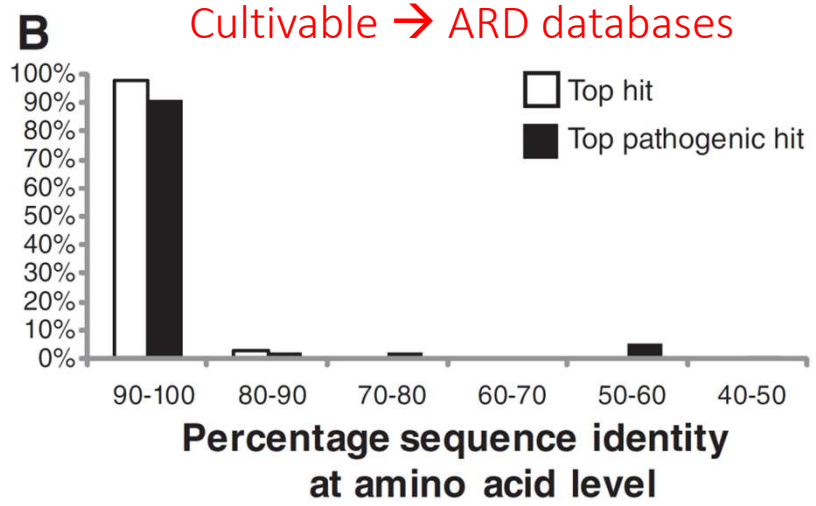


1. Huge number of bacterial cells ($3.8x10^{13}$), pathogens are subdominant
2. Great diversity (estimated hundreds of species)
3. Mostly un(hardly)culturable bacteria

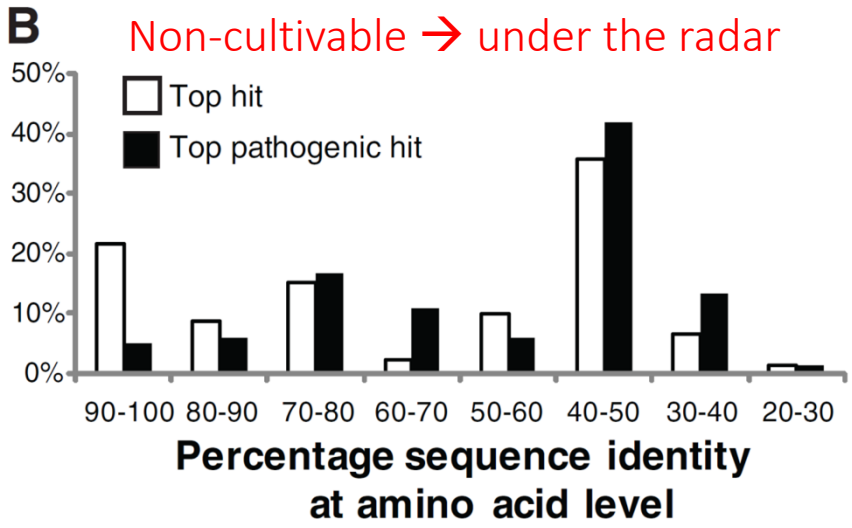
Lasken RS, McLean JS. *Nature Reviews Genetics*. 2014; 15(9):577–584. 1. **Li, J.** *et al. Nat. Biotechnol.* **32,** 834–841 (2014). **Sender, R.** *et al. PLoS Biol.* **14,** e1002533 (2016).

