# The exploitation of probiotics; a potential therapy to modulate and eliminate human gut resistome?



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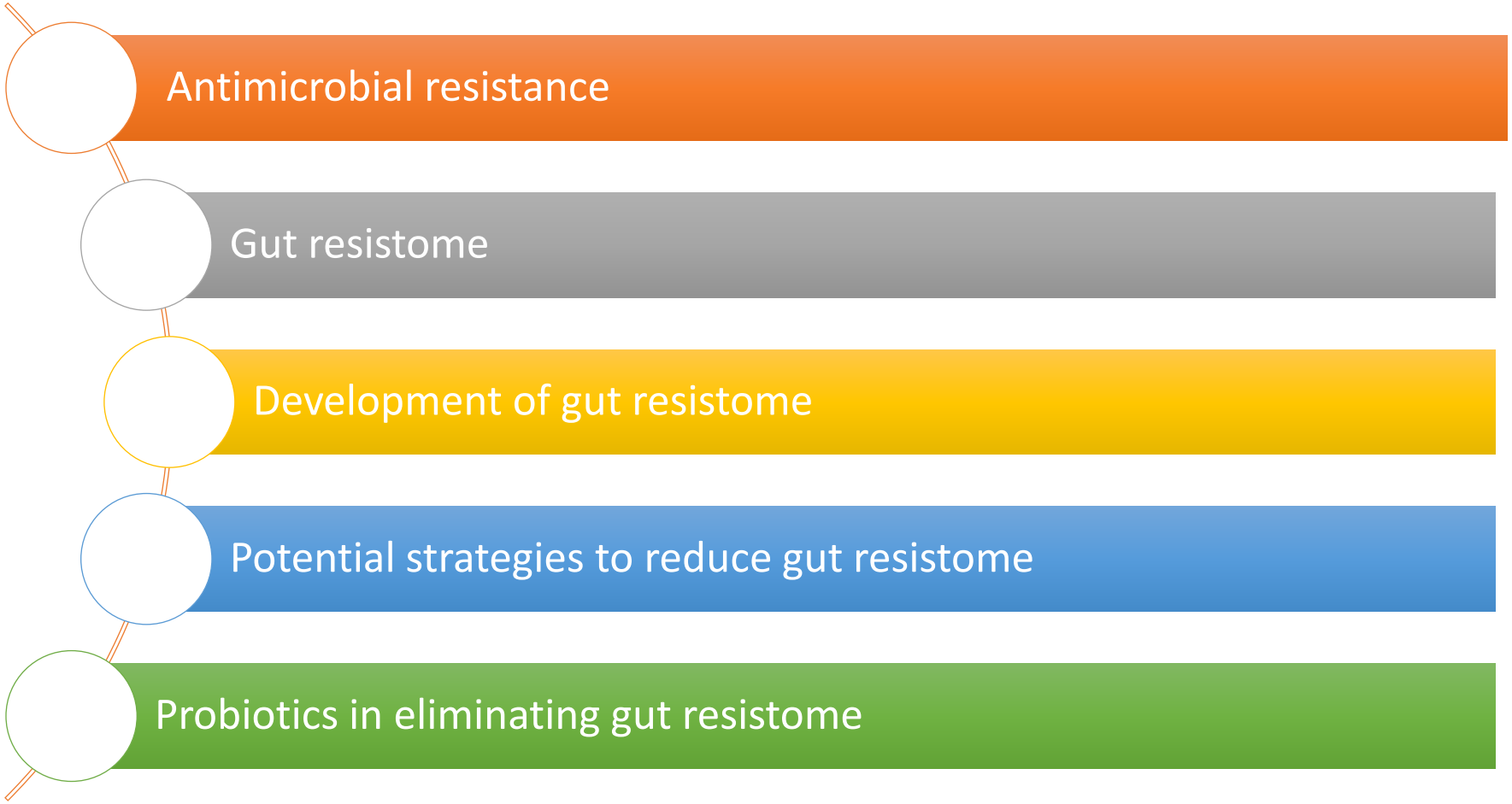
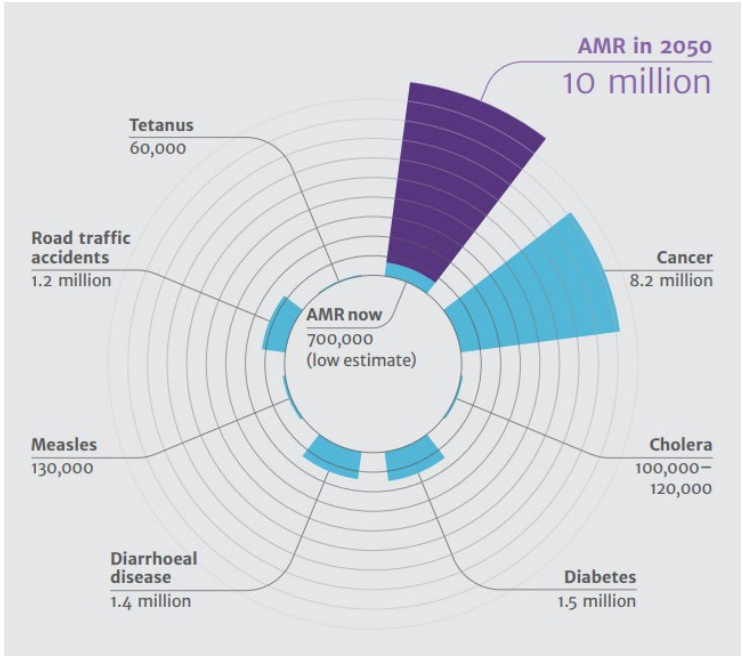
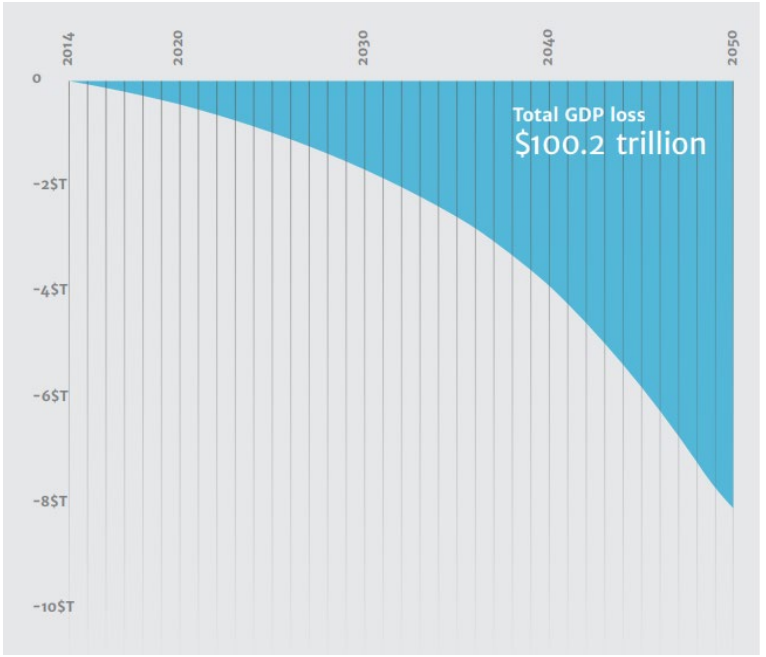
# Outline

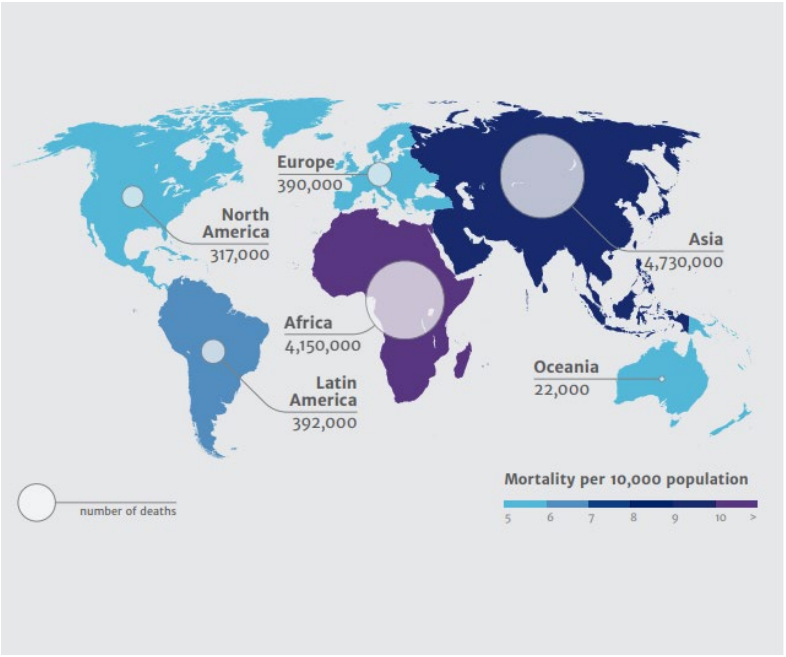

Antimicrobial resistance
Gut resistome
Development of gut resistome
Potential strategies to reduce gut resistome
Probiotics in eliminating gut resistome

# Antimicrobial resistance (AMR)

- One of the top ten global public health challenges
- Responsible for more than 700,000 deaths per year
- Projected to increase to up to 10 million by 2050
- Cost of AMR to the economy is significant
- Success of modern medicine would be at increased risk

