The microbiome and antibiotic resistance: opportunities for the microbiome business.

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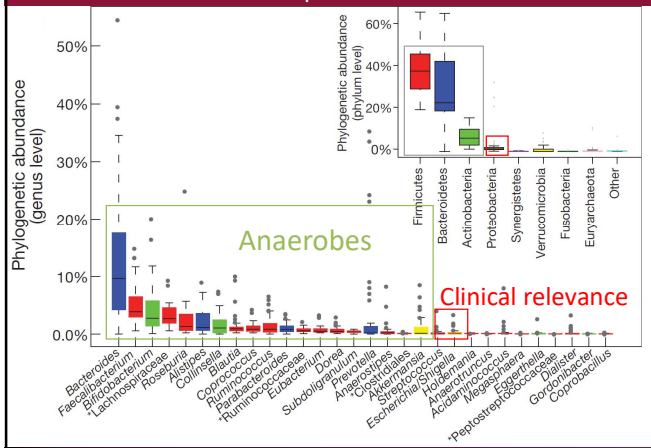
The intestinal microbiota

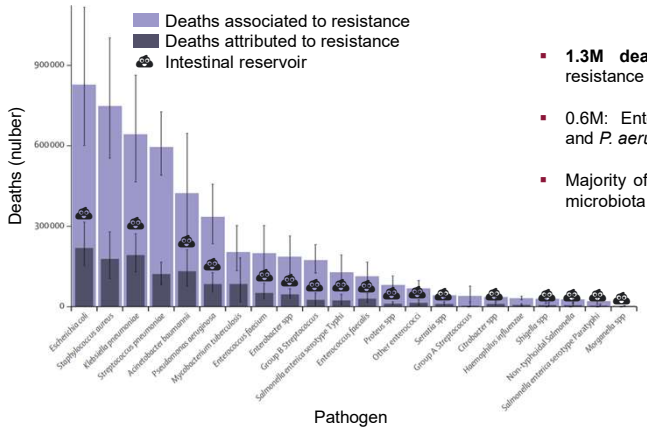
Composition of the intestinal microbiota



- **High number of bacteria nombre** ($3x10^{13}$)[1]
- **High diversity** (hundreds of species)
- Most bacteria are harldy culturable
- Pathogenic and antibiotic resistant bacteria are **sub-dominant : need for culture**

Figure from **Arumugam M.** Nature. (2011). [1]**Sender, R.** et al. *PLoS Biol* (2016);

The threat of antibiotic resistance lies in the gut



- **1.3M deaths** attributed to antibiotic resistance
- 0.6M: Enterobacterales, *A. baumannii* and *P. aeruginosa* (Proteobacteria)
- Majority of bacteria **present in the gut** microbiota before causing infections

Adapted from **Antimicrobial Resistance Collaborators**. et al. *The Lancet* **399**, 629–655 (2022).

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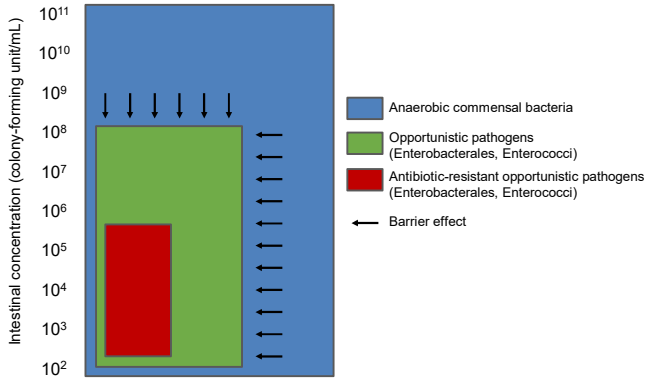
Let's see the microbiota from a quantitative perspective

The microbiota opposes sustainable colonization by exogenous bacteria: barrier effect or resistance to colonization.



From **Grall N et Ruppé E.** EMC Biologie, 2017.