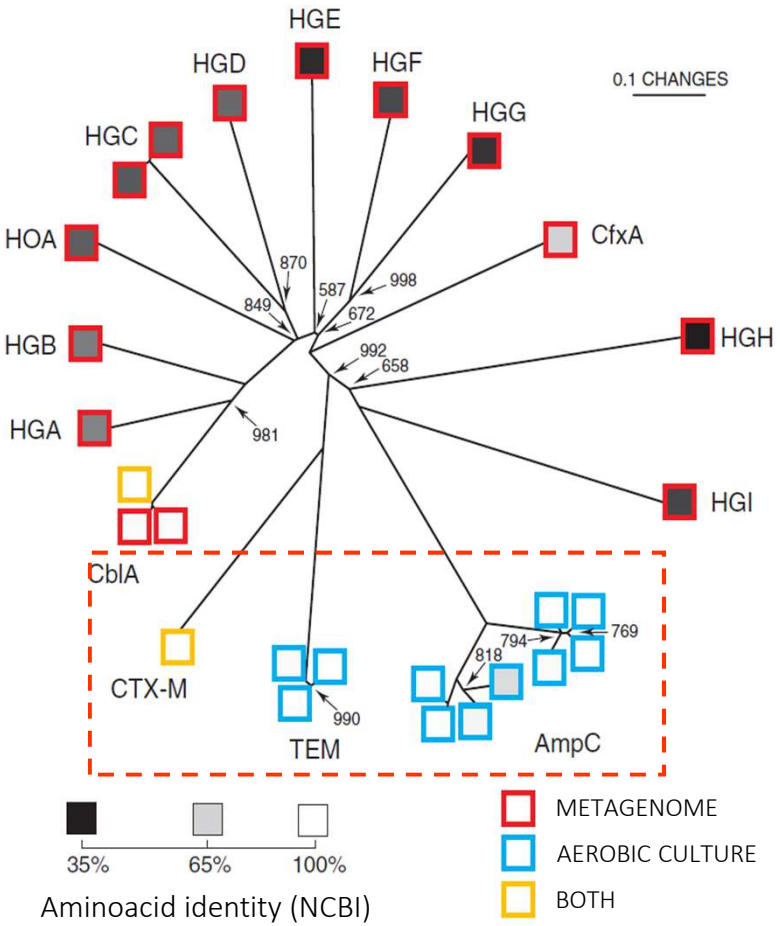
A clue from functional metagenomics… ARDs from the intestinal microbiota might differ from those in the databases

