ARLG
Antibacterial Resistance Leadership Group

ARLG Grand Rounds
The microbiota and resistome in clinical trials: opportunities and challenges

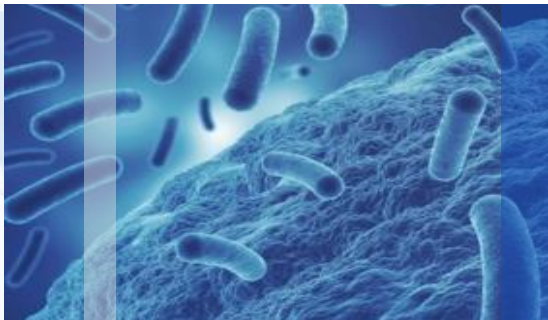
Melinda M. Pettigrew, PhD

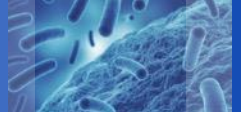
Deputy Dean and Anna M. R. Lauder Professor of Epidemiology

Yale School of Public Health

Moderator: Henry Chip Chambers, MD



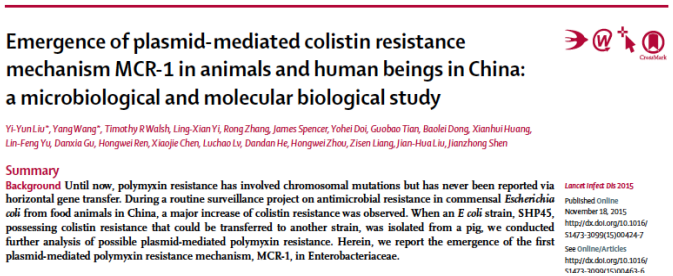




Questions for your consideration

- Should the microbiome and antibiotic resistome be evaluated as a key part of clinical trials for antibacterial drugs?
- What information can be gained?
- What would it take to get us there?

Antibiotic resistance is a major global public health threat



Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

Yi-Yun Liu*, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchao Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen

Summary

Background Until now, polymyxin resistance has involved chromosomal mutations but has never been reported via horizontal gene transfer. During a routine surveillance project on antimicrobial resistance in commensal *Escherichia coli* from food animals in China, a major increase of colistin resistance was observed. When an *E coli* strain, SHP45, possessing colistin resistance that could be transferred to another strain, was isolated from a pig, we conducted further analysis of possible plasmid-mediated polymyxin resistance. Herein, we report the emergence of the first plasmid-mediated polymyxin resistance mechanism, MCR-1, in Enterobacteriaceae.

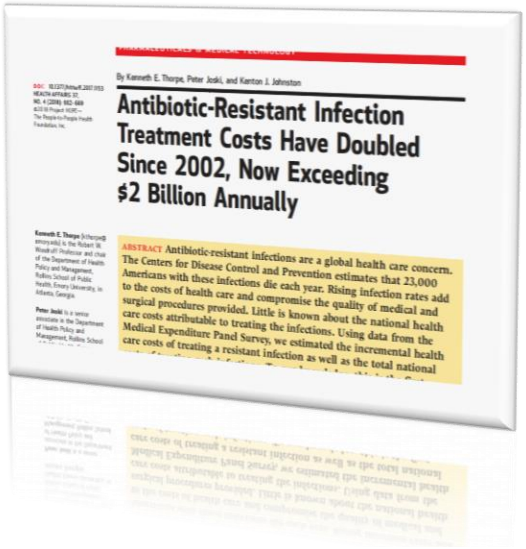
Lancet Infect Dis 2015
Published Online November 18, 2015
http://dx.doi.org/10.1016/S1473-3099(15)00424-7
See Online/Articles
http://dx.doi.org/10.1016/S1473-3099(15)00463-6

GERMINATION *A Blog by Maryn McKenna*

Apocalypse Pig: The Last Antibiotic Begins to Fail

POSTED SAT, 11/21/2015

A pig being home-raised for a festival in China. PHOTOGRAPH BY CLEMSON FUCHS (CC)

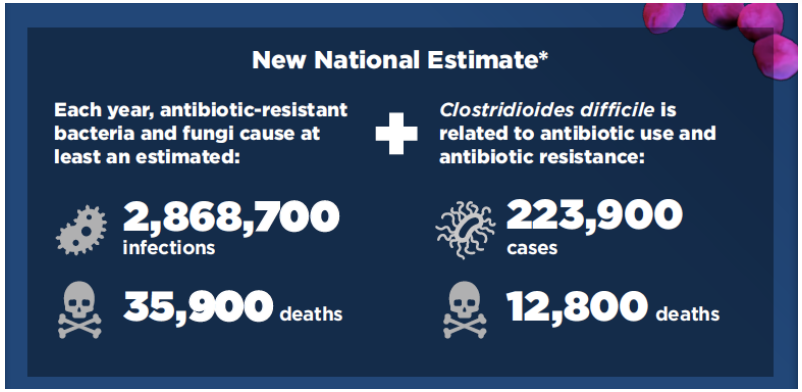


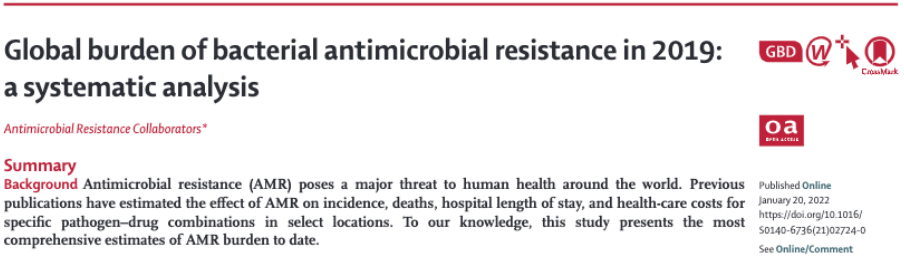
By Kenneth E. Thorpe, Peter Joski, and Kenton J. Johnston

Antibiotic-Resistant Infection Treatment Costs Have Doubled Since 2002, Now Exceeding $2 Billion Annually

ABSTRACT Antibiotic-resistant infections are a global health care concern. The Centers for Disease Control and Prevention estimates that 23,000 Americans with these infections die each year. Rising infection rates add to the costs of health care and compromise the quality of medical and surgical procedures provided. Little is known about the national health care costs attributable to treating the infections. Using data from the Medical Expenditure Panel Survey, we estimated the incremental health care costs of treating a resistant infection as well as the total national

CDC Report on Antibiotic Resistance Threats in the US, 2019





Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis

Antimicrobial Resistance Collaborators*

Summary

Background Antimicrobial resistance (AMR) poses a major threat to human health around the world. Previous publications have estimated the effect of AMR on incidence, deaths, hospital length of stay, and health-care costs for specific pathogen–drug combinations in select locations. To our knowledge, this study presents the most comprehensive estimates of AMR burden to date.

Published Online January 20, 2022
https://doi.org/10.1016/S0140-6736(21)02724-0
See Online/Comment

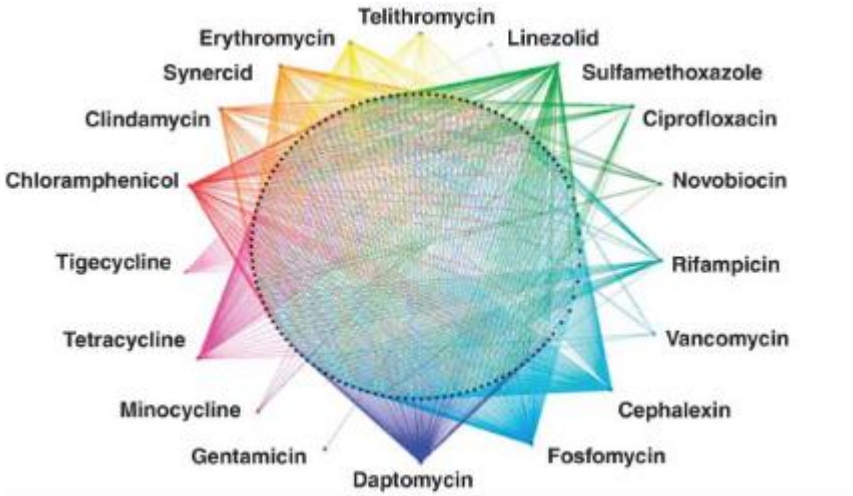
4.95 million deaths associated with AMR
1.27 million deaths directly attributable to AMR

The term “antibiotic resistome” was first used in 2006

Sampling the Antibiotic Resistome

Vanessa M. D'Costa,[1] Katherine M. McGrann,[1] Donald W. Hughes,[2] Gerard D. Wright[1]*

Microbial resistance to antibiotics currently spans all known classes of natural and synthetic compounds. It has not only hindered our treatment of infections but also dramatically reshaped drug discovery, yet its origins have not been systematically studied. Soil-dwelling bacteria produce and encounter a myriad of antibiotics, evolving corresponding sensing and evading strategies. They are a reservoir of resistance determinants that can be mobilized into the microbial community. Study of this reservoir could provide an early warning system for future clinically relevant antibiotic resistance mechanisms.