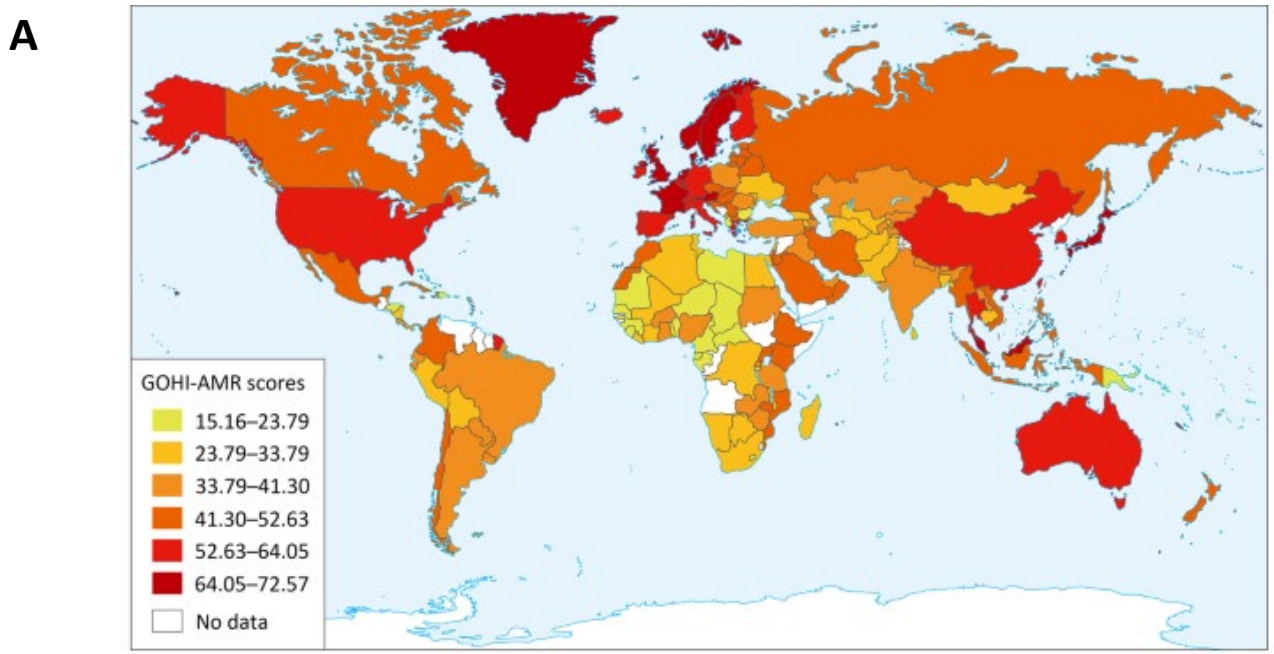



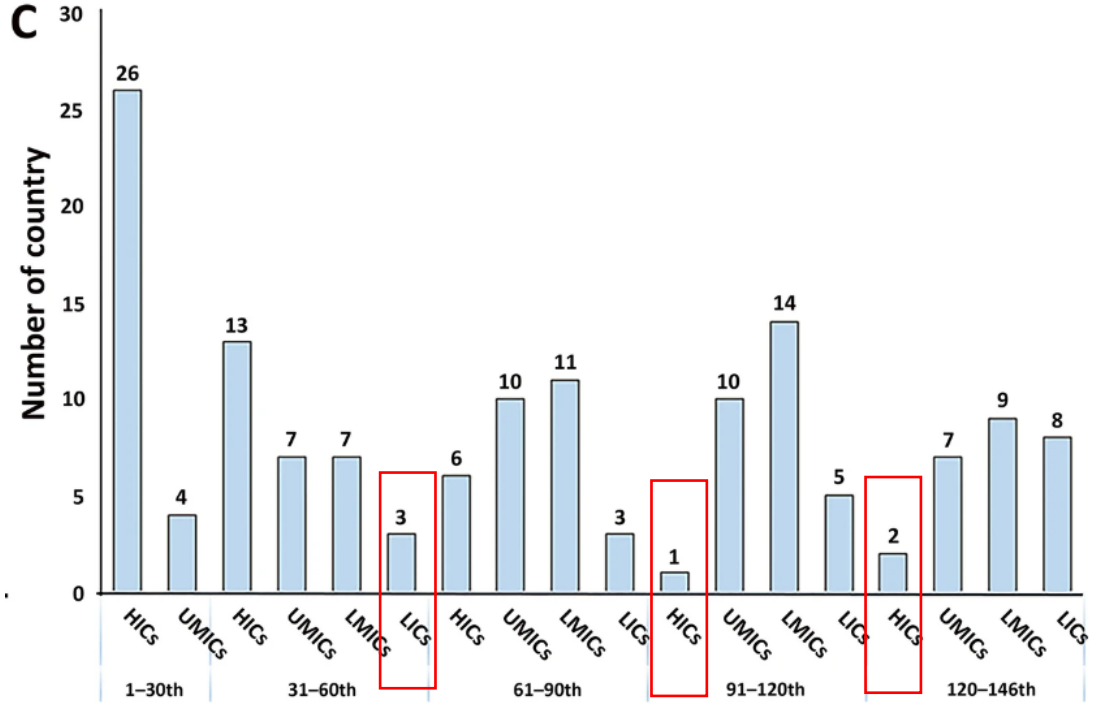

WHO, 2021

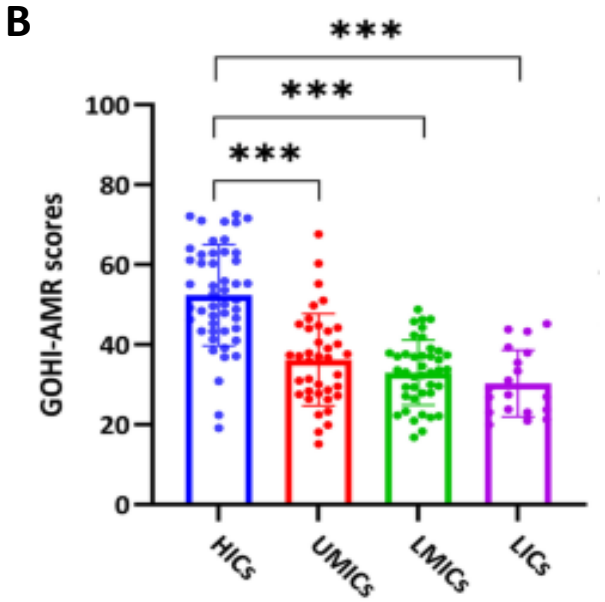
Gerard D. Wright, 2007. *Nature Reviews Microbiology*

Review on Antimicrobial Resistance, 2014

Antimicrobial resistance. biomerieuxconnection.com



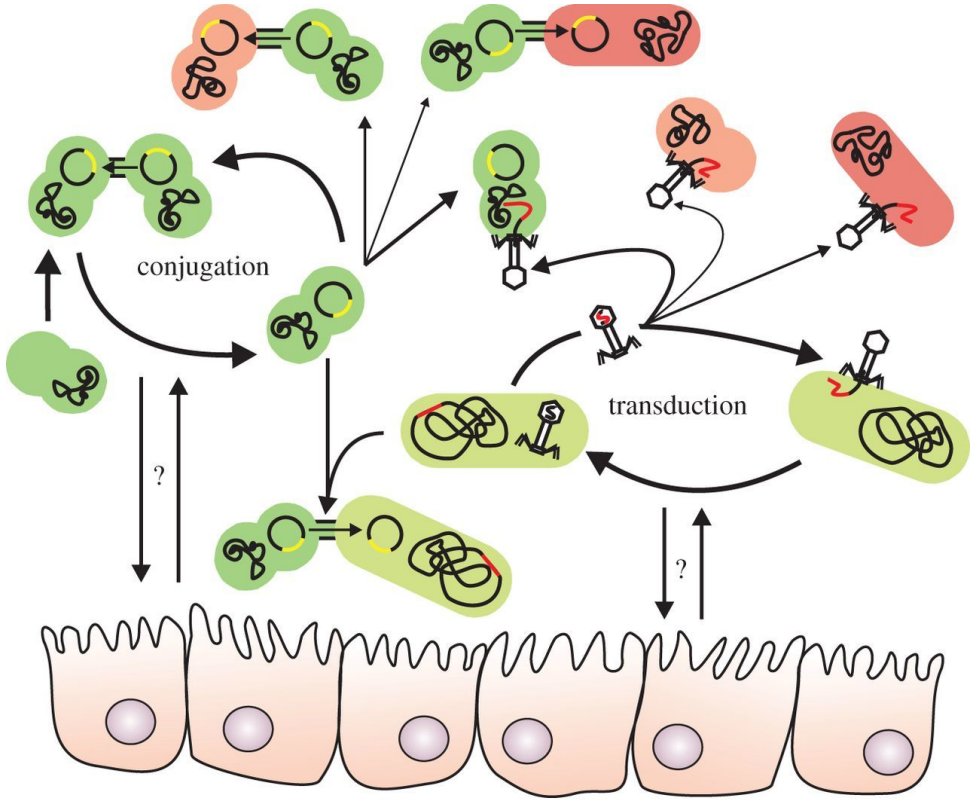




The summarized global GOHI-AMR scores among the four income nation groups. **A)** worldwide distribution map of the GOHI-AMR overall scores. **B)** statistical analysis of the GOHI-AMR scores in each of the four income groups. **C)** Distribution of overall GOHI-AMR scores among four groupings of high-income countries. (*GOHI-AMR Antimicrobial resistance in Global One Health Index, HICs high-income countries, UMICs upper-middle-income countries, LMICs lower-middle-income countries, LICs low-income countries)*

Zhou et al., 2022. *Infectious diseases of poverty*

# Gut resistome

- Collection of genes or genetic material that confers antimicrobial resistance constitutes the gut resistome,
- Considered a reservoir for the potential spread of resistance genes from commensals to pathogens,
- Diversity of this gut resistome is influenced by various environmental factors including diet and antibiotic exposure




Van Schaik, 2015. *Philosophical Transactions of the Royal Society B: Biological Sciences*