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Opportunity #1: lower the concentrations of drug-resistant pathogens

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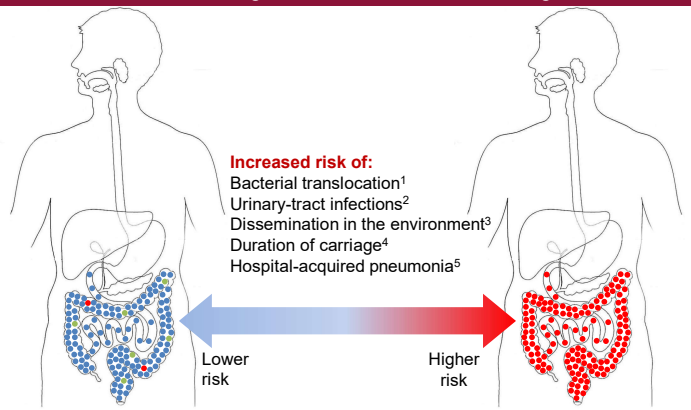
Quantification of multidrug-resistant bacteria in the gut: it matters!



1.Taur, Y. et al. Clin. Infect. Dis. 55, 905–914 (2012); 2.Ruppé, E. et al. Antimicrob. Agents Chemother. 57, 4512–4517 (2013); 3.Donskey, C.J. et al. N. Engl. J. Med. 343, 1925–1932 (2000); 4.Ruppé, E. et al. Clin. Infect. Dis. 61, 593–600 (2015); 5. Andremont, O. et al. Intensive Care Med (2020).

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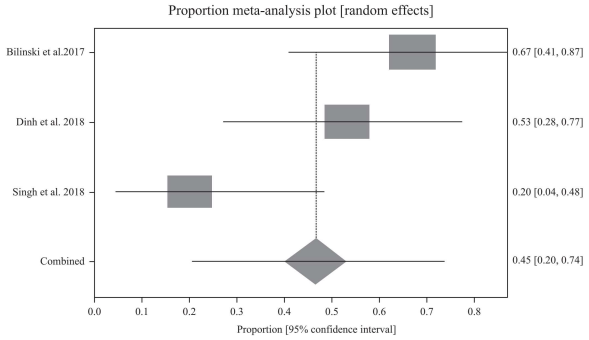
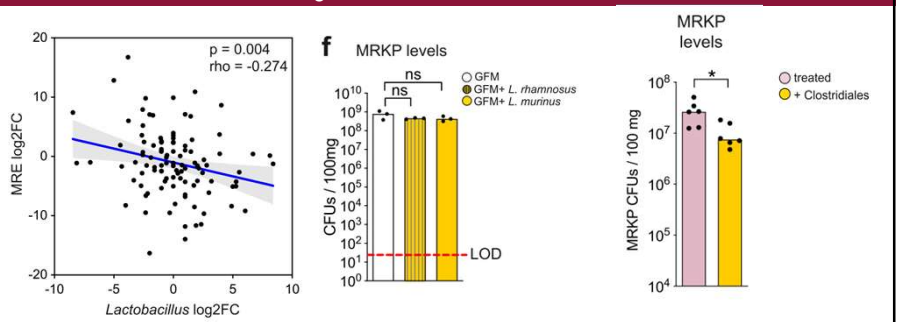
Fecal microbiota transplantation restores the barrier effect



Current evidence supports a better efficacy for *P. aeruginosa*, lower for *K. pneumoniae*. Observational studies: need for a randomized control trial

LBP potential: *Lactobacillus* supports Clostridiales to restrict gut colonization by multidrug-resistant Enterobacteriaceae



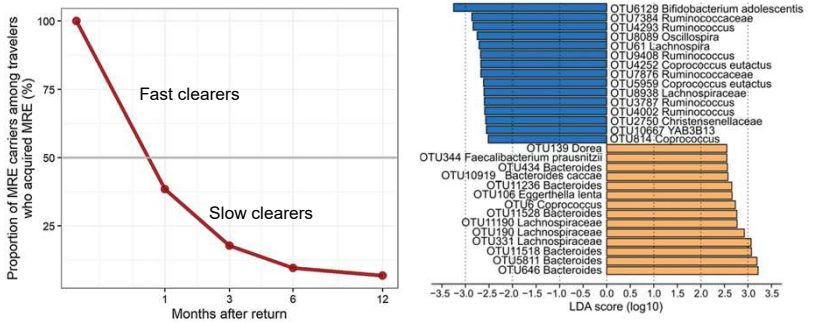
- *Lactobacillus* spp. is required but is not sufficient to restrict multidrug-resistant *K. pneumoniae* (MRKP) gut colonization in mice
- *Lactobacillus* promote the expansion of Clostridiales commensals which restricts MRKP gut colonization in mice.