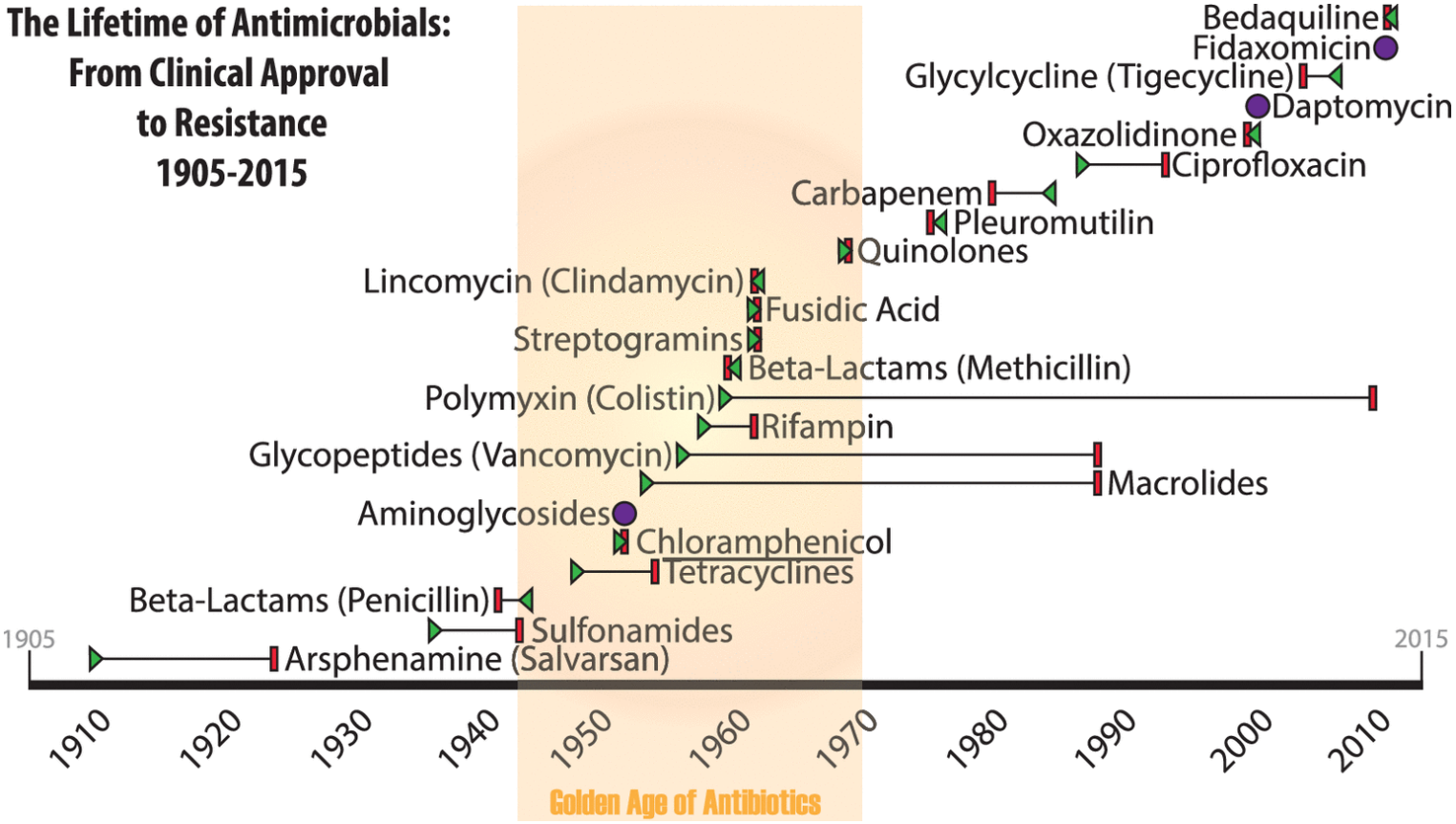


- Library of 480 morphologically diverse spore-forming soil bacteria screened against 21 antibiotics
- Each strain was resistant, 7-8 antibiotics on average, range 2-21 drugs
- 200 different resistance profiles

all antibiotic resistance genes, including genes associated with non-pathogens and including cryptic and precursor genes

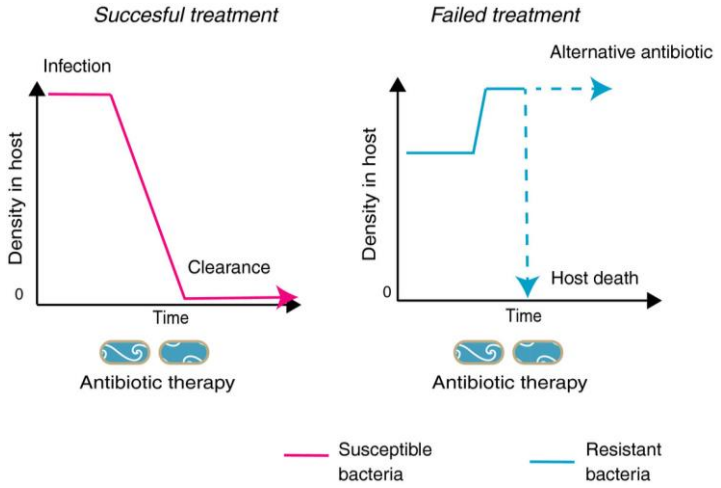
D’Costa, VM et al. (2006) *Science*. 311: 374-377

Resistance develops soon after antibiotics are introduced: the resistome is a source of ARGs

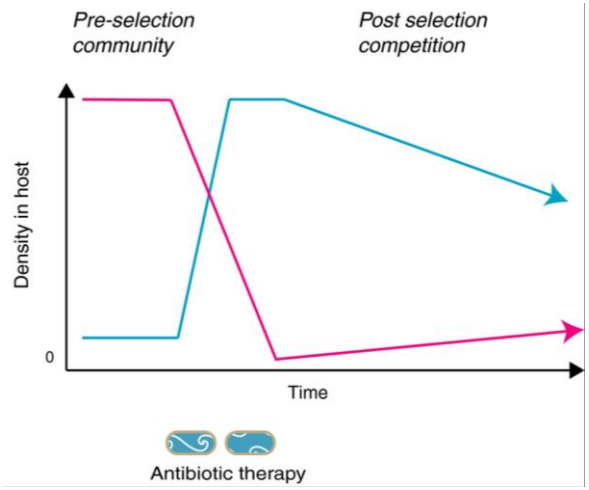


We often focus on pathogens: Antibiotics impact pathogens *and* commensals

Pathogen selection



Microbiota/commensal selection



Depends on:

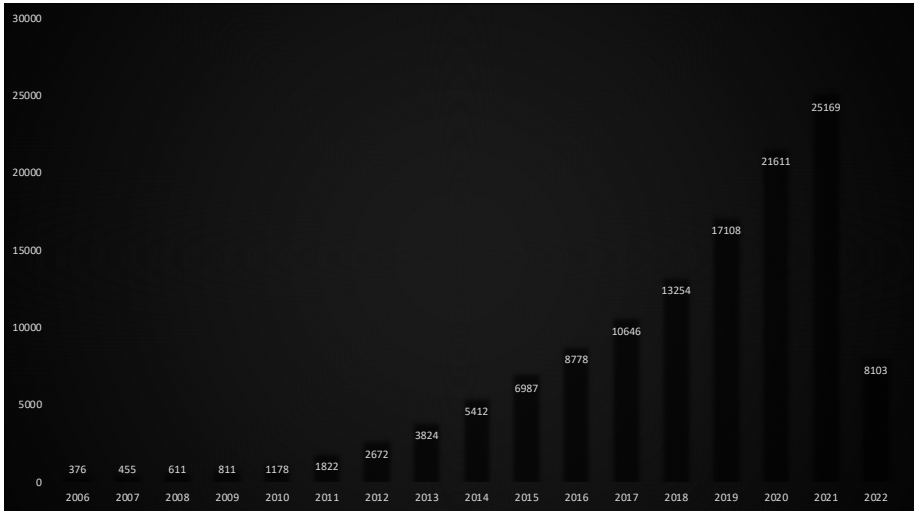
- Concentrations achieved in a particular body site
- Susceptibility of the resident microbiota (species and resistance genes expressed)
- Interactions between species

This is relevant because:

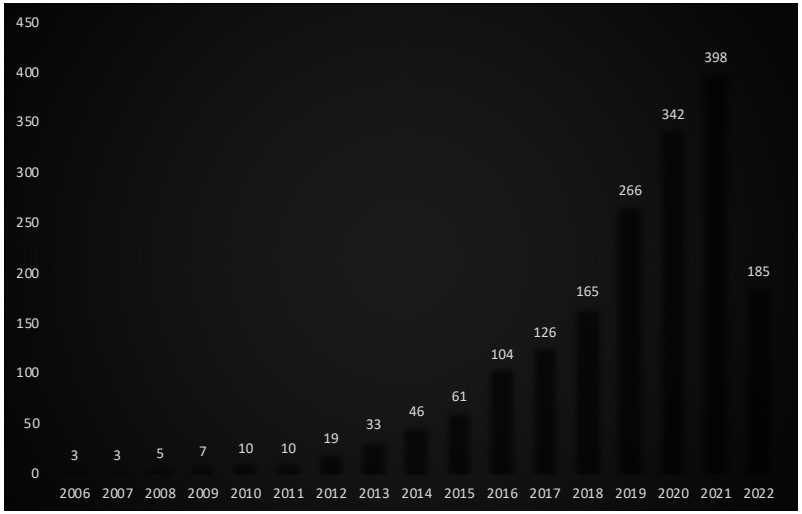
- Commensals can be “pathogens” and resistant strains/genes can be transmitted