# Factors Shaping the gut resistome

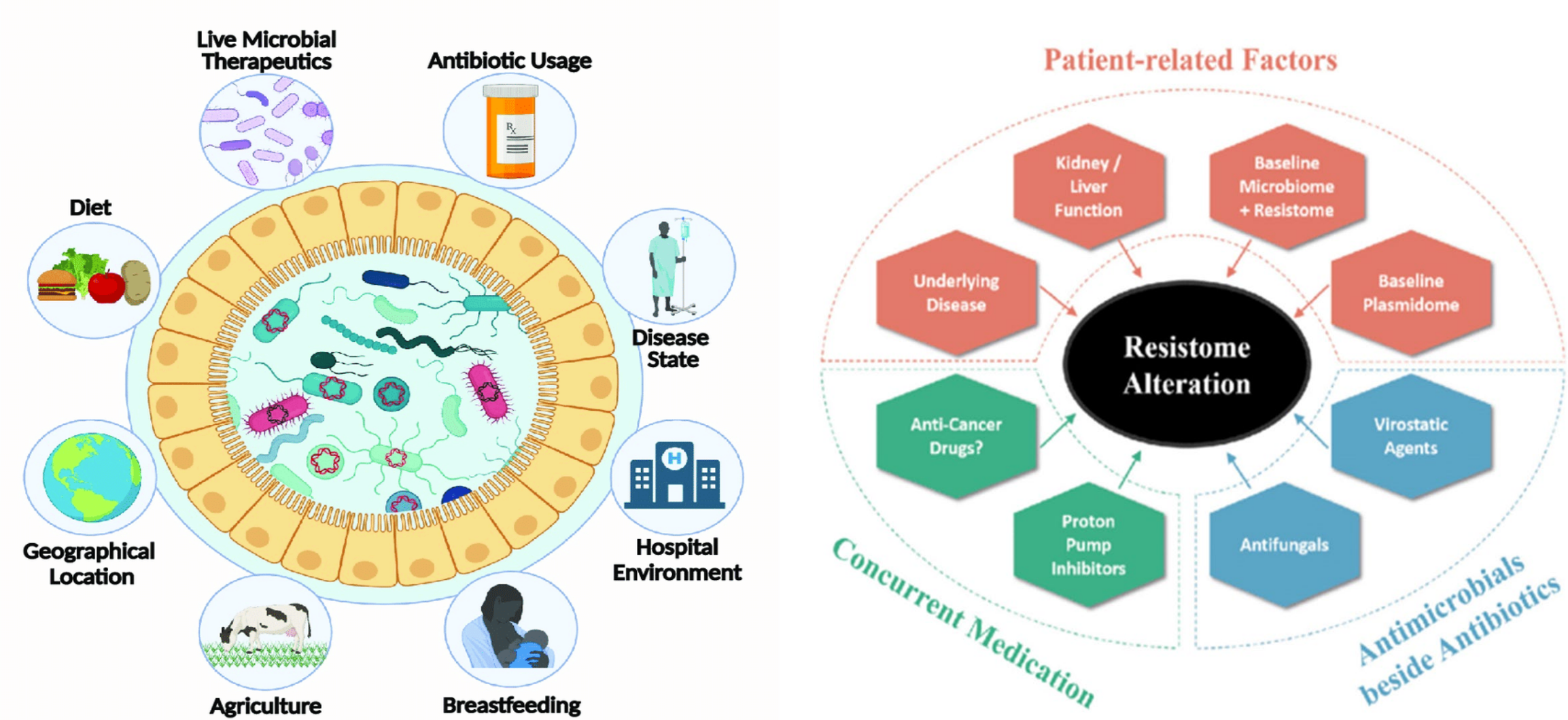
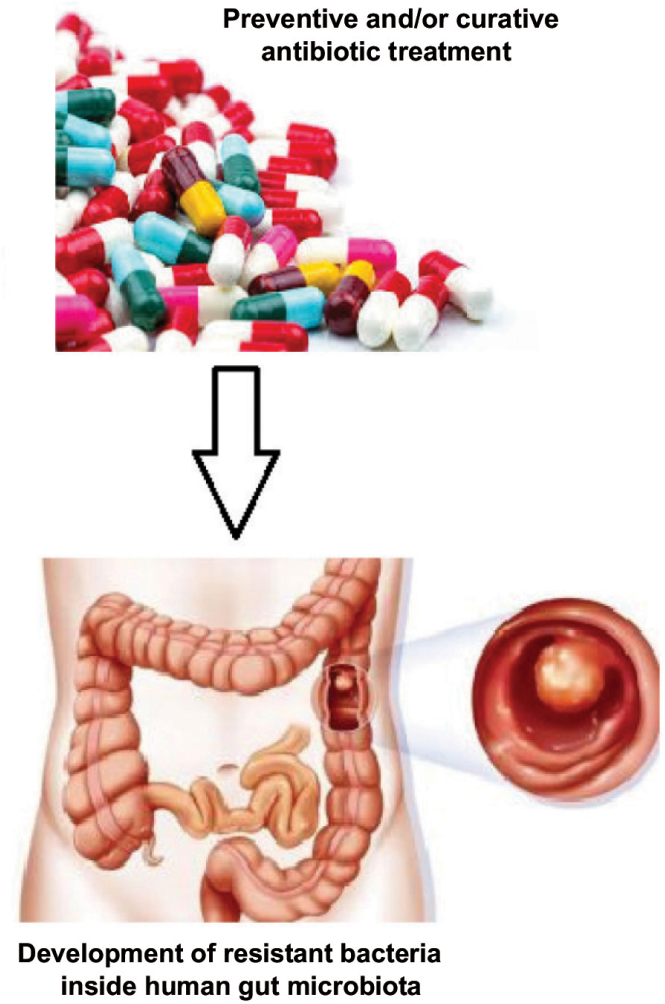
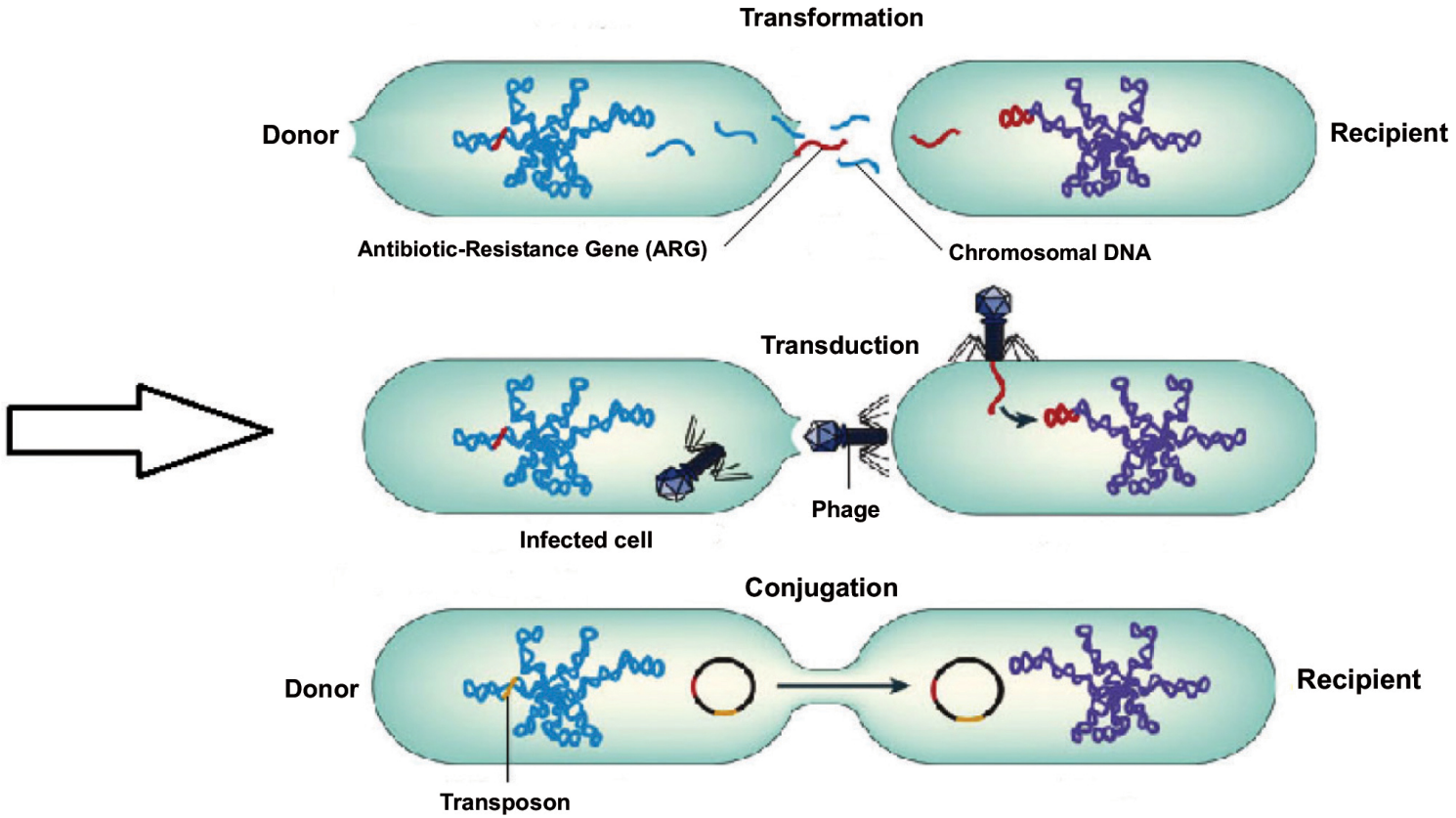



**Figure: Host and Environmental Factors that Impact the Gut Resistome**

Crits-Christoph et al. 2022. *Gut Microbes*

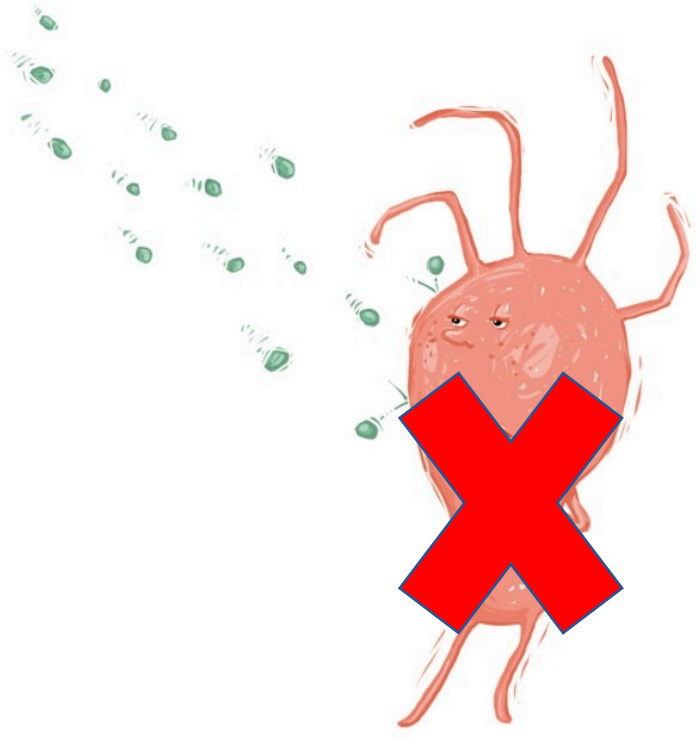
# Gut microbiota in antimicrobial resistance