Cultivable → ARD databases



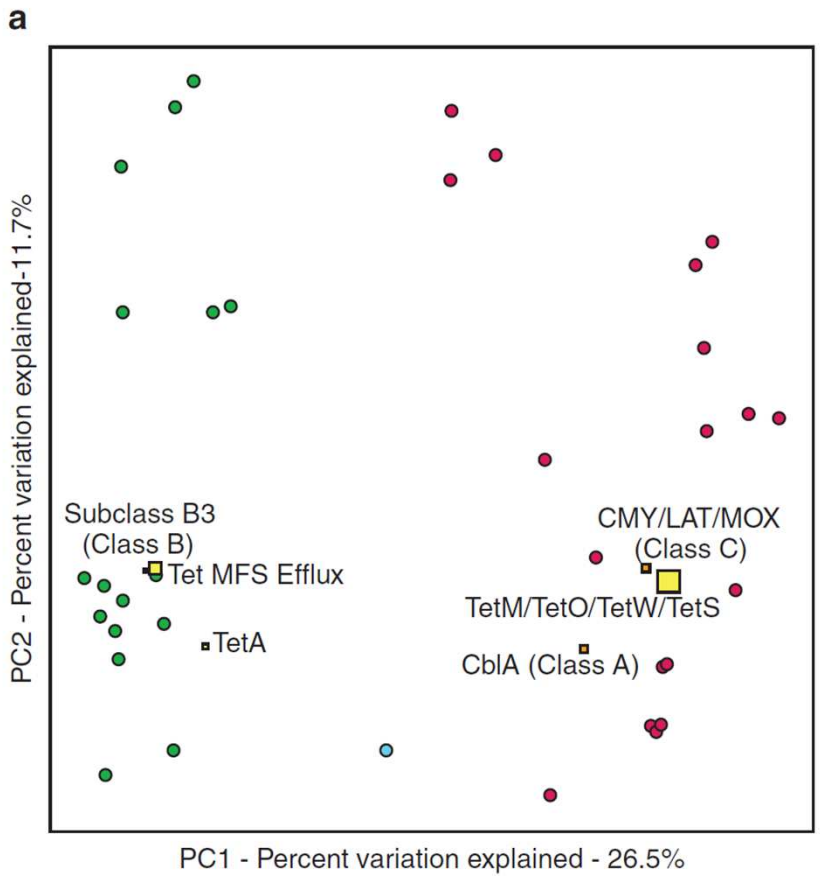
Non-cultivable → under the radar

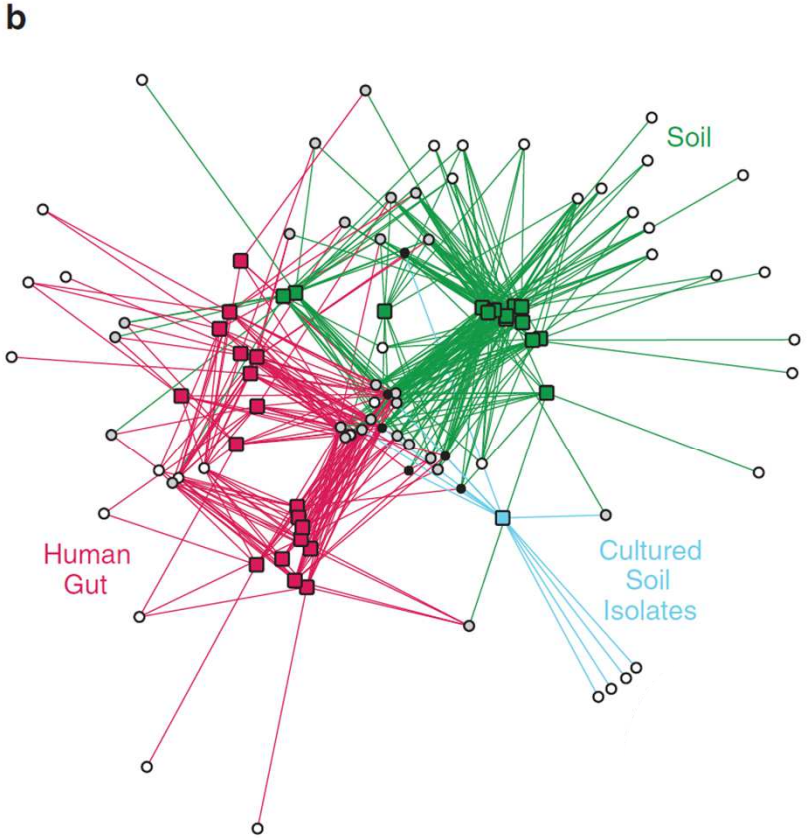




Sommer, M.O., *et al. Science* 325, 1128-1131 (2009).

The human resistome does not resemble that of soils





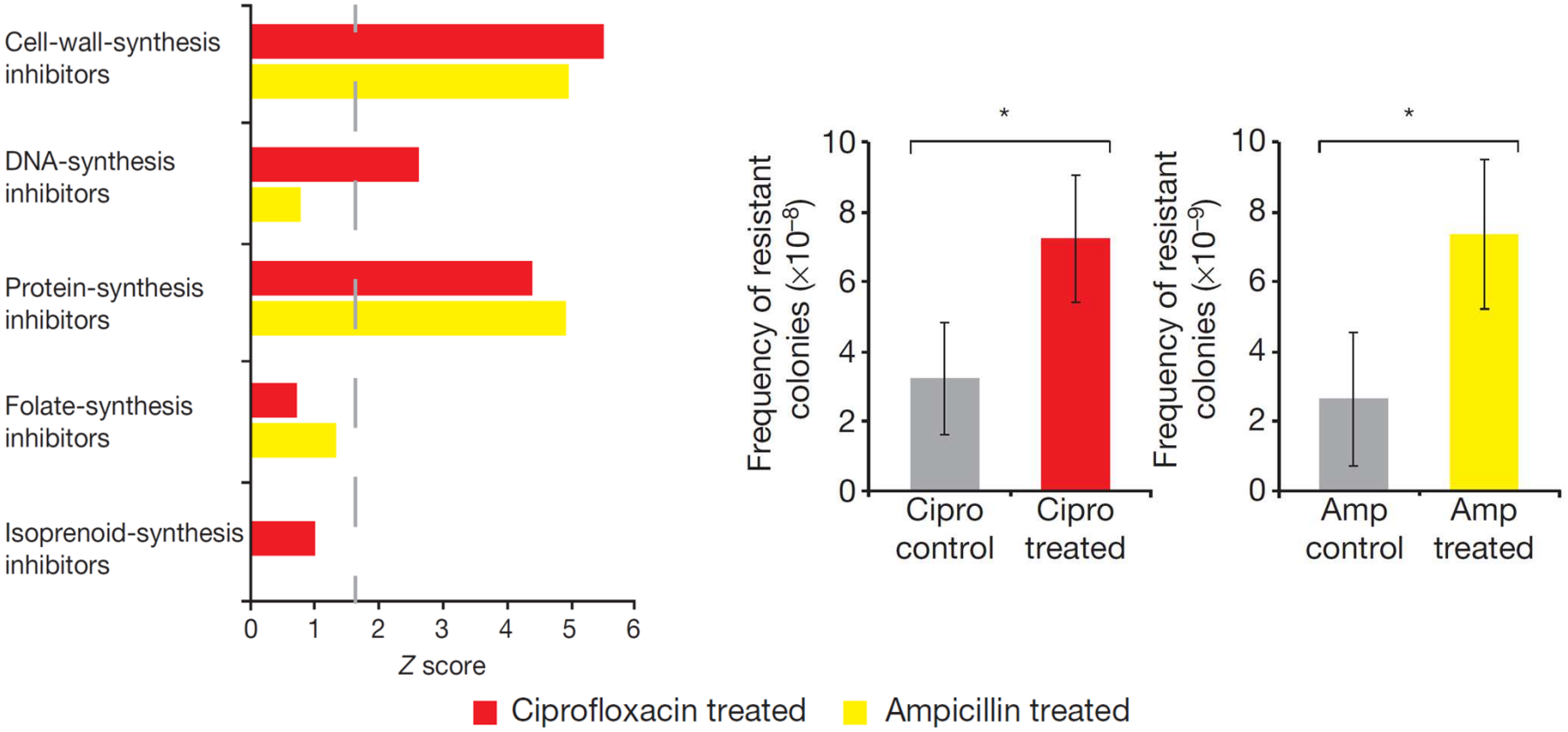
- Main drivers of the difference between human, soils and isolates resistome
- Sample