Papers in PubMed 2006-2022

Microbiome



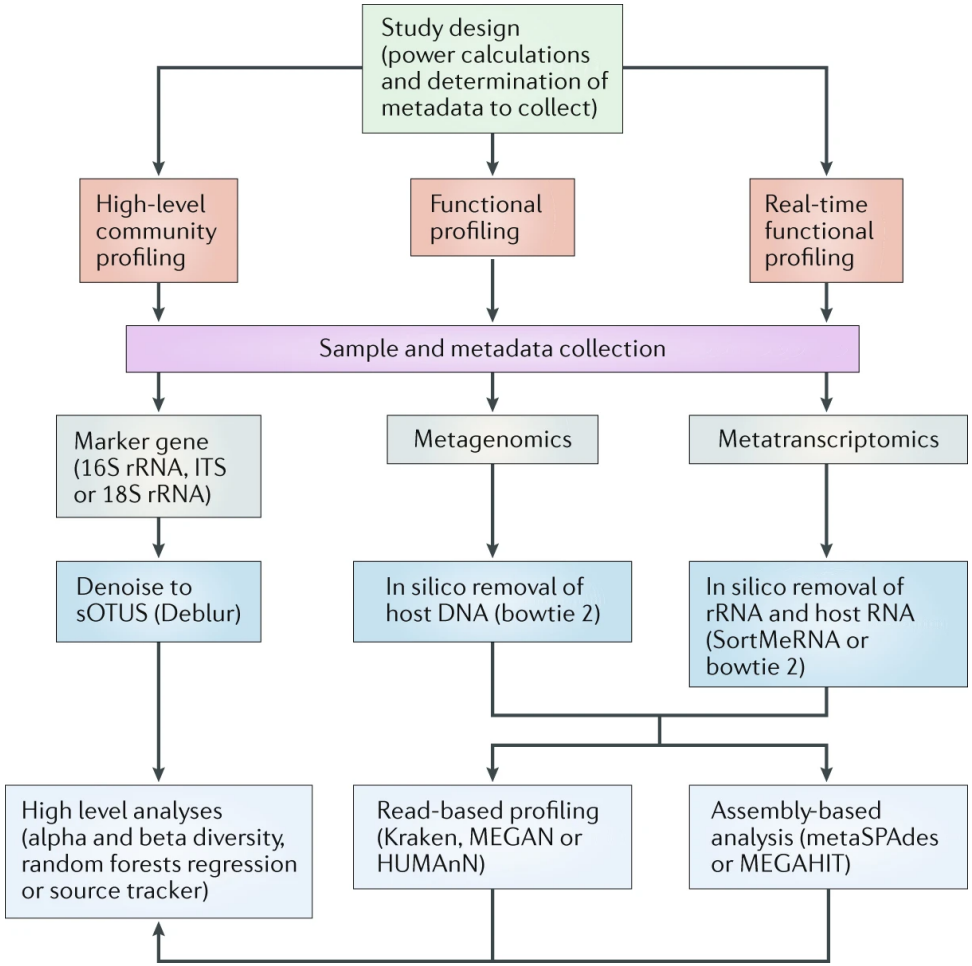
Resistome



Challenges for microbiome and resistome studies: One-size does not fit all

- Lack of standardization in methods, definitions, and terminology
- Majority of studies focus on the gut and are cross-sectional and/or observational
- High inter-individual, geographic, and temporal variation
- Data and analytic challenges associated with technical variability, high-dimensionality, and sparsity/zero inflation
- Resources
  - trade-offs in coverage and cost
  - specimen collection
- Requires tailored computational and bioinformatic expertise, familiar with molecular and population data

Workflow for microbiome profiling



D'Costa, VM et al. (2006) Science. 311: 374-377

Should you perform 16S rRNA profiling or shotgun metagenomic sequencing?

frontiers in Microbiology

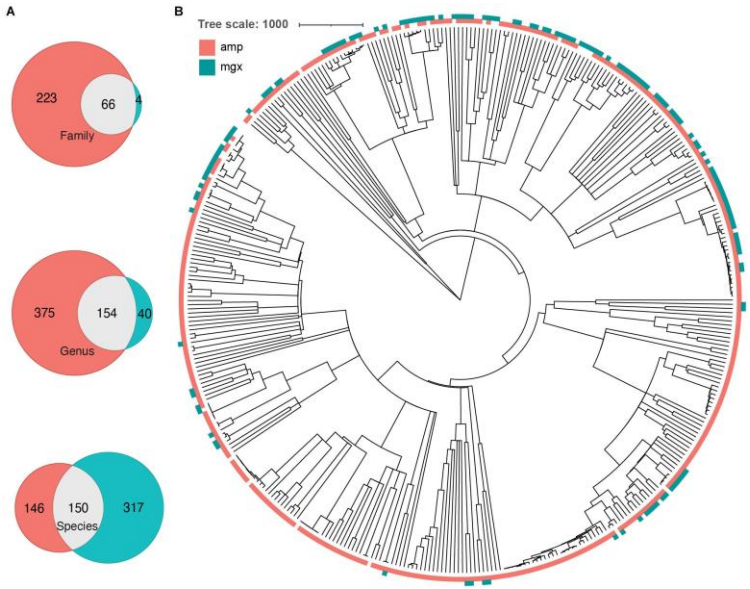
ORIGINAL RESEARCH
published: 15 July 2021
doi: 10.3389/fmicb.2021.670336

Comparative Analysis of 16S rRNA Gene and Metagenome Sequencing in Pediatric Gut Microbiomes

*Danielle Peterson[1], Kevin S. Bonham[1], Sophie Rowland[1], Cassandra W. Pattanayak[2], RESONANCE Consortium and Vanja Klepac-Ceraj[1]**

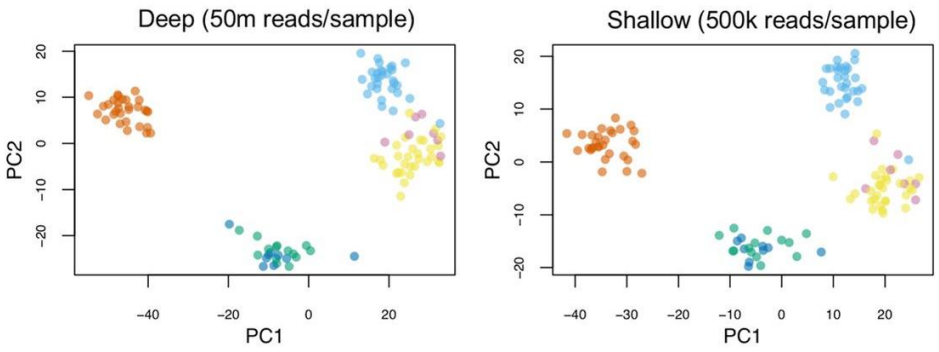
[1] Department of Biological Sciences, Wellesley College, Wellesley, MA, United States, [2] Department of Mathematics, Quantitative Reasoning Program, and the Quantitative Analysis Institute at Wellesley College, Wellesley, MA, United States

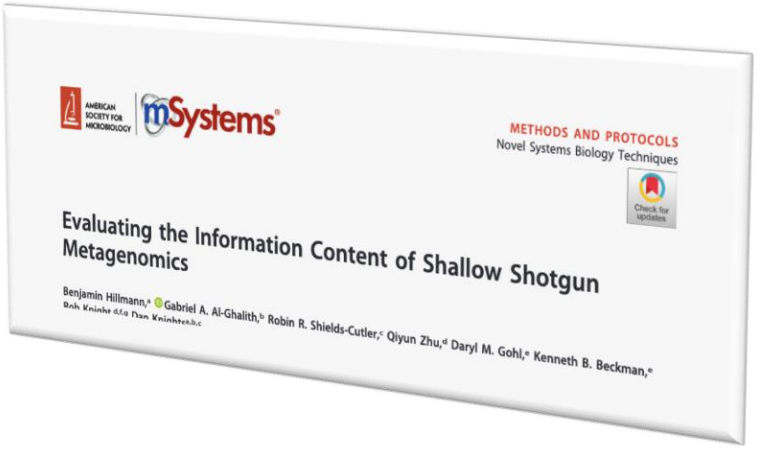
OPEN ACCESS

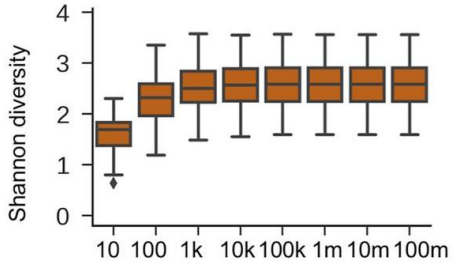


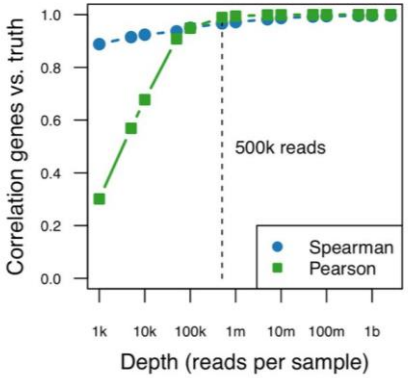
- 16S rRNA sequencing is cost-effective, limited taxonomic resolution
- Cannot profile non-bacterial members of the community or AMR
- 16S rRNA identifies more taxa at the genus level, relative abundances are systematically lower
- *Bifidobacterium* and *Enterobacter* under-represented in 16S rRNA sequencing

Shallow metagenomic sequencing may be appropriate for some purposes



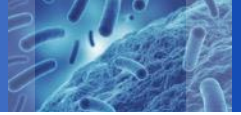






- Shallow sequencing does not perform well in low biomass samples or w/high host DNA contamination (e.g., blood)
- Does not allow for *de novo* assembly of genes and genomes

Hillman, B. et al.. (2018) *mSysttems*. 3:00069-18.



There are >47 antibiotic resistance gene databases

Databases	Last Modified	Notes
ARDB and ARG-ANNOT	Archived, 2009 and 2018	Not actively updated
ARGminer	2019	Ensemble database from other sources (CARD, ARDB, SARG etc.), machine learning and crowdsourcing to refine
CARD	2021	Comprehensive, sequences must be in GenBank w/published experimental validation
FARME	2019	Based on metagenomic studies, predicted AMR
MEGAres	2019	Assembled from multiple sources, biocide and metal resistance, designed for abundance-based analysis from metagenomic data
Mustard	2018	Gut resistome
NDARO	2021	Curated by NCBI; AMR, stress response, and virulence genes for clinically important pathogens
PATRIC	2017	Genome sequence data and metadata
ResFam	2015	Not actively updated
ResFinder/ PointFinder	2021	Acquired resistance genes/mutations
SARG	2019	Hierarchical database based on CARD and ARDB, acquired resistance, two levels-type (e.g., vancomycin) and gene

Papp, M and Solymosi (2022) Antibiotics. 11:339.