Firoozeh and Zibaei, 2022. *Anti-Infective Agents*

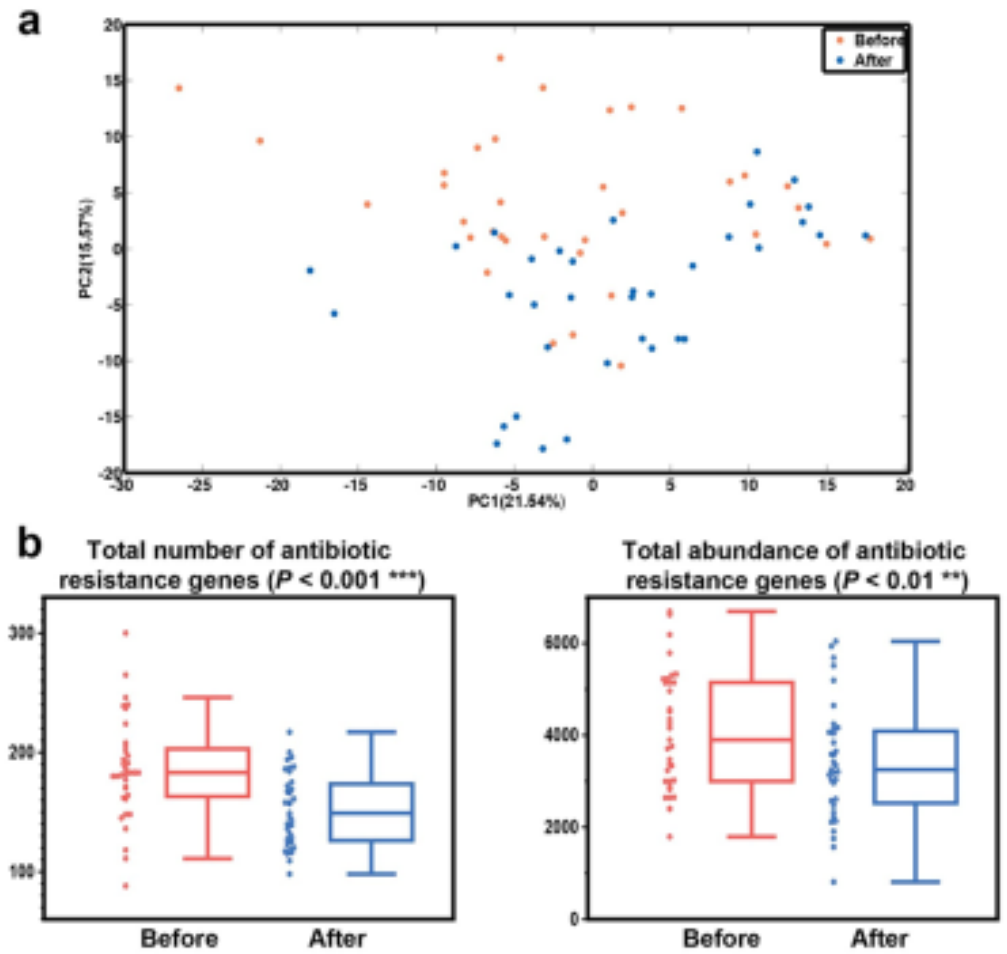
# Strategies to control or eliminate gut resistome



- Use of antibiotics for the sole purpose of growth promotion in the agricultural livestock industry since 2006 in the EU (Cogliani et al., 2011).
- EFSA has instituted guidelines on the use of food additives in animal products (Panel, 2012).
- US has strongly opposed restrictions on antibiotic use
- Promoting the use of probiotics as a substitute for antibiotics in both the medical field and livestock agriculture

# Strategies to control or eliminate gut resistome

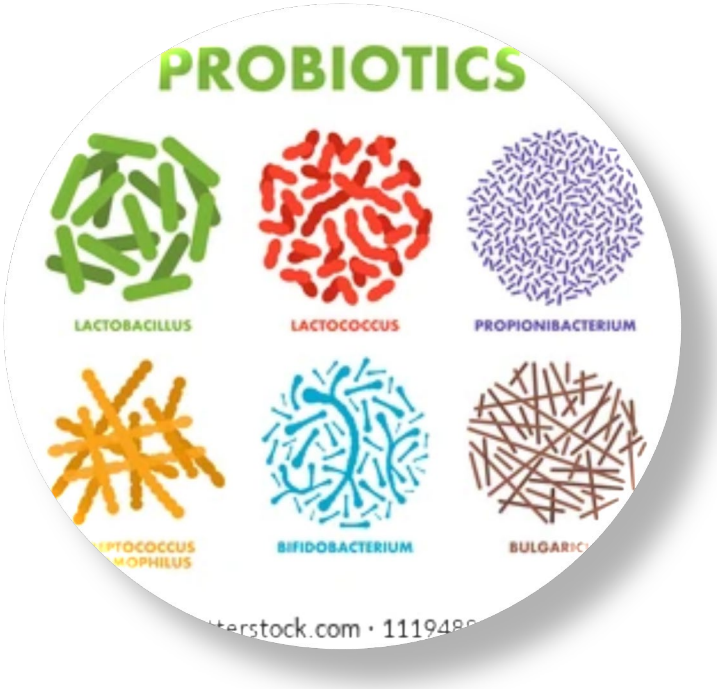
Diminution of the gut resistome after a gut microbiota-targeted dietary intervention in obese children (Wu et al., 2016)




**(a)** The PCA score plot based on the profile of 399 ARGs showing significant segregation between the samples before and after dietary intervention (log-transformed, PERMONOVA $P = 0.0187$, permutations = 9999). **(b)** The total number and abundance of ARGs. The boxes denote the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively), and the line inside the boxes denotes the median. The whiskers denote the lowest and highest values within 1.5 times of the IQR from the first and third quartiles, respectively.

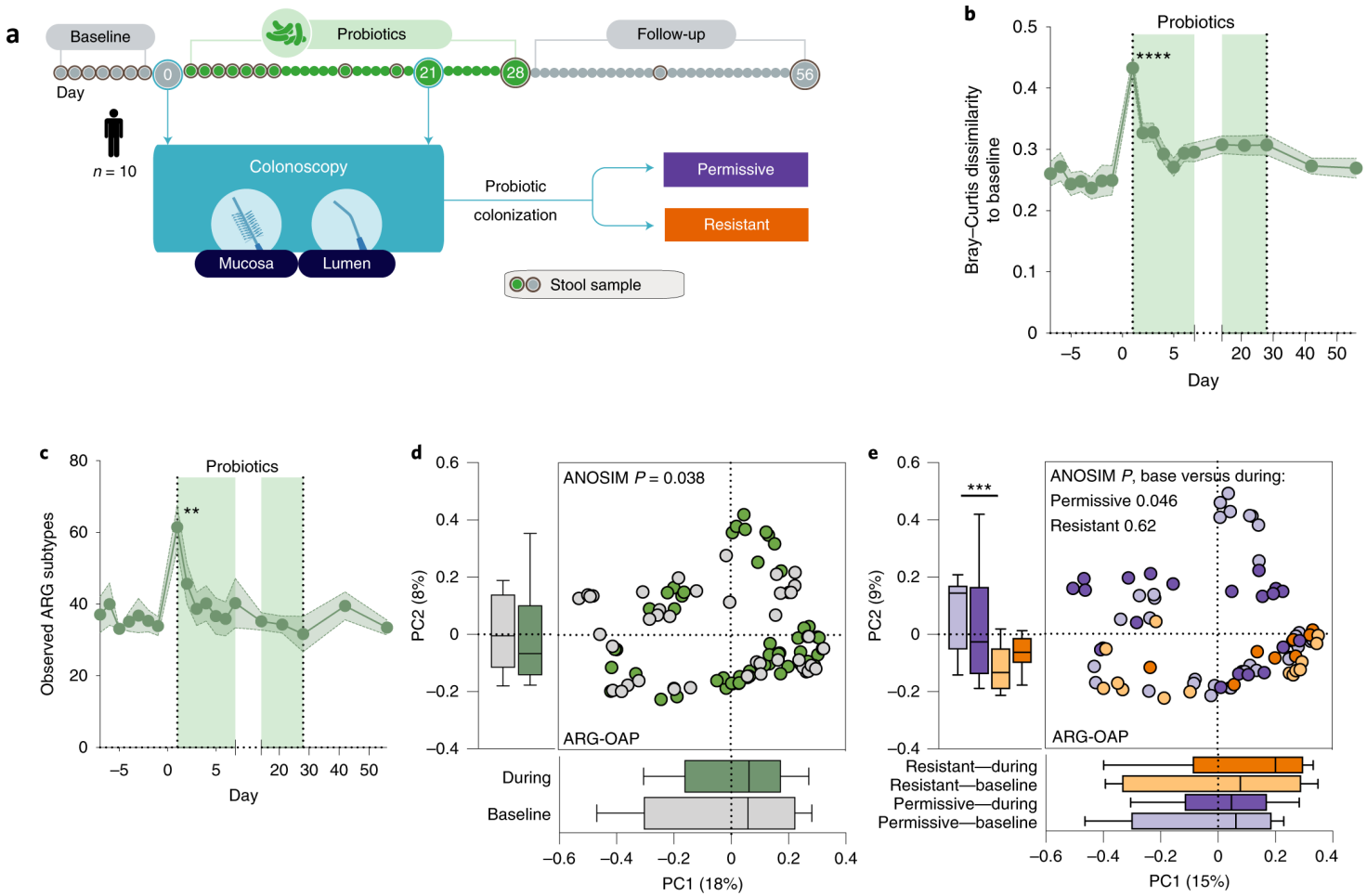
Tsigalou et al., 2020. *Frontiers in Microbiology*

# Strategies to control or eliminate gut resistome




- Probiotic strains present antimicrobial activity and inhibit the growth and displace the adhesion of potential pathogens to human mucus.
- Collado et al. 2007, showed that probiotic strains were able to inhibit and displace the adhesion of *Bacteroides, Clostridium, Staphylococcus*, and *Enterobacter*.
- Probiotics restore mucosal homeostasis and they also play a pivotal role in the prevention and treatment of *H. pylori*