Bad bugs travel with travellers

574 travellers to tropical regions
(screened negative for multidrug-resistant Enterobacteriaceae before departure)
MRE acquisition rate 51% (n=293)

Bad bugs travel with travellers

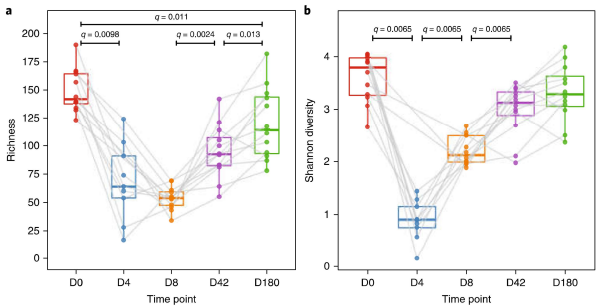
Travelers who got cleared of MRE 1 month after returning from a tropical region have a different microbiota from those who did not.



Opportunity #2: prevent from damaging the microbiota

Effects of a short high exposure to antibiotics

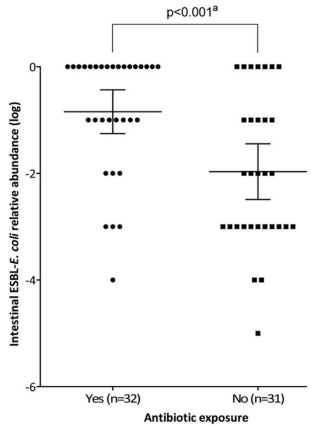
12 healthy volunteers, 4 day-course of oral meropenem, vancomycin and gentamicin.



Pace of recovery: **still unclear**

Palleja, A. et al. *Nature Microbiology* (2018).

Antibiotics increase the relative abundance of ESBL-*E. coli*

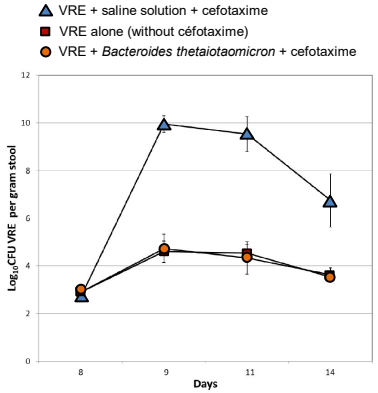


ESBL-*E. coli* relative abundance = [*ESBL-E. coli*] / [Total Gram-negative bacilli]

The intestinal relative abundance of ESBL-*E. coli* increases after antibiotic exposure

Ruppé, E. et al. *Antimicrob. Agents Chemother.* **57**, 4512–4517 (2013). ESBL: extended-spectrum beta-lactamase

Some bacteria degrade antibiotics and protect the microbiota



- Administration of *Bacteroides thetaiotaomicron* (beta-lactamase producer) prevents colonization by vancomycin-resistant *Enterococcus faecium* (*vanB*) upon exposure to cefotaxime.
- Mechanism: degradation of cefotaxime residues by beta-lactamase.

From **Stiefel, U.** *et al.* 2014 Aug;58(8):4535-42

Inactivate the antibiotic in the colon using engineered beta-lactamases

Synthetic BIOLOGICS

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

Less *C. difficile* infections

	Placebo (N=206)	Ribaxamase (N=206)
Local laboratory-confirmed *C difficile* 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 *C difficile* 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 *C difficile* 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

Data are until the 4-week follow-up. *Represents a 71% reduction in the relative risk of *C difficile* infection; CI is based on the Newcombe-Wilson CI. †Based on the pre-specified Z test. ‡Received treatment with oral vancomycin, metronidazole, fidaxomicin, or a combination, specifically to treat a *C difficile* infection.