Geographical differences in the gut antibiotic resistome

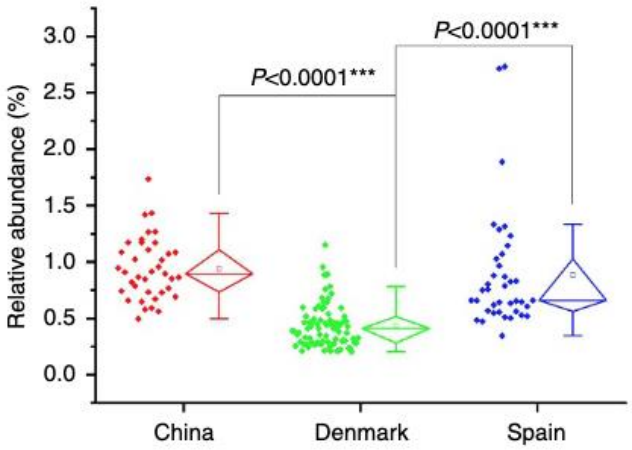
ARTICLE

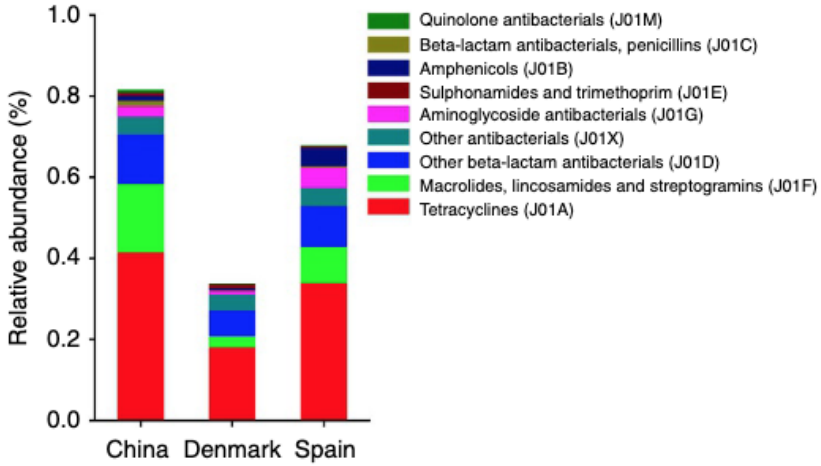
Received 21 Feb 2013 | Accepted 13 Jun 2013 | Published 23 Jul 2013

DOI: 10.1038/ncomms3151

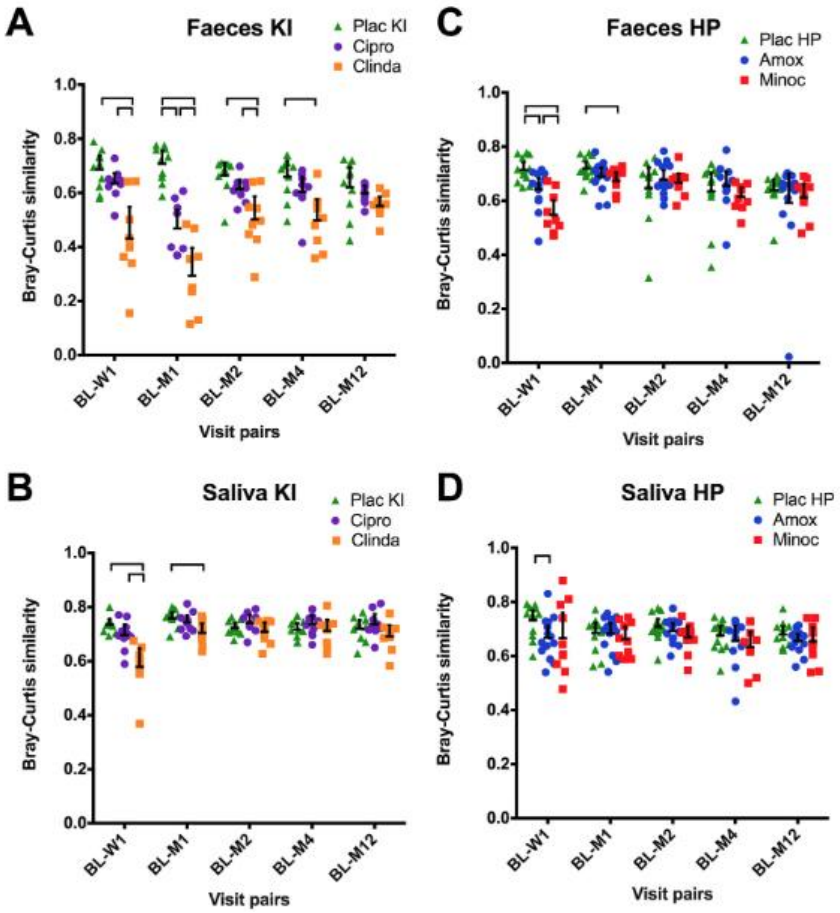
Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota

Yongfei Hu[1,*], Xi Yang[1,*], Junjie Qin[2], Na Lu[1], Gong Cheng[1], Na Wu[1], Yuanlong Pan[1], Jing Li[1], Liying Zhu[3], Xin Wang[3], Zhiqi Meng[3], Fangqing Zhao[4], Di Liu[1], Juncai Ma[1], Nan Qin[5], Chunsheng Xiang[5], Yonghong Xiao[5], Lanjuan Li[5], Huanming Yang[2], Jian Wang[2], Ruifu Yang[6], George F. Gao[1,7], Jun Wang[2] & Baoli Zhu[1]





The microbiome's response to antibiotics differs by body site and antibiotic type



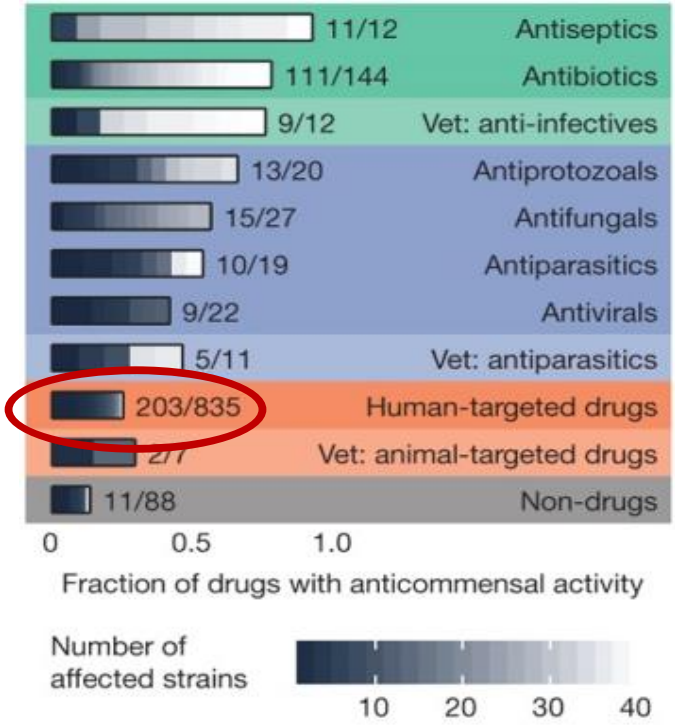
- Salivary microbiome recovers more quickly than the fecal microbiome
- Clindamycin resulted in the most profound microbiota shifts
  - 4 months in stool
  - 1 month in saliva

Karolinska Institute, Sweden (KI)
Helperby, United Kingdon (HP)

Many drug classes impact the microbiome

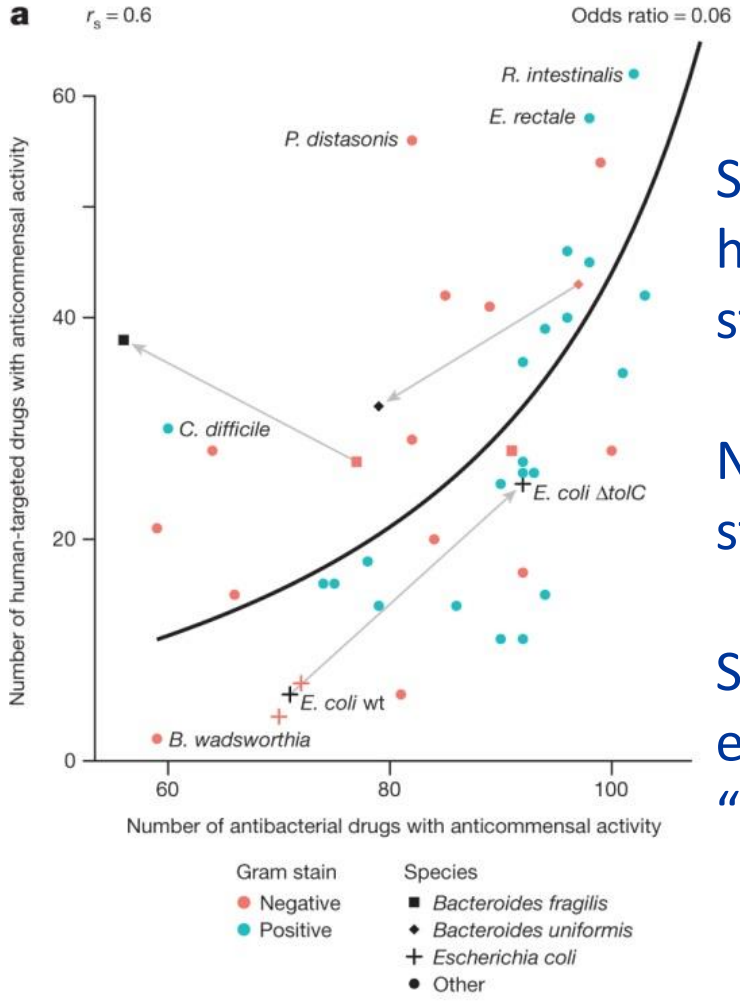

ARTICLE

Extensive impact of non-antibiotic drugs on human gut bacteria



- Examined 1,197 marketed drugs screened against 40 gut bacteria
  - 835 human targets (i.e., not anti-infectives)
- 27% of non-antibiotics inhibited the growth of at least one strain (i.e., many drugs impact the microbiota)
  - antipsychotics are overrepresented

Antibiotic resistance mechanisms protect against human-targeted drugs



Susceptibility to antibacterial agents and human-targeted drugs correlates across strains

Not dependent on cell membrane structure

Suggests common mechanism (e.g., efflux pumps) rather than traditional “resistance genes”

Maier, L. et al. (2018) Nature. 555: 623-628

PERSPECTIVE
https://doi.org/10.1038/s41591-021-01258-0
nature medicine

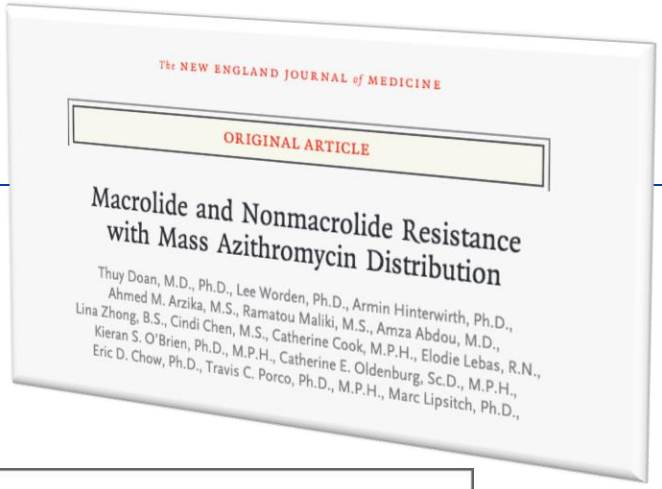
A framework for microbiome science in public health

Jeremy E. Wilkinson[1,2,22], Eric A. Franzosa[1,2,3,22], Christine Everett[1,4], Chengchen Li[1,2], HCMPH researchers and trainees*, HCMPH investigators*, Frank B. Hu[1,4,5,6], Dyann F. Wirth[1,3,7], Mingyang Song[1,5,6,8,9], Andrew T. Chan[1,3,4,7,8,9], Eric Rimm[1,4,5,6], Wendy S. Garrett[1,7,10,11,23] ✉ and Curtis Huttenhower[1,2,3,7,23] ✉

"While microbiome studies in human populations have the luxury of being amenable to interventional designs (unlike genetics), the vast majority of studies to date are observational…such studies also run the risk of conflating causation and correlation…."