- Sample
- Function unique to one ecosystem
- Function shared between 2 ecosystems
- Function shared between 3 ecosystems

Gibson, *et al*. *ISME J* **9,** 207–216 (2015).

Do phages participate in the exchange of ARDs in the microbiota?

Exposition of mice to ampicillin or ciprofloxacin
Metagenomic sequencing of the phage fraction of feces.
More ARDs in the phageome of mice exposed to antibiotics… really?



Modi, S. R., et al. *Nature* **499,** 219–222 (2013).

Do phages really participate in the exchange of ARDs in the microbiota?

Phages rarely encode antibiotic resistance genes: a cautionary tale for virome analyses

François Enault[1,2], Arnaud Briet[3], Léa Bouteille[3], Simon Roux[4], Matthew B Sullivan[4,5] and Marie-Agnès Petit[3]

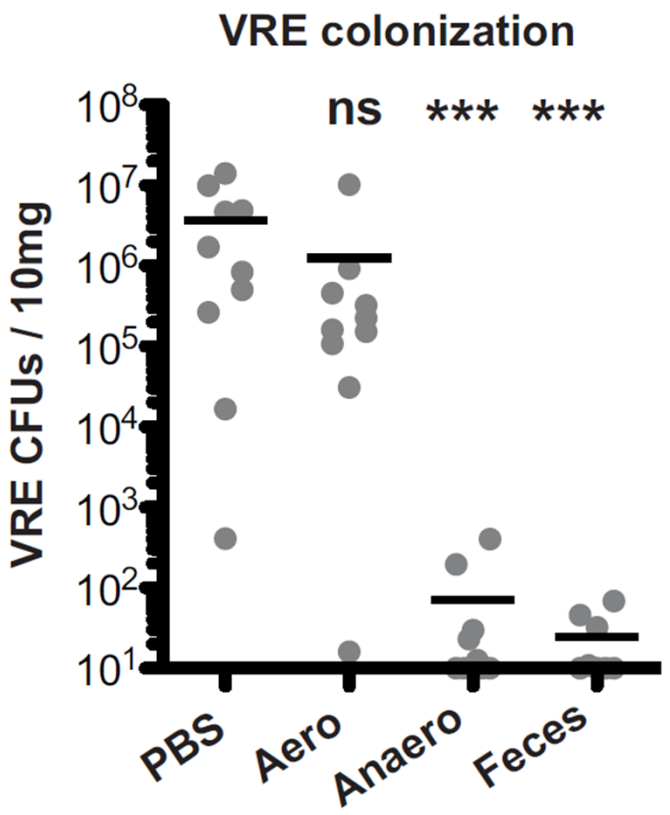
[1]*Clermont Université, Université Blaise Pascal, Laboratoire 'Microorganismes: Génome et Environnement', Clermont-Ferrand, France;* [2]*CNRS UMR 6023, LMGE, Aubière, France;* [3]*Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France;* [4]*Department of Microbiology, The Ohio State University, Columbus, OH, USA and* [5]*Department of Civil, Environmental and Geodetic Engineering, The Ohio State University, Columbus, OH, USA*