Table 2: **Number of patients with *Clostridium difficile* infections and being actively treated for these infections, and the risk reduction associated with ribaxamase treatment**

	Placebo	Ribaxamase	p value
Clostridium difficile			
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

The number of patients with samples taken per treatment group varied by collection time: at screening, samples were provided by 206 patients from each group; at the end of treatment period 2 (72 h after the last dose of ceftriaxone), samples were provided by 186 patients in the placebo group and 185 patients in the ribaxamase group; and at the 4-week follow-up visit, samples were provided by 157 patients in the placebo group and 198 patients in the ribaxamase group. p values are one-sided and based on the prespecified Z test. Data from the end of treatment period 2 and the follow-up visit were cumulative data for new colonisation (ie, patients with a negative faecal sample at screening, then a positive result at these timepoints).

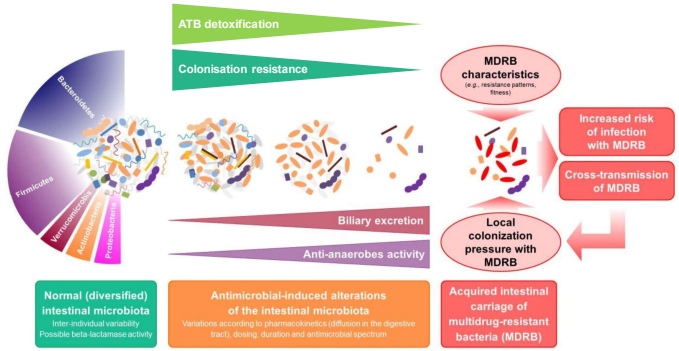
Table 3: **Number of patients positive for faecal colonisation by opportunistic pathogens at prespecified collection times**

Less acquisition of VRE

Kokai-Kun, J.F. et al. *Lancet Infect Dis* **9**, 487–496 (2019).

#Opportunity 3: Rank antibiotics according to their impact

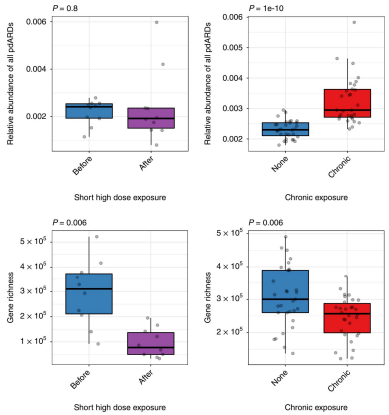
The impact of antibiotics on the microbiota is multifaceted



- Difficult to rank antibiotics according to their impact (which criterion?)
- Mainly spectrum × intestinal excretion (× putative degradation)?

Adapted from **Woerther, P.-L** *et al. Int. J. Antimicrob. Agents* (2018). 6

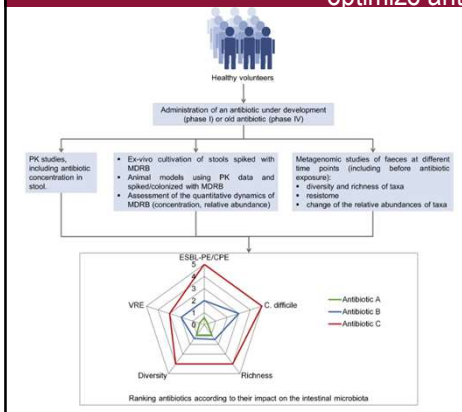
The resistome expands after iterative antibiotic exposures



- N=6095 predicted resistance genes (0.2% of the 3.9M genes in the catalog tested)
- Average of 1377 resistance genes per subject (min. 258, max. 2367)
- Low identity with known resistance genes (average 29.8% in amino acids)
- Chromosomal location
- Impact cannot only be assessed by a single exposure!

Ruppé, E. et al. *Nature Microbiol* **4**, 112–123 (2019).