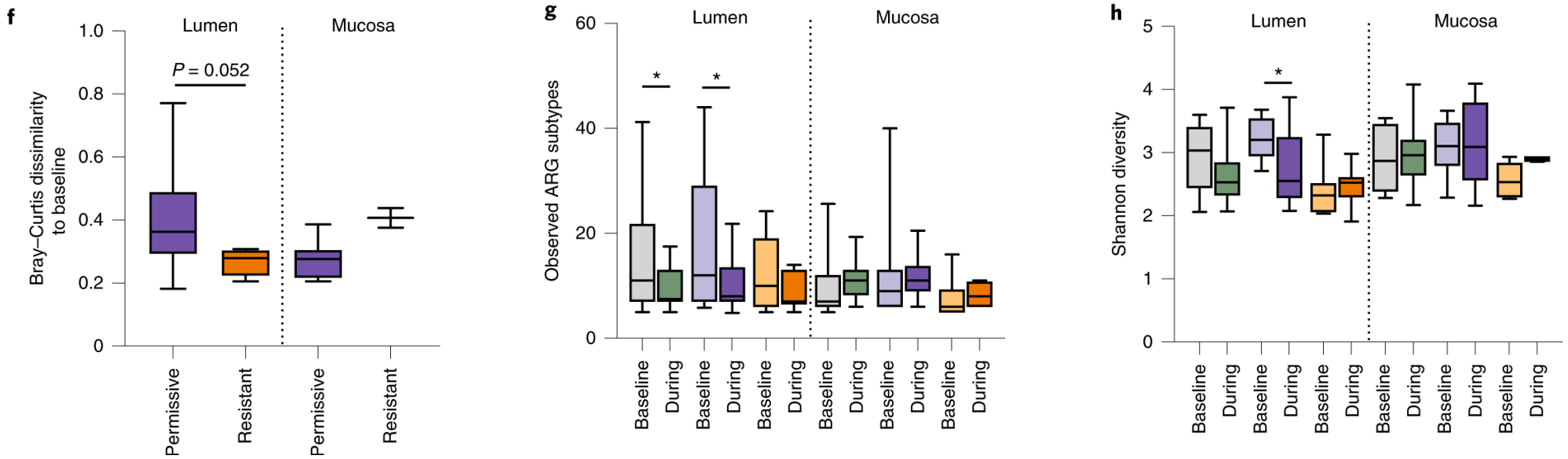
# Probiotic colonization is associated with a reduced ARG load




Ten men and women provided samples before, after, and during 28 d of supplementation with a probiotic **a**, Experimental design, **b**, Bray–Curtis dissimilarity (Stool ARG subtypes to baseline. **c**, Observed ARG subtypes in stool over time, **d**, Bray–Curtis dissimilarity of ARGs collected before (grey) or during supplementation (day 21, green). **e**, Same as **d** but based on ARG subtypes and colour-coded according to probiotic colonization permissiveness (purple, $n = 6$) or resistance (orange, $n = 4$) and time point (before, light; during, dark). PC2 permissive versus resistant baseline $P = 0.0004$.

Montassier et al., 2021. *Nature Microbiology*

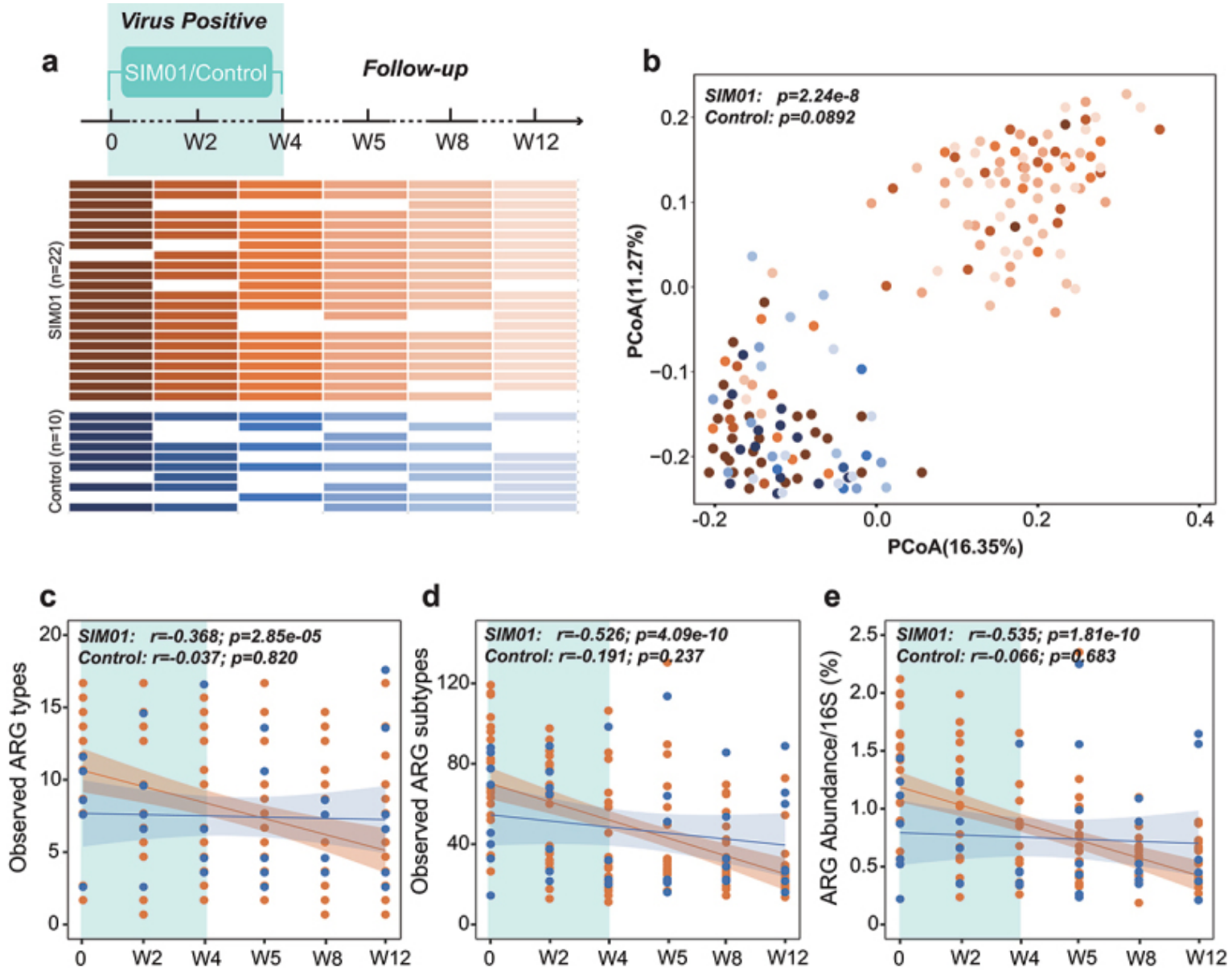
# Probiotic colonization is associated with a reduced ARG load




**f**, Per-person Bray–Curtis dissimilarity to baseline calculated in all participants or in the two subsets based on ARG subtypes. Lumen $P = 0.052$. **g**,**h**, Alpha diversity measurements (**g**), observed ARGs (subtypes) or Shannon diversity index in endoscopic samples (**h**) of permissive and resistant individuals, compared either to the baseline of each subset or between subsets. In **g**, lumen, all samples baseline versus during $P = 0.035$, permissive baseline versus during $P = 0.0223$. In **h**, lumen, permissive baseline versus during $P = 0.0226$. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$.

Montassier et al., 2021. *Nature Microbiology*

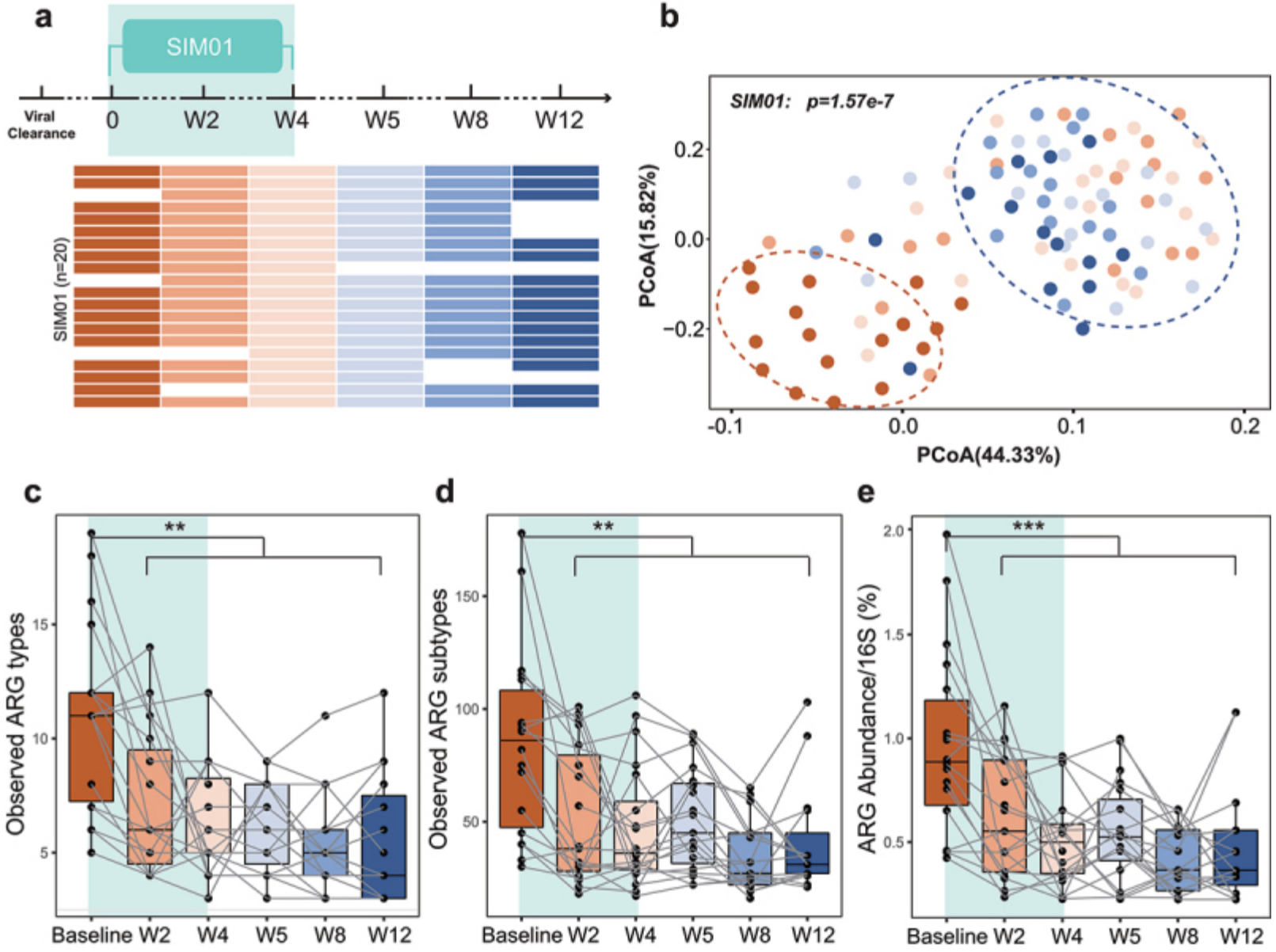
# Probiotics reduced ARGs in COVID-19 positive patient




(a) Schematic overview of the study design, depicting the total number of samples and participants from whom data were available. The horizontal bars represent the sample collected at a specific time point. (b) Probiotics were associated with an increased dissimilarity of resistome configuration compared with pre-treatment ARGs. The observed ARG types (C), subtypes (d), and abundance in COVID-19 patients exhibited a significant decrease after taking probiotics, but no significant trend was found in the control group (Pearson Correlation).