Antibiotic resistance genes (ARGs) are pervasive in gut microbiota, but it remains unclear how often ARGs are transferred, particularly to pathogens. Traditionally, ARG spread is attributed to horizontal transfer mediated either by DNA transformation, bacterial conjugation or generalized transduction. However, recent viral metagenome (virome) analyses suggest that ARGs are frequently carried by phages, which is inconsistent with the traditional view that phage genomes rarely encode ARGs. Here we used exploratory and conservative bioinformatic strategies found in the literature to detect ARGs in phage genomes, and experimentally assessed a subset of ARG predicted using exploratory thresholds. ARG abundances in 1181 phage genomes were vastly overestimated using exploratory thresholds (421 predicted vs 2 known), due to low similarities and matches to protein unrelated to antibiotic resistance. Consistent with this, four ARGs predicted using exploratory thresholds were experimentally evaluated and failed to confer antibiotic resistance in Escherichia coli. Reanalysis of available human- or mouse-associated viromes for ARGs and their genomic context suggested that bona fide ARG attributed to phages in viromes were previously overestimated. These findings provide guidance for documentation of ARG in viromes, and reassert that ARGs are rarely encoded in phages.

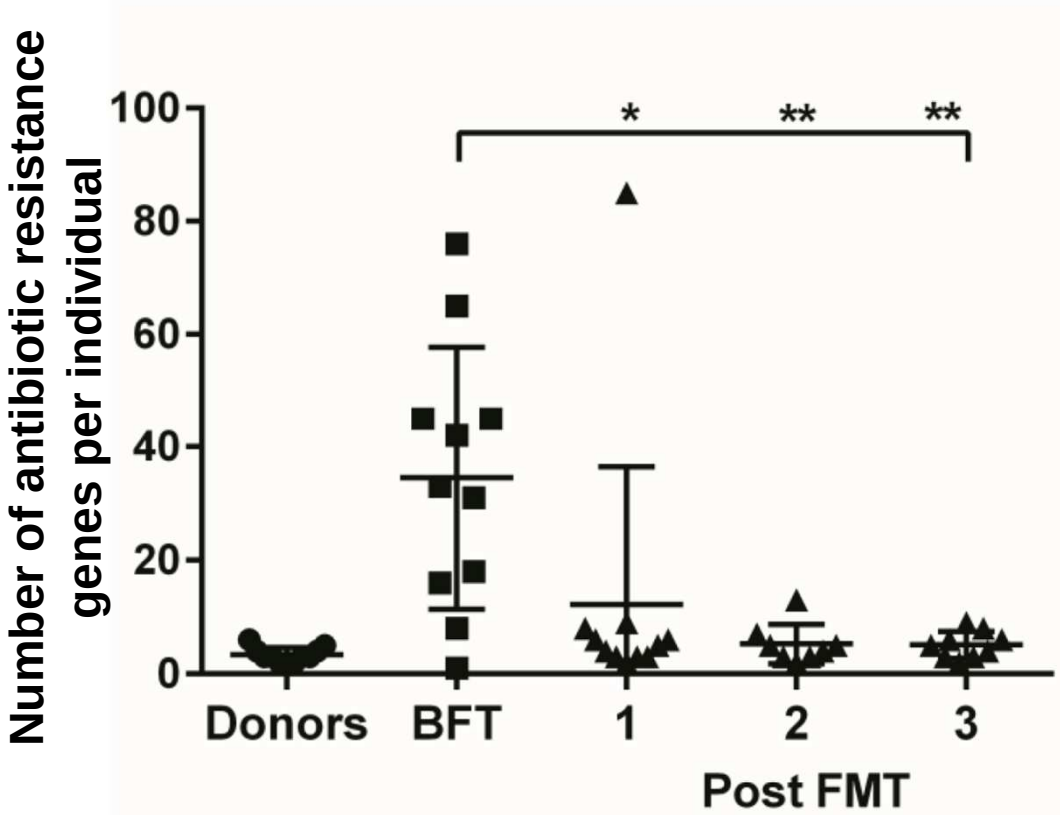
Enault, F. *et al. ISME J* (2016). doi:10.1038/ismej.2016.90

How about the interplay between the intestinal microbiota and multidrug resistant bacteria?