MORDOR TRIAL

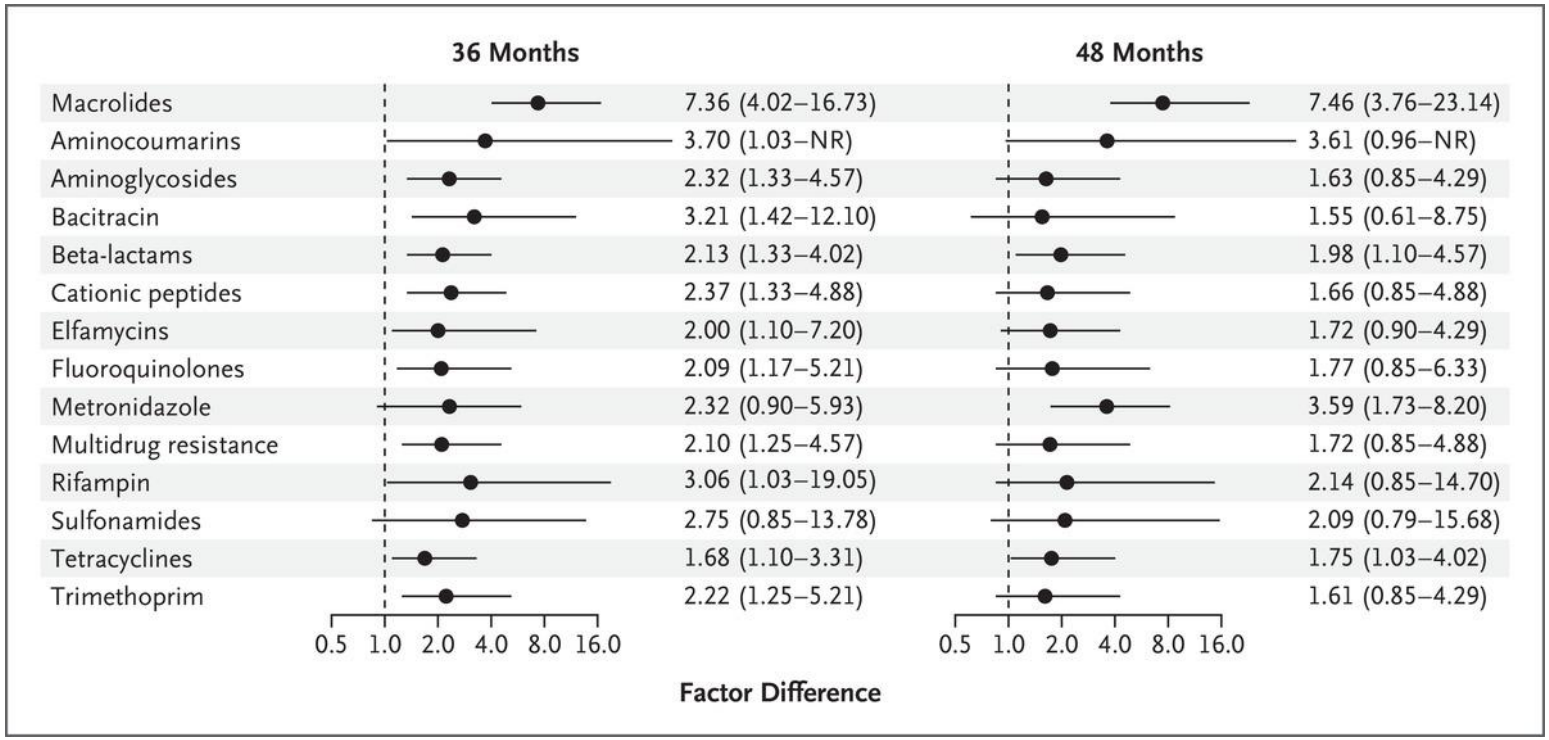
Twice yearly azithromycin or placebo for 4 years to address infant and child mortality

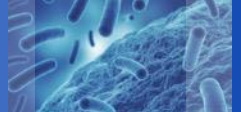

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Macrolide and Nonmacrolide Resistance with Mass Azithromycin Distribution

Thuy Doan, M.D., Ph.D., Lee Worden, Ph.D., Armin Hinterwirth, Ph.D., Ahmed M. Arzika, M.S., Ramatou Maliki, M.S., Amza Abdou, M.D., Lina Zhong, B.S., Cindi Chen, M.S., Catherine Cook, M.P.H., Elodie Lebas, R.N., Kieran S. O'Brien, Ph.D., M.P.H., Catherine E. Oldenburg, Sc.D., M.P.H., Eric D. Chow, Ph.D., Travis C. Porco, Ph.D., M.P.H., Marc Lipsitch, Ph.D.,

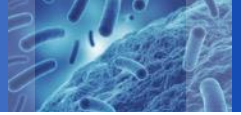




Short vs. Standard Course Outpatient Therapy of Community Acquired Pneumonia in Children (SCOUT-CAP)

Response Adjusted for Days of Antibiotic Risk (RADAR)

Desirability of Outcome Ranking (DOOR)



SCOUT-CAP and STAR

- Is there a better way to design clinical trials to more effectively address antibiotic resistance?

- Can we safely shorten the duration of antibiotic therapy to effectively treat patients and help address antibiotic resistance?

- Does the duration of antibiotic therapy affect the resistome and microbiota dysbiosis?

PIDS and IDSA guidelines for Community Acquired Pneumonia (CAP) in children

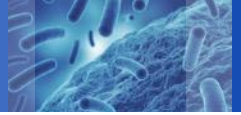
CAP is a common childhood infection and leading reason for hospitalization

Treatment courses of 10 days have been best studied, although shorter courses may be as effective…

-Strong recommendation, moderate quality evidence

Treatment for the shortest effective duration will minimize exposure of both pathogens and the normal microbiota to antimicrobials and minimize selection for resistance

-Strong recommendation, low quality evidence



Isn't the answer obvious?

The effect of antibiotics on the risk of resistance is not the same as the effect of an intervention to reduce antibiotic use on the risk of resistance

- Fitness gains of resistance may be offset by fitness costs of taxa carrying antibiotic resistance
- Assumes the absence of an antibiotic has the same but opposite effect as its presence
- Antibiotics can have lasting impacts (e.g., changes to the genetic level can change the fitness landscape)
- The presence of clinical concentrations of antibiotics more strongly selects for resistance than the absence selects for reversion to sensitivity

Smith, HW. 1975. Nature. 258: 628-30.
Spangnolo F. et al. 2021. mBio. 12: e01966-21

SCOUT-CAP DOOR step 1: Categorization of participants by overall clinical outcome

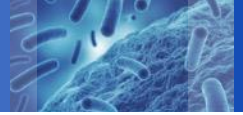
THREE COMPONENTS, EIGHT ORDINAL LEVELS RANKED IN DESCENDING ORDER

	Adequate Clinical Response	Resolution of Pneumonia Symptoms	Maximal Antibiotic Side Effects
1	Yes	Resolved	None
2	Yes	Resolved	Mild
3	Yes	Resolved	Moderate
4	Yes	Resolved	Severe
5	Yes	Persistent Symptoms	Any
6	No, ED/clinic visit only	Any	Any
7	No, hospitalization	Any	Any
8	Death from any cause	Any	Any

Step 2: DOOR and Response Adjusted for Days of Antibiotic Risk (RADAR)

- Composite endpoint, ranks clinical response, resolution of symptoms, adverse events, and number of antibiotic days

- DOOR is constructed using two rules:
  - Comparisons of two patients with different clinical outcomes
    - patient with the better clinical outcome receives a higher rank
  - Within each door rank (i.e., two patients with the same clinical outcome)
    - patient with a shorter ***actual*** duration of antibiotic use receives a higher rank

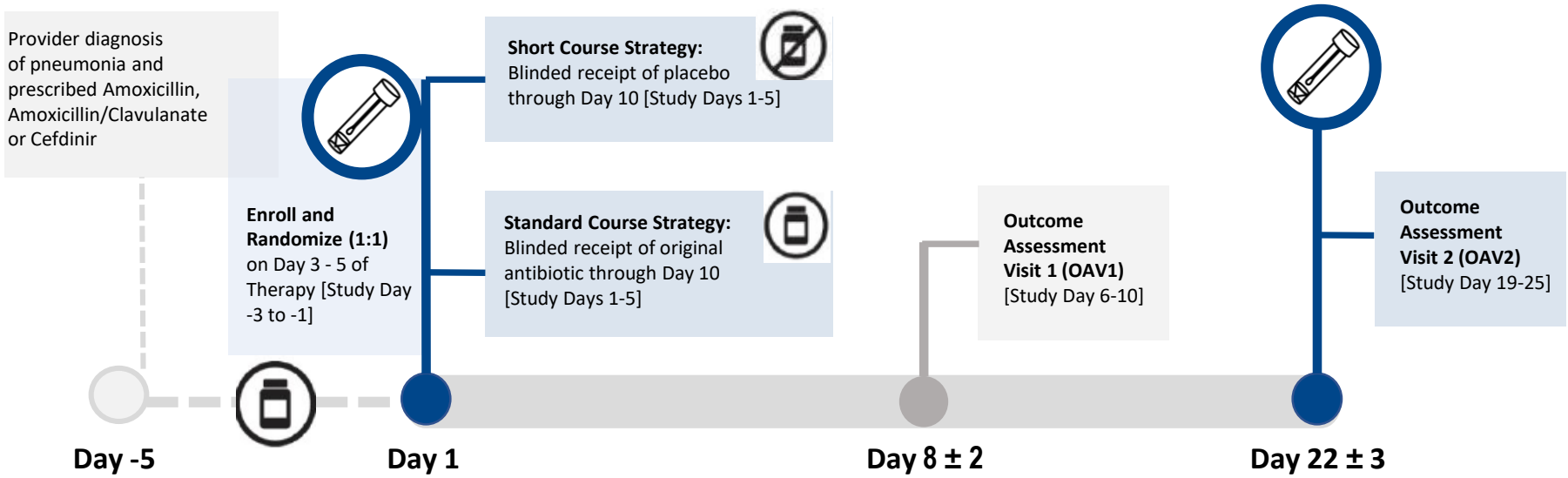


STEP 3: Evaluate superiority of RADAR

- Estimation: probability that a randomly selected patient will have a better outcome if assigned the new strategy relative to the control