Su et al., 2022. *Gut microbes*

# Probiotics reduced ARGs in COVID-19 after clearance of virus




(a) Schematic overview of the study design, depicting the total number of samples and participants from whom data were available. The horizontal bars represent the sample collected at a specific time point. (b) Bray–Curtis dissimilarities clearly separated the resistome at baseline and that after probiotics supplementation in subjects taking SIM01. The dynamics of the observed ARG types (c), subtypes (d), and abundance (e) in subjects taking SIM01 from baseline to week 12. *P < .05, **P < .01, ***P < .001, ****P < .0001, Kruskal–Wallis and Dunn's tests (all panels).

Su et al., 2022. *Gut microbes*

Influence of antibiotic exposure (broad vs. narrow after first week of life) on abundance of antibiotic resistance genes (ARGs) in probiotic supplemented extremely preterm (PEP) infants.

| | 28 days | | | | 120 days | | | |
|---|---|---|---|---|---|---|---|---|
| | **Broad[*] ($n$ = 5[**])** | **Narrow ($n$ = 12[**])** | ***P*** | **FDR Q** | **Broad[*] ($n$ = 7[**])** | **Narrow ($n$ = 11[**])** | ***P*** | **FDR Q** |
| Class A Beta lactamase | 0.00 | 0.00 | 0.799 | 0.846 | 1.43 | 3.01 | 0.596 | 0.867 |
| Class C Beta lactamase | 45.96 | 0.00 | 0.009 | 0.162 | 9.11 | 9.52 | 0.328 | 0.875 |
| Aminoglycoside phosphotransferase | 6.14 | 0.00 | 0.082 | 0.369 | – | – | – | – |
| Aminoglycoside nucleotidyltransferase | 0.93 | 0.00 | 0.104 | 0.312 | 0.00 | 0.00 | 0.860 | |
| Tetracycline efflux | 29.55 | 0.00 | 0.019 | 0.171 | 7.92 | 7.92 | 0.375 | 0.857 |
| Tetracycline ribosomal protection | 6.49 | 0.00 | 0.082 | 0.369 | 11.68 | 28.48 | 0.246 | 0.787 |
| Quinolone resistance | 29.75 | 7.08 | 0.506 | 0.828 | 9.40 | 9.40 | 0.425 | 0.85 |
| ABC efflux pump | 3.23 | 0.43 | 0.279 | 0.628 | 0.70 | 1.10 | 0.479 | 0.852 |
| RND antibiotic efflux | 312.10 | 19.81 | 0.799 | 0.900 | 94.00 | 93.09 | 0.536 | 0.858 |
| MFS antibiotic efflux | 272.36 | 79.67 | 0.506 | 0.759 | 70.92 | 111.28 | 0.860 | 0.917 |
| Multidrug efflux pump activity | 22.08 | 24.71 | 0.879 | 0.879 | 19.08 | 6.55 | 0.647 | 0.863 |
| Multidrug resistance efflux pump | 0.00 | 0.00 | 0.234 | 0.602 | 3.02 | 3.02 | 0.069 | 0.368 |
| Gene modulating antibiotic efflux | 75.30 | 13.81 | 0.328 | 0.656 | 19.65 | 24.88 | 0.008 | 0.128 |
| SMR antibiotic efflux | 0.00 | 0.00 | 0.506 | 0.759 | – | – | – | – |

Esaiassen et al., al 2018. *Frontiers*

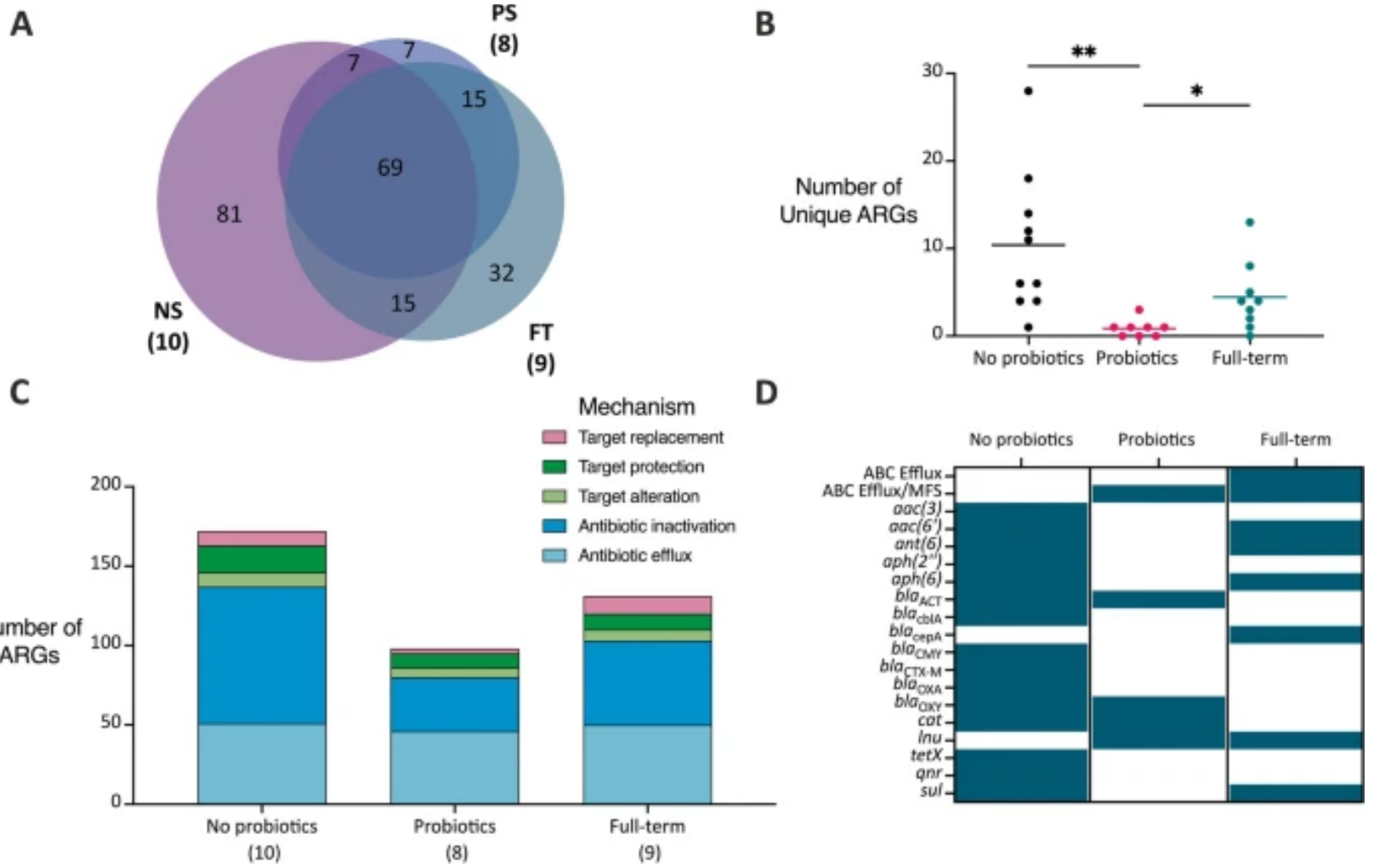
Influence of antibiotic exposure (broad vs. narrow after first week of life) on abundance of antibiotic resistance genes (ARGs) in extremely preterm (PEP) infants.

| | 28 days | | | | 120 days | | | |
|---|---|---|---|---|---|---|---|---|
| | ($n = 7^{**}$) | ($n = 15^{**}$) | | | ($n = 9^{**}$) | ($n = 13^{**}$) | | |
| Class A Beta lactamase | 0.00 | 0.00 | 0.447 | 0.731 | 5.00 | 3.01 | 0.324 | 0.864 |
| Class C Beta lactamase | 44.96 | 0.00 | **0.021** | **0.095** | 9.11 | 8.16 | 0.235 | 0.752 |
| Aminoglycoside phosphotransferase | 6.14 | 0.00 | 0.078 | 0.281 | – | – | – | – |
| Aminoglycoside nucleotidyltransferase | 0.93 | 0.00 | **0.008** | **0.072** | 0.00 | 0.00 | 0.794 | 0.851 |
| Tetracycline efflux | 52.29 | 0.00 | **0.014** | **0.084** | 7.92 | 0.00 | 0.235 | 0.94 |
| Tetracycline ribosomal protection | 5.97 | 0.00 | 0.210 | 0.540 | 11.68 | 2.17 | 0.393 | 0.886 |
| Quinolone resistance | 29.75 | 9.43 | 0.298 | 0.671 | 9.40 | 8.34 | 0.357 | 0.816 |
| ABC efflux pump | 3.23 | 1.07 | 0.392 | 0.784 | 0.70 | 0.64 | 0.471 | 0.814 |
| RND antibiotic efflux | 312.10 | 37.73 | 0.875 | 0.875 | 94.00 | 84.96 | 0.393 | 0.63 |
| MFS antibiotic efflux | 272.36 | 117.02 | 0.490 | 0.68 | 119.50 | 107.51 | 0.404 | 0.59 |
| Multidrug efflux pump activity | 22.08 | 26.53 | 0.581 | 0.70 | 19.08 | 13.63 | 0.647 | 0.69 |
| Multidrug resistance efflux pump | 0.00 | 0.00 | 0.162 | 0.486 | 3.02 | 0.00 | 0.017 | 0.272 |
| Gene modulating antibiotic efflux | 75.30 | 15.53 | 0.490 | 0.73 | 19.65 | 20.86 | 0.393 | 0.63 |
| SMR antibiotic efflux | 0.00 | 0.00 | 0.447 | 0.805 | – | – | – | – |
| Antibiotic target | 1.70 | 0.00 | **0.002** | **0.030** | 2.36 | 0.00 | 0.096 | 0.512 |

Probiotic-supplemented extremely preterm (PEP) infants had a lower abundance of ARGs compared to only antibiotics-treated infants. Probiotic supplementation may induce colonization resistance and alleviate harmful effects of antibiotics on the gut microbiota and antibiotic resistome.