Mice[1]



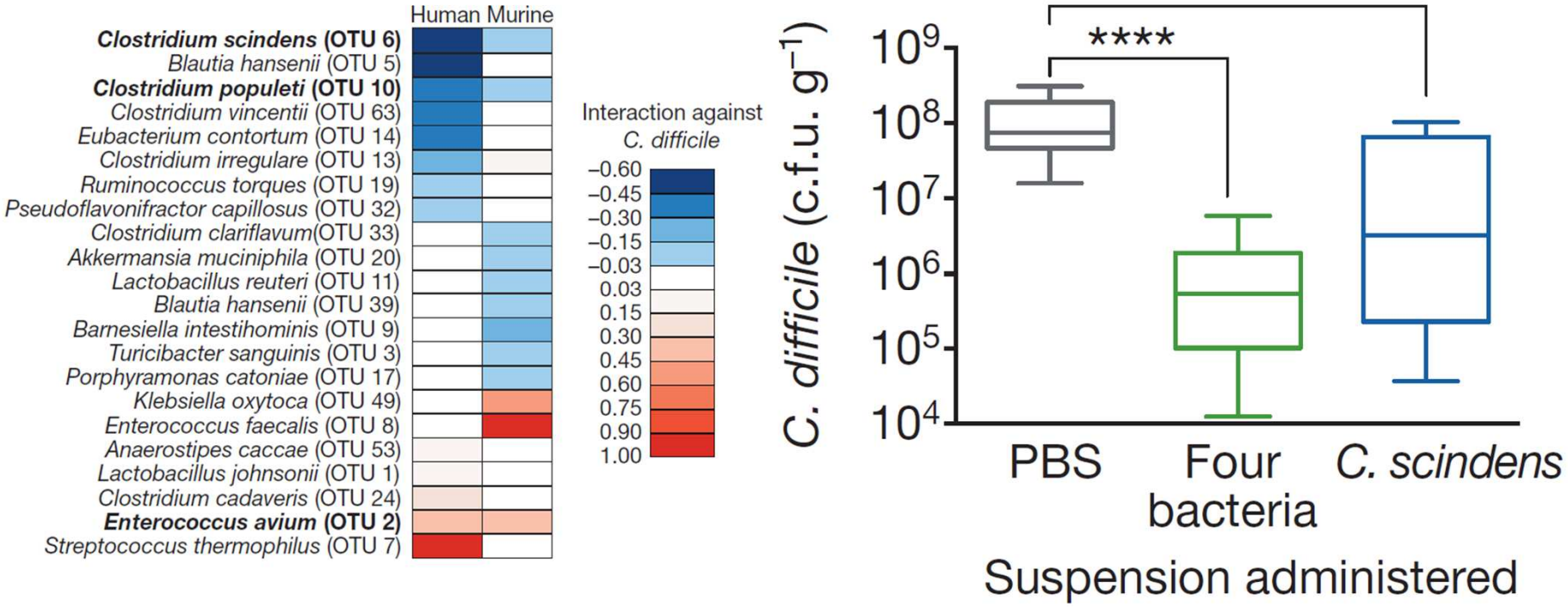
Human[2]



[1]Ubeda, C. et al. *Infect. Immun.* 81, 965–973 (2013); [2]Millan, B. *et al. Clin. Infect. Dis.* (2016). doi:10.1093/cid/ciw185

Some From metagenomic studies to a combination of bacteria that can repress the germination of *Clostridium difficile*

- 16S data (from human and mice): identification of OTUs associated with the repression of the growth of Cdif.
- In vivo, these strains indeed repress the groth of Cdif (through the production of secondary bile acids)



Buffie, C. G. *et al. Nature* (2014). doi:10.1038/nature13828; OTUs: operational taxonomic units

Take-home messages

Interplay between pathogens, ATB, resistance and commensals is far from being understood

The intestinal microbiota is indeed a major reservoir of ARDs

The transfer of ARDs between intestinal bacteria and pathogens is (for now) poorly supported.

Metagenomics can help us in identifying the key bacteria that can prevent from the growth from resistant bacteria, and/or favour their clearance

Yet, let's be cautious about the findings of metagenomic studies!

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See you in Geneva for more metagenomics!

International Conference on Clinical Metagenomics

October 19-20 2017