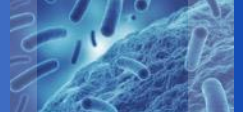
- Hypothesis testing
  - null: no difference in RADAR
    - the probability that a patient assigned to the new strategy will have a better outcome than if assigned to the control is 50%
  - alternative: 60% probability of a more desirable RADAR for the short course strategy
    - the probability that a patient assigned to the short-course strategy will have a better outcome than if assigned to the standard strategy is 60%

SCOUT-CAP and STAR study design and timeline



SCOUT-CAP results

- 380 children with non-severe pneumonia, ages 6-71 months (short course=189; standard course=191)
- No differences between strategies in DOOR
- Short course strategy: 69% (95% CI 63%-75%) probability of a more desirable RADAR outcome compared to the standard course strategy
- 5-day antibiotic strategy superior to a 10-day strategy
  - similar clinical response and antibiotic adverse effects
  - reduced antibiotic exposure



ARLG-STAR objectives

Primary Objective: To compare the antibiotic resistome in children receiving short course vs. standard course antibiotic therapy for CAP

Primary Hypothesis: The relative abundance of antibiotic resistance genes will be lower in children receiving short course vs. standard course antibiotic therapy

Secondary Objective: To identify changes in the gastrointestinal microbiome associated with antibiotic-associated diarrhea in children receiving short course vs. standard course antibiotic therapy for CAP

ARLG-STAR methods

- Intention to treat cohort (ITT): all randomized subjects that were still eligible on Day 1 of the study
  - 171 subjects with analyzed throat swab samples

- Shotgun metagenomic sequencing of enrollment visit and OAV2 throat samples
  - identification of antibiotic resistance genes
  - species level taxonomic identification

- 16S rRNA gene sequencing of throat and stool samples from enrollment, OAV1 and OAV2
  - taxonomic identification of bacteria at the genus level

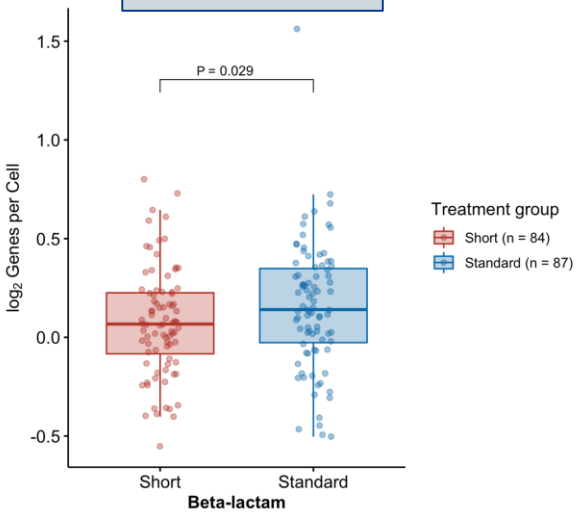
ARLG-STAR characteristics of the ITT population

Characteristic	Short (n = 84)	Standard (n = 87)	Total (n = 171)
Age			
6–23 mo	26 (31)	30 (34)	56 (33)
24–71 mo	58 (69)	57 (66)	115 (67)
Sex			
Female	46 (55)	38 (44)	84 (49)
Male	38 (45)	49 (56)	87 (51)
Race			
Asian	4 (5)	1 (1)	5 (3)
Black or African American	19 (23)	23 (26)	42 (25)
Multiracial	8 (10)	4 (5)	12 (7)
White	51 (61)	58 (67)	109 (64)
Initial antibiotic			
Amoxicillin	78 (93)	78 (90)	156 (91)
Amoxicillin-clav. or Cefdinir	6 (7)	9 (10)	15 (8)

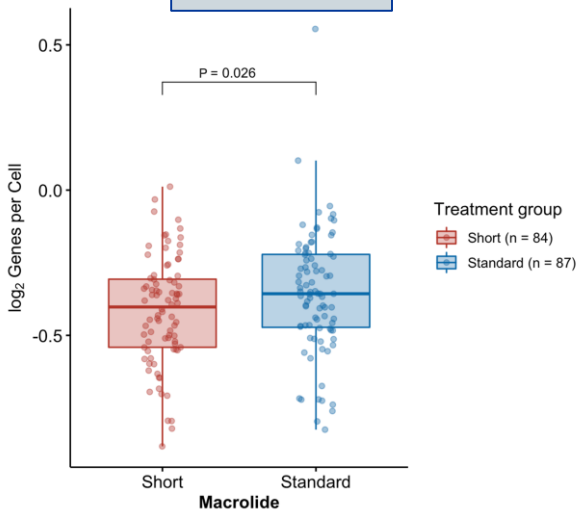
Resistance genes per prokaryotic cell (RGPC) were higher in the standard strategy group at OAV2 (N=171)

Resistance examined for 10 clinically relevant antibiotics

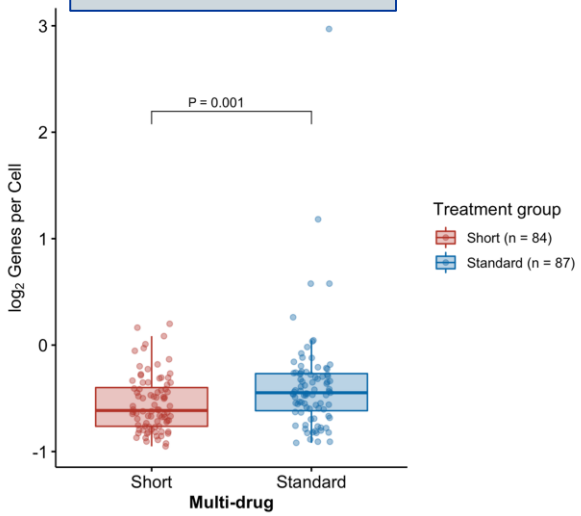
Beta-lactams



Macrolides



Multi-drug efflux

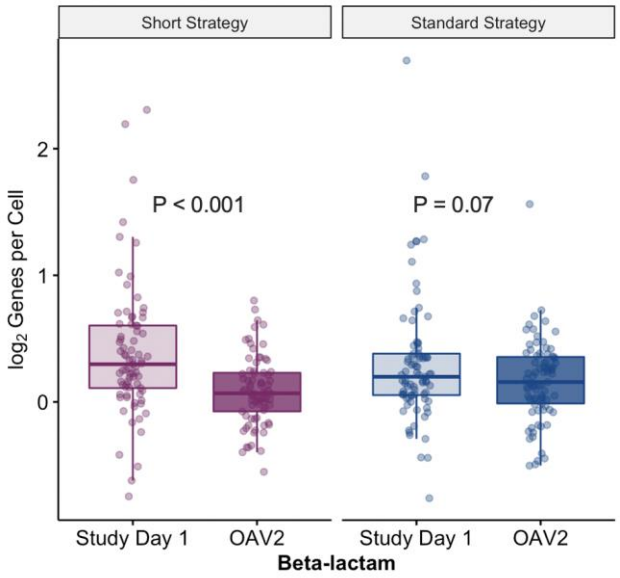


Wilcoxon-Rank sum test

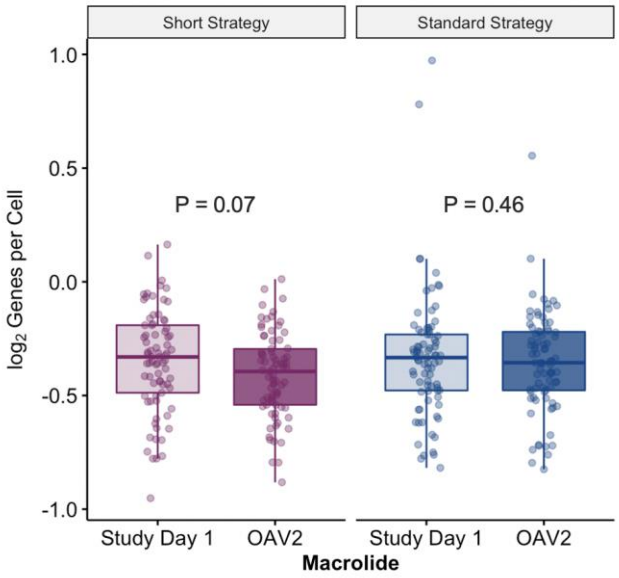
RGPC at enrollment and OAV2 by treatment strategy (N=158)

No significant differences in RGPC by treatment strategy at enrollment for any of the 10 antibiotic types