Esaiassen et al., al 2018. *Frontiers*

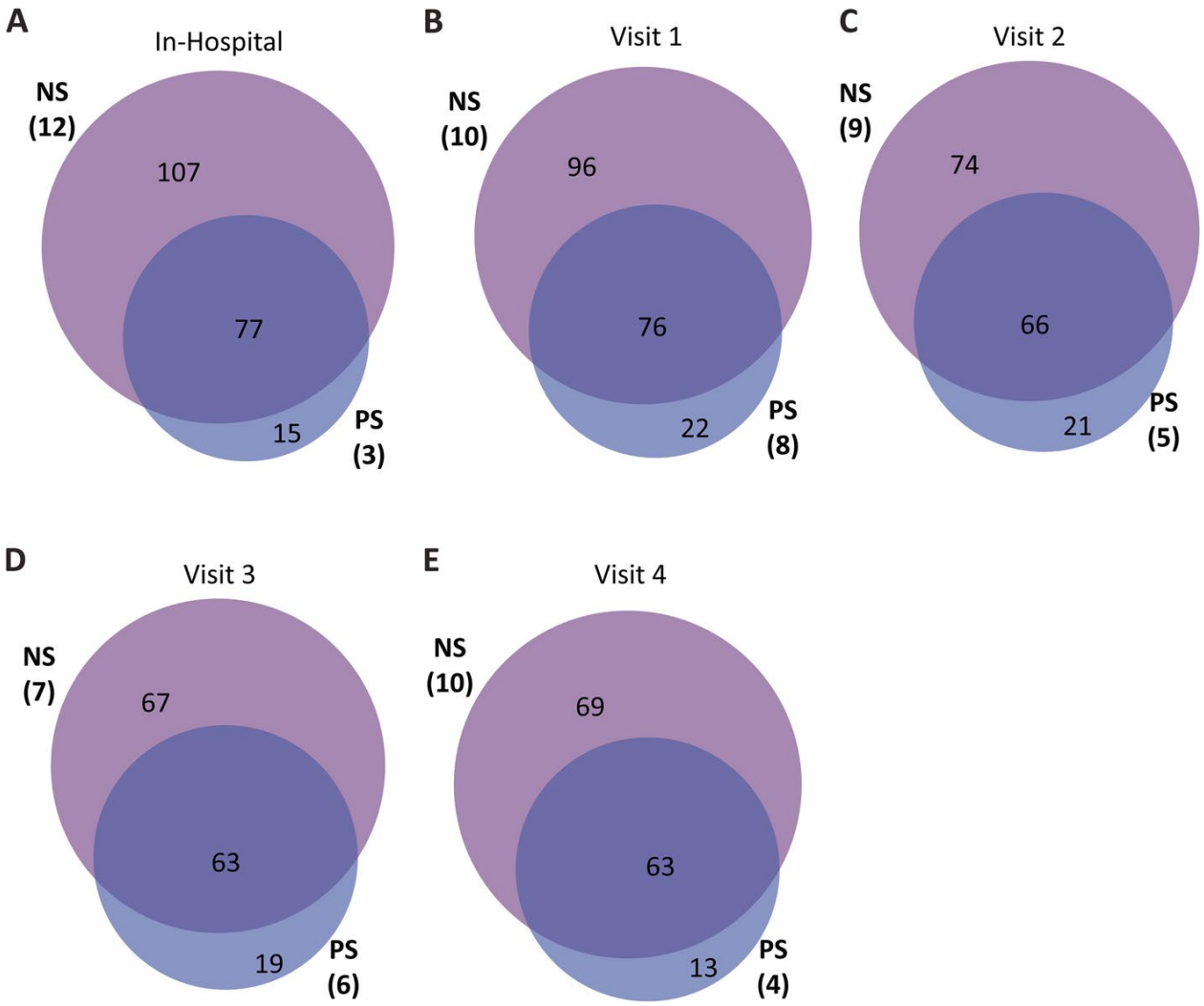
# Preterm infants not supplemented with probiotics have a greater diversity of antibiotic resistance genes




A) Unique and overlapping ARGs identified in each infant group. The number of infants is shown next to the sample type. B) The number of unique ARGs identified in each infant. Significant differences are denoted by a line and asterisk(s) C) A breakdown of the mechanisms of antibiotic resistance identified in each infant group D) The presence or absence of selected AMR gene families in each infant group. A teal box indicates that at least one gene from that AMR gene family was identified in any of the infant samples (*NS* = not supplemented preterm, *PS* = probiotic-supplemented preterm, and FT = full-term infants)

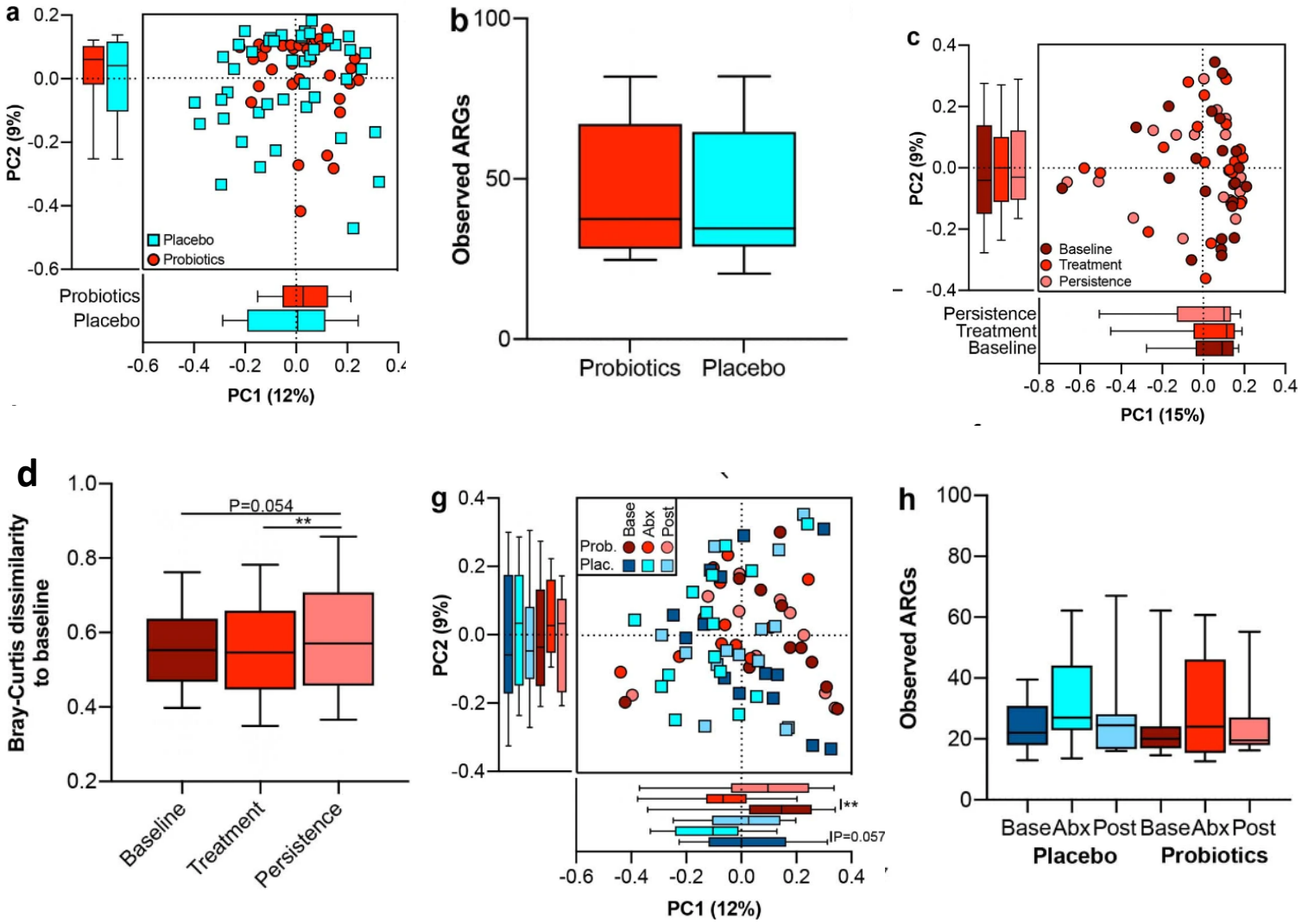
Guitor et al., 2022. *Microbiome*

# Probiotics reduce the diversity of the preterm gut resistome up to 5 months of age




Number of unique genes in preterm infants at various time-points. These gene counts are from mapping reads to CARD using bowtie2 and counting the number of genes with at least 100 reads. Data are from NS and PS infants at the inhospital collection (A), visit 1 (B), visit 2 (C), visit 3 (D), and visit 4 (E) time-points. The number of infants included in each time-point is indicated (NS = non-probiotic-supplemented preterm, PS = probiotic-supplemented preterm)

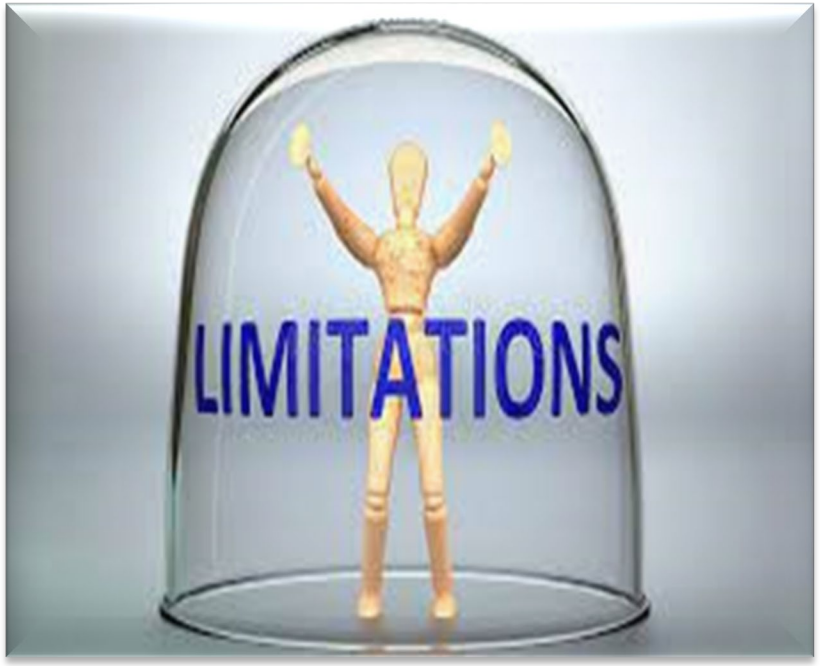
# Effect of probiotics and antibiotics on resistome in publicly available datasets.