Impact of antibiotics on the intestinal microbiota needs to be re-defined to optimize antibiotic usage



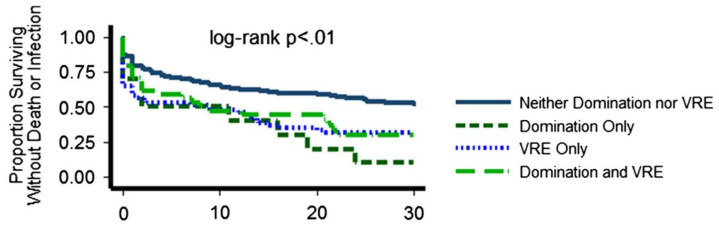
Standardization in testing the impact of antibiotics on the microbiota would help!

Ruppé, E. et al. *Clin Microbiol Infect* **24**, 3–5 (2018).

Opportunity #4: Develop microbiota biomarkers

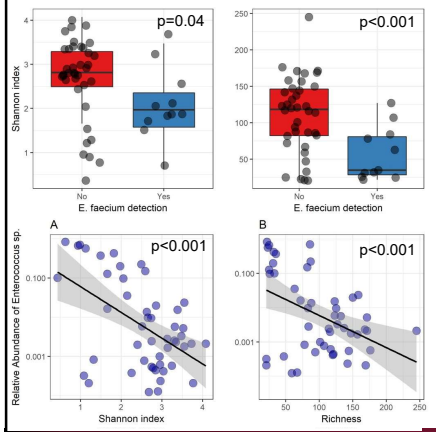
Dominance of enterococci on admission associated with prognosis

Dominance of enterococci (>30% reads) on admission to the ICU is associated with higher mortality.



Freedberg, D.E. et al. *Intensive Care Med* (2018).

Enterococci are associated with dysbiosis



Patients colonised by *E. faecium* have **lower richness** and **diversity**

16S sequencing: significant association between intestinal **richness and diversity** and relative abundance of **enterococci**.

Fontaine, C. et al. *Plos ONE*, (2020).

The NOSOCOVID study

	HR	95% CI		p
OROPHARYNX				
Enterococcus spp. quantitative (log)	1.100	1.010	1.197	0.0281
Antibiotic treatment active against Enterococcus/MRSA #	2.872	1.704	4.841	<.0001
Candida spp. quantitative (log)	1.183	1.065	1.313	0.0017
Antifungal treatment active against Candida spp. #	1.499	0.846	2.655	0.1650
S. aureus quantitative (log)	1.265	1.112	1.440	0.0004
Antibiotic treatment active against anaerobic bacteria #	2.240	1.343	3.737	0.0020
Age*	1.026	1.004	1.049	0.0230
Chronic diseases**	2.047	1.273	3.291	0.0031
Daily SOFA score*	1.172	1.105	1.243	<.0001
RECTAL				
Enterococcus spp. quantitative (log)	1.156	1.052	1.270	0.0026
Antibiotic treatment active against Enterococcus/MRSA #	2.253	1.296	3.915	0.0040
Candida spp. quantitative (log)	1.182	1.059	1.320	0.0029
Antifungal treatment active against Candida spp. #	0.991	0.558	1.759	0.9740
Antibiotic treatment active against anaerobic bacteria #	2.842	1.706	4.733	<.0001
S. aureus quantitative (log)	1.470	1.207	1.789	0.0001
Age*	1.030	1.008	1.053	0.0078
Chronic diseases**	2.071	1.267	3.386	0.0037
Daily SOFA score*	1.226	1.157	1.299	<.0001

March-September 2020: **95 COVID-19 patients** admitted to the medical intensive care unit at Bichat Hospital (Paris)

Throat and rectal swabs **at admission, then 2x per week**

Quantitative cultures+16S sequencing on rectal swabs

Rectal and oropharyngeal concentrations of *Enterococcus*, *Staphylococcus aureus* and *Candida* associated with mortality

Patrier, J. et al. *Crit Care* **26**, 300 (2022).

Take-home messages

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

Yet new ideas to combat multidrug-resistance emerge from a better understanding of this interplay

Especially, preserving/restoring the barrier effect in order to prevent the overgrowth of resistant bacteria

Indeed, lowering the concentrations of resistant bacteria could have various benefits

Need for a metrics!

Exciting times!!!

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