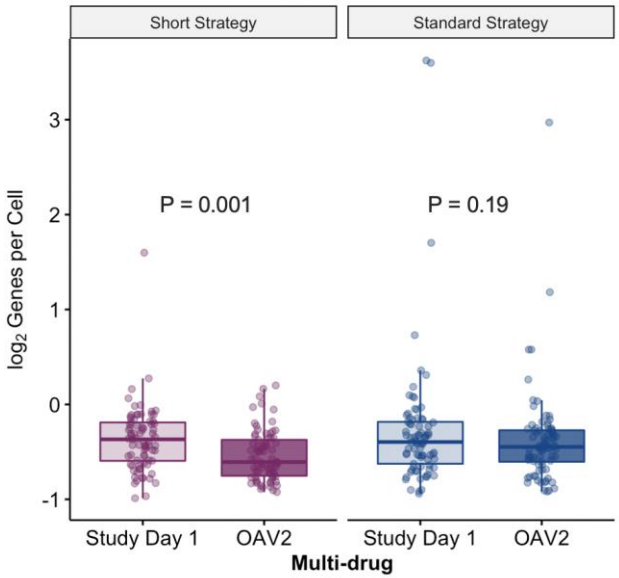
Beta-lactams



Macrolides

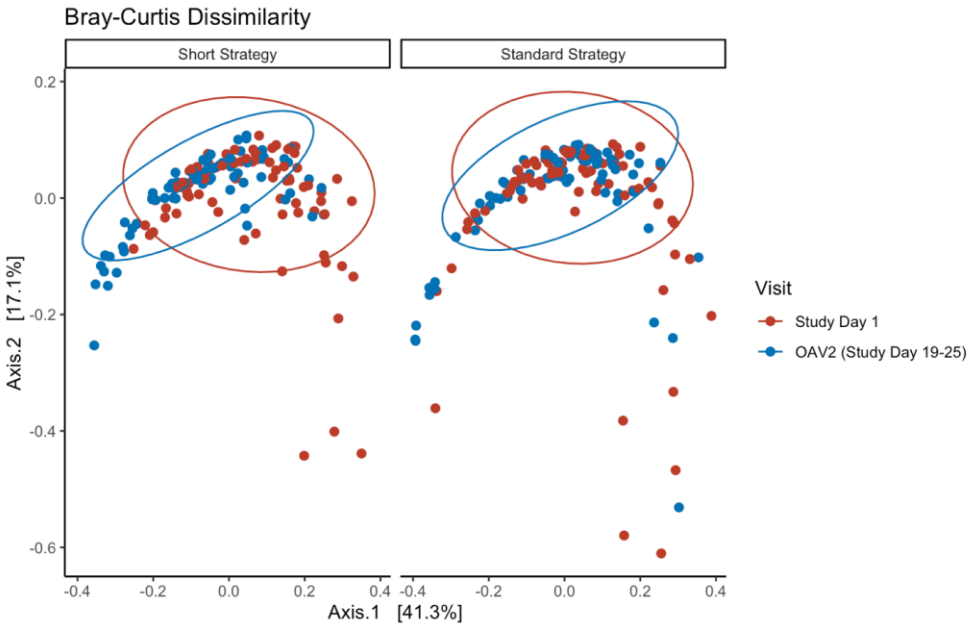


Multi-drug efflux



Wilcoxon-Rank sum test, FDR adjusted P values

Beta diversity in ARGs differs by visit and treatment strategy



- ARG composition differed by visit (PERMANOVA, P=0.001) but not by treatment group
- Interaction between treatment strategy and visit
- Compositional ARG profile differed at enrollment and OAV2 in the short course strategy group (P<0.001)
- No statistically significant difference by visit for the standard treatment group

Commensal taxa differ in abundance by treatment strategy

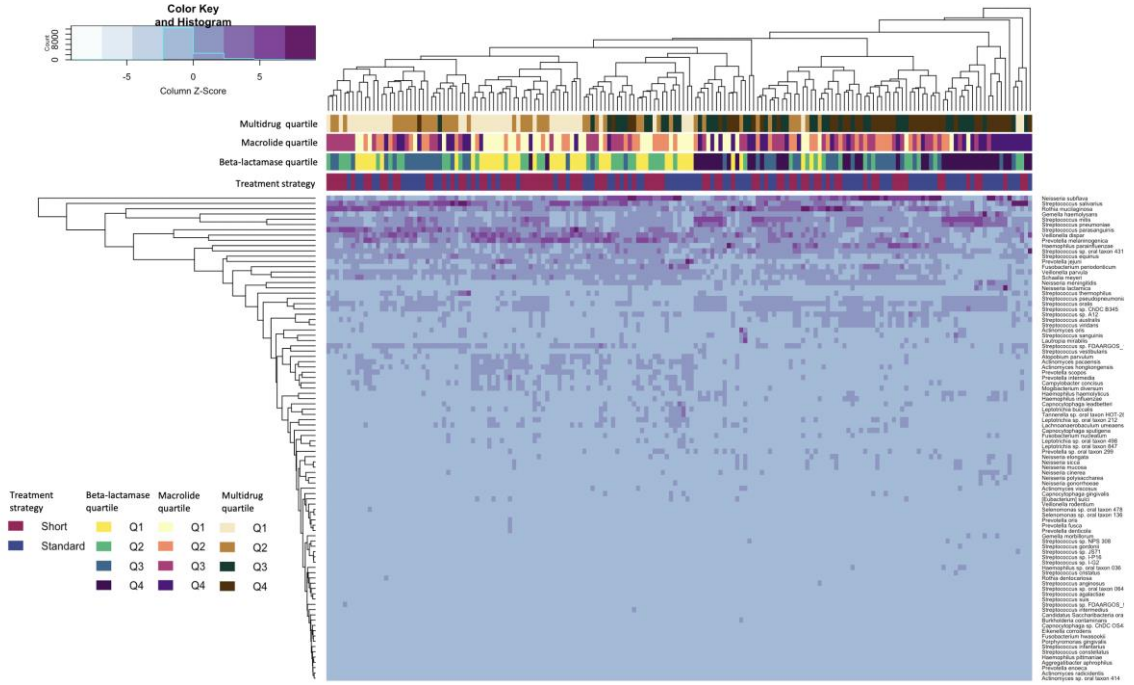
- 4,494 unique species

Higher relative abundance in the standard strategy group

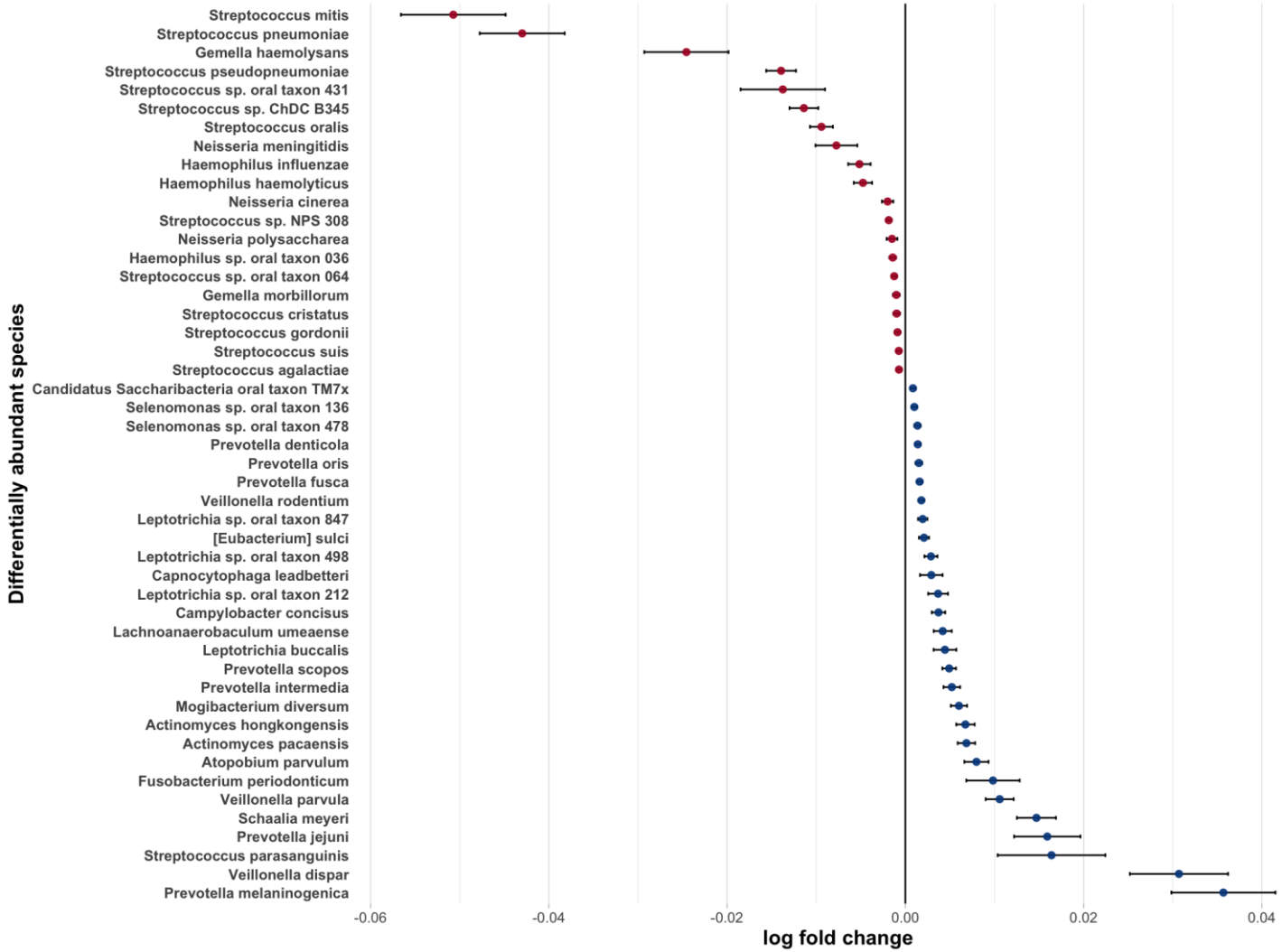
- *Neisseria subflava*
- *Capnocytophaga*

Higher relative abundance in the short strategy group

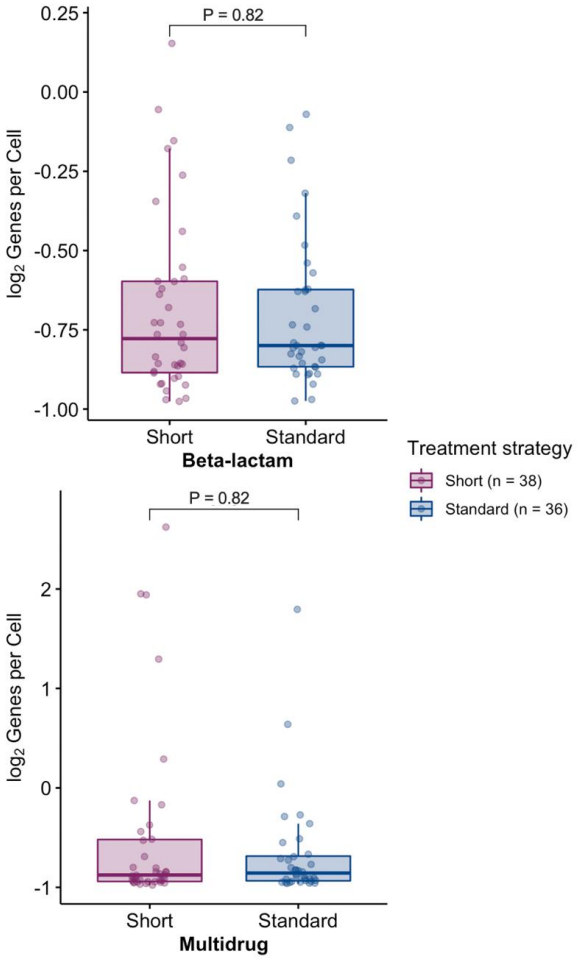
- *Prevotella scopos*
- *P. oris*
- *P. jejuni*
- *Veillonella parvulla*