**a-b**, Study of sailors on long sea placebo (blue) vs probiotics red); **c-d**, 22 participants treated with placebo vs probiotics (baseline dark red, last day probiotics red, and follow-up light red); **g-h**, Cohort with diabetes placebo (blue) vs probiotics (red) after 1-week antibiotics treatment (samples at baseline are dark colored after antibiotics and intervention are light-colored). **(a,c,d,g)** Beta diversity is based on Bray Curtis dissimilarities. (**b,h)** Observed ARGs. **d**, Persistence vs. treatment

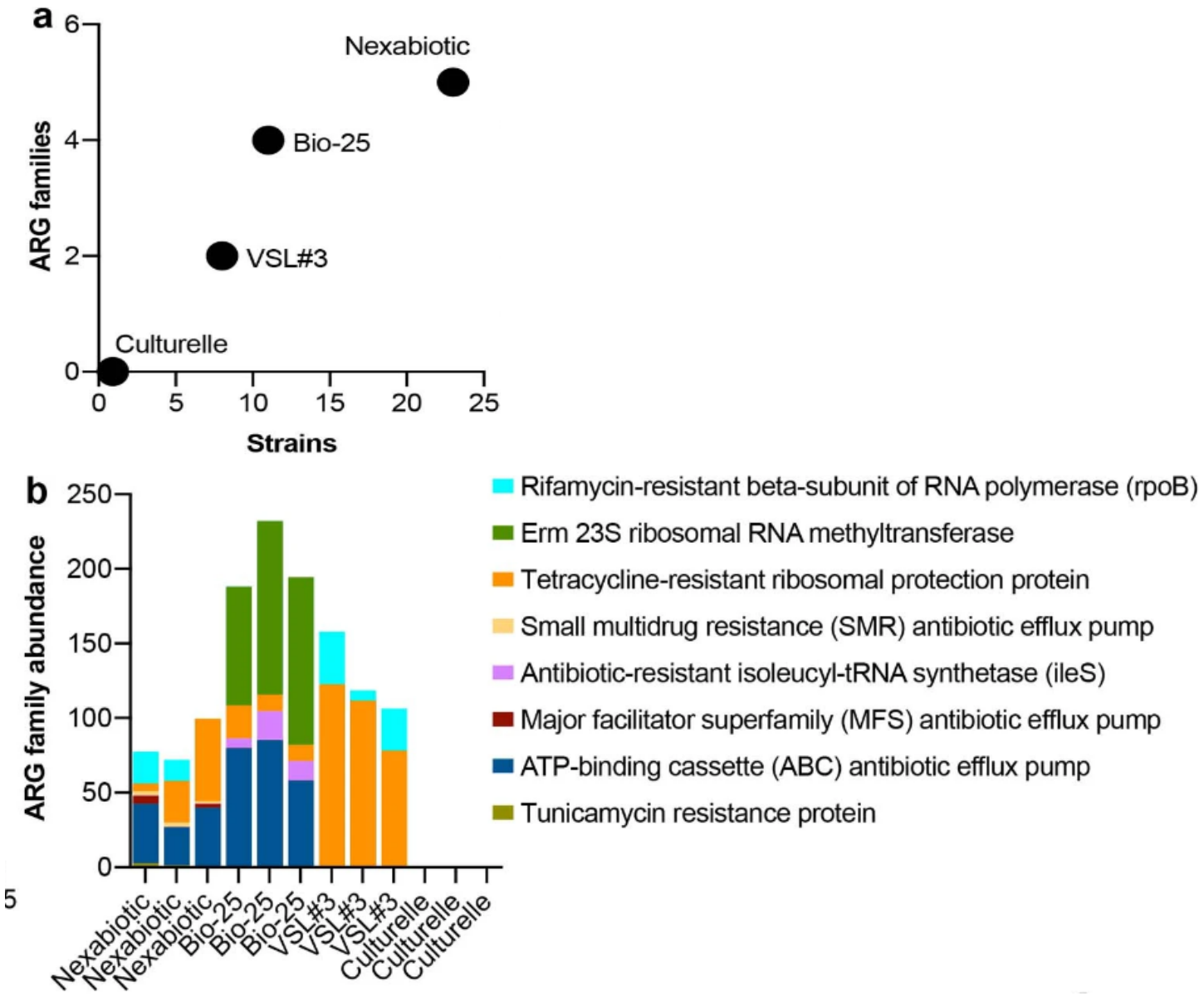
Montassier et al., 2021. *Nature Microbiology*

# Limitation of use of probiotics




- The use of bacterial probiotics to reduce antibiotic-associated side effects has several potential limitations including;
- Development of probiotic strains resistant to antibiotics,
- Passage of antibiotic-resistant genes to pathogenic bacteria through horizontal gene transfer.

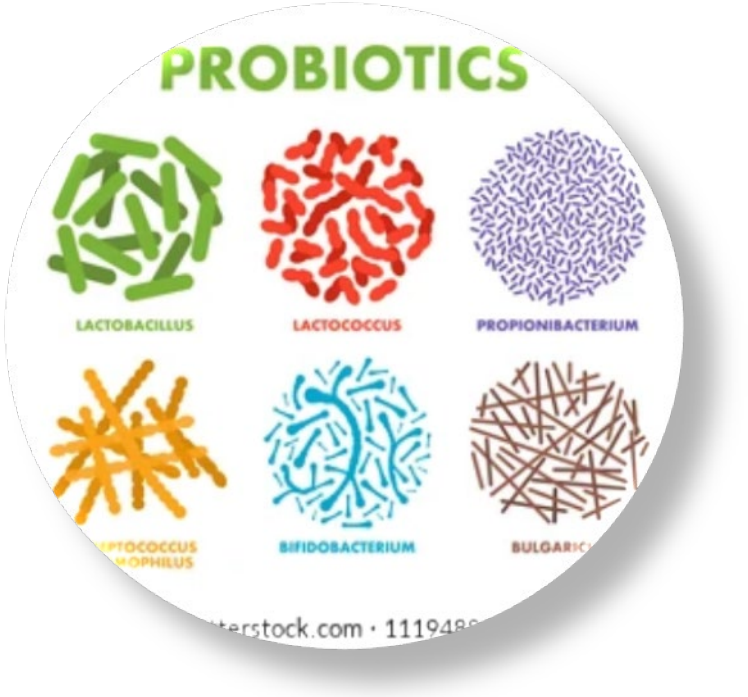
# Abundance of resistant genes in Probiotics




Single-end shotgun metagenomics sequencing was performed on 4 commercially available probiotic products (Bio25, Culturelle, Nexabiotic and VSL#3; 3 pills per product): **a**, Abundance of ARG families correlated with the number of strains in the supplement. **b**, Observed ARG families.

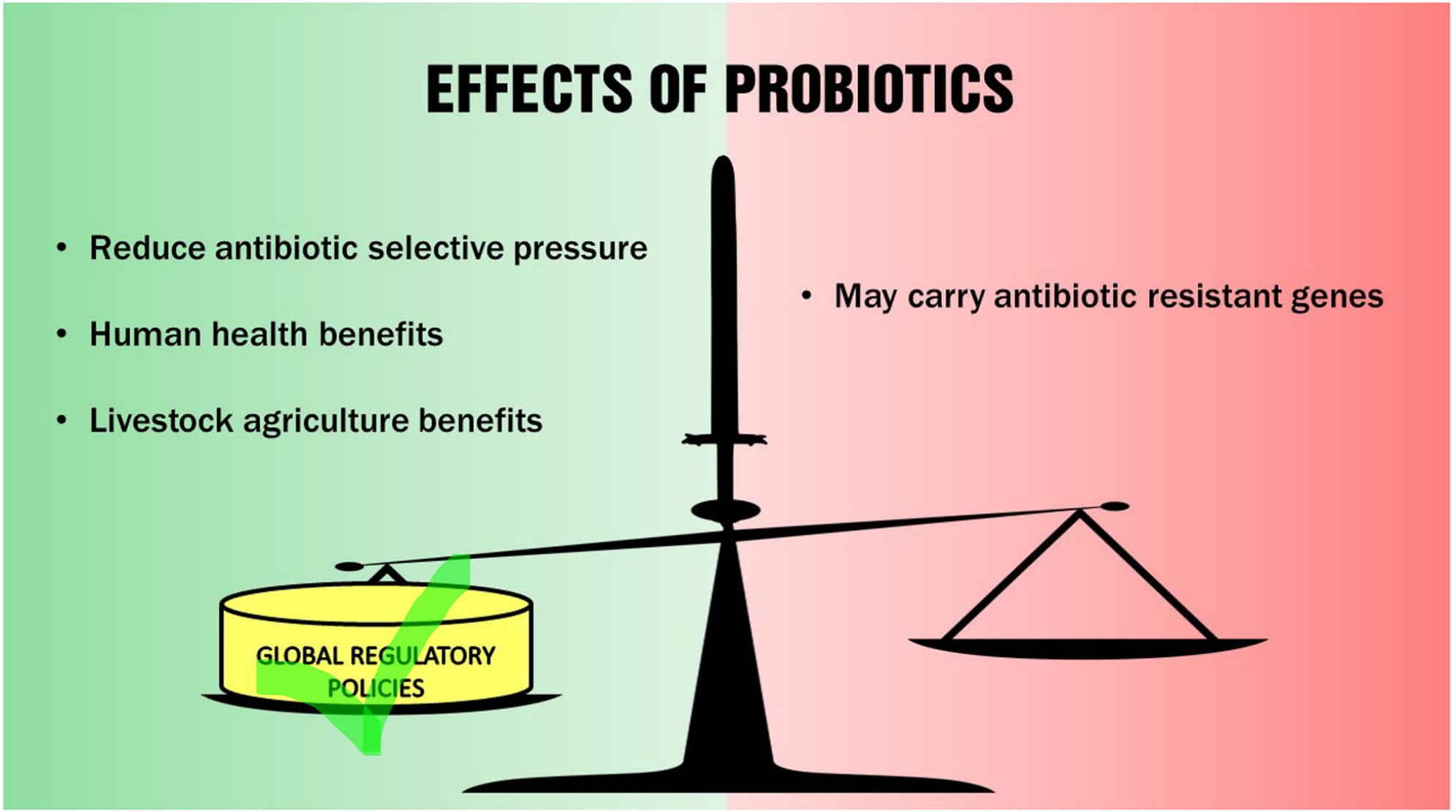
Montassier et al., 2021. Nature Microbiology

# Take home message




- Some probiotic strains carry antibiotic-resistance genes and have the potential to pass antibiotic-resistance genes;
- Still, probiotics are of good choice to reduce selectively antibiotic pressure
- Need to screen probiotic strains that are used in both livestock and human applications
- Human health benefits
- Livestock agricultural benefits

# Take home message



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