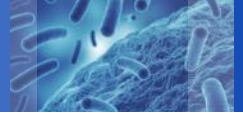
Pathogens are among 48 differentially abundant taxa in samples with a high vs. low beta-lactam ARGs



No differences in the gut resistome by treatment strategy

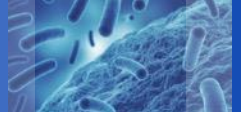


- Study likely underpowered (n=74 total)
- Participants characteristics differed from the SCOUT-CAP by race and age



Conclusions

- Small differences in the duration of therapy make a difference in the abundance of antibiotic resistance genes
- Standard beta-lactam treatment is associated with higher abundances of beta-lactam, macrolide, and multi-drug efflux resistance genes (co-selection of AMR genes)
- Children receiving standard beta-lactam therapy have higher abundances of ARGs for longer
- Limitations include the lack of swabs prior to study entry and after long-term follow up
  - randomized designed helps control for this



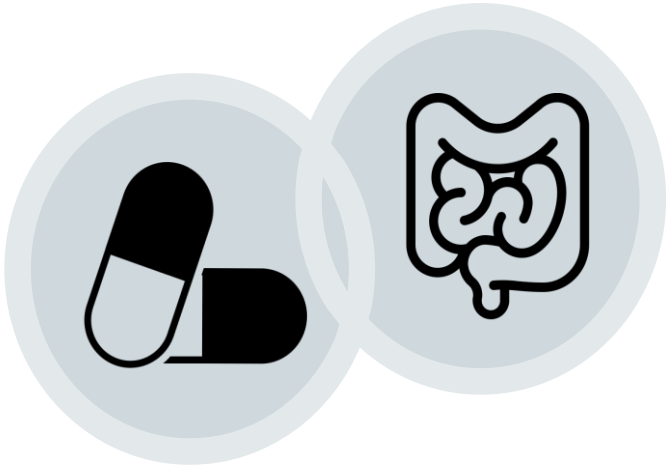
What are the potential public health implications?

- 931,748 (627,845-1,235,652) antibiotic prescriptions for pediatric pneumonia in 2015

- Widespread adoption of a 5-day beta-lactam strategy for the treatment of pediatric CAP could lead to a reduction in antibiotic exposure of ~ 5 million antibiotic days in US children

Collection of microbiome data allows for examination of other outcomes: Antibiotic associated diarrhea (AAD)

- 1 to 3 loose stools in 24–48-hour window with exposure to antibiotics
- ~30% in children on oral antibiotics
- May last up to 8 weeks after the end of therapy



What's causing it?

Multiple etiologies, *C. difficile*?

- adults: 15-25% of AAD
- children: less well studied

Antibiotic treatment → disturbs the gut flora & function → diarrhea

Kwon, J. et al. (2022) *J Infect Dis*. Jiac082.

Characteristics of the study population

- Children with stool samples for all 3 visits (n=67)
- Median diarrhea duration: 3 (2.0 , 5.5) days
- Diarrhea occurred sporadically

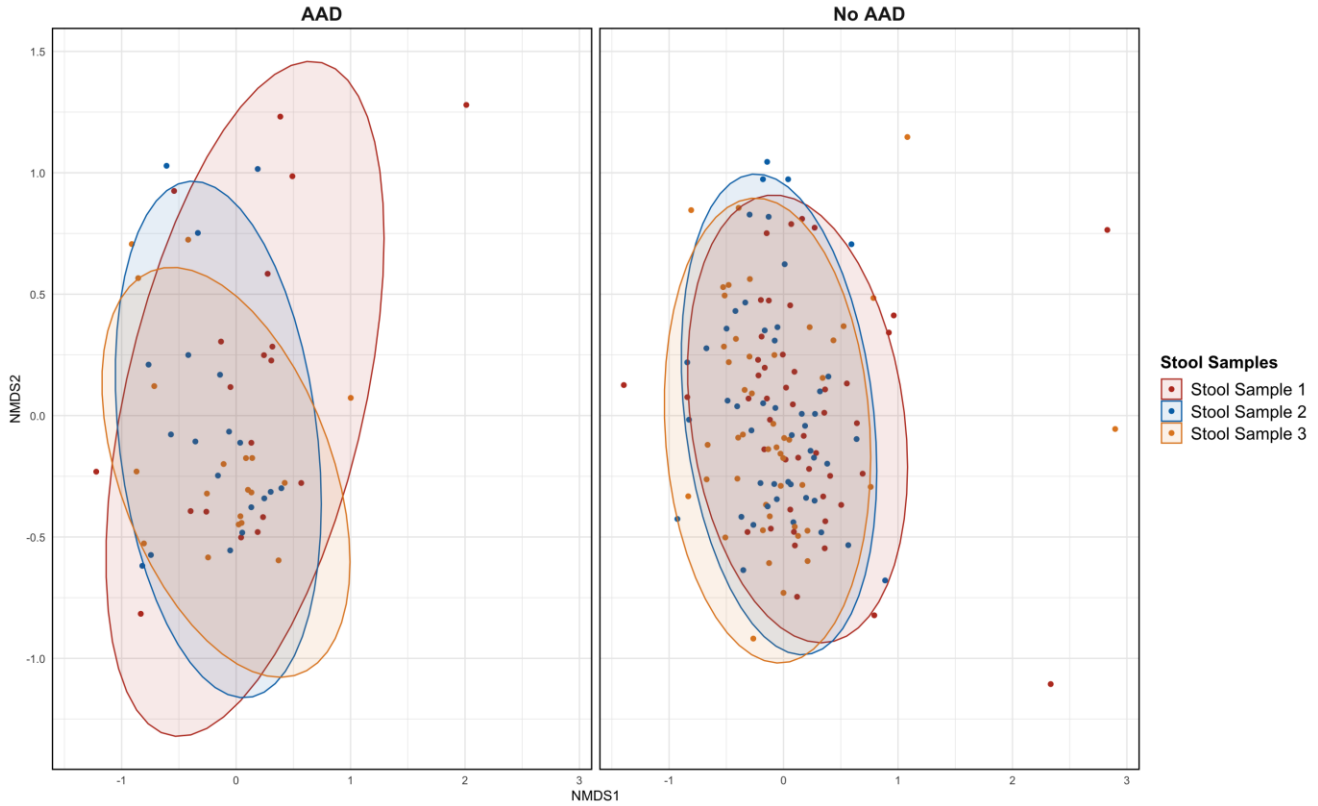
n = 19

n = 48

- Groups differed from SCOUT-CAP
  - age and race
- Higher proportion of males with AAD (Fisher's Exact Test, P = 0.05)

Sex	AAD	No AAD
Male	15 (79%)	24 (50%)
Female	4 (21%)	24 (50%)

Beta diversity differs by AAD and over time

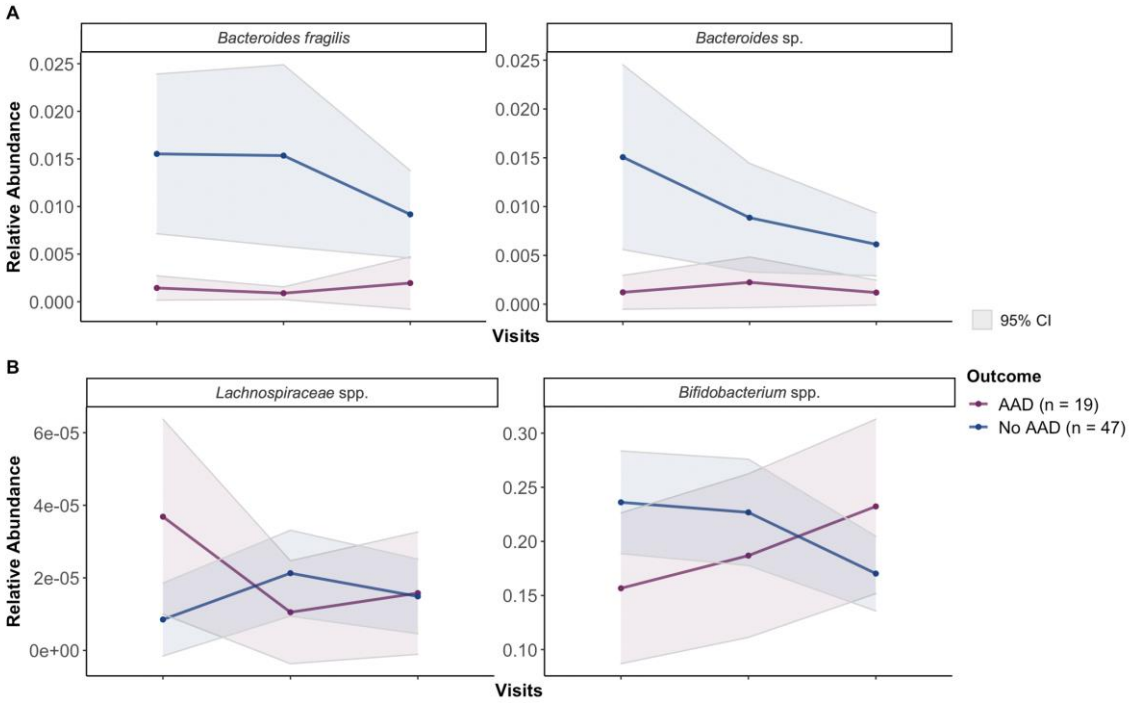


GI microbiota differs by outcome (i.e., AAD yes/no) (PERMANOVA, P = 0.03) and over time (PERMANOVA, P <0.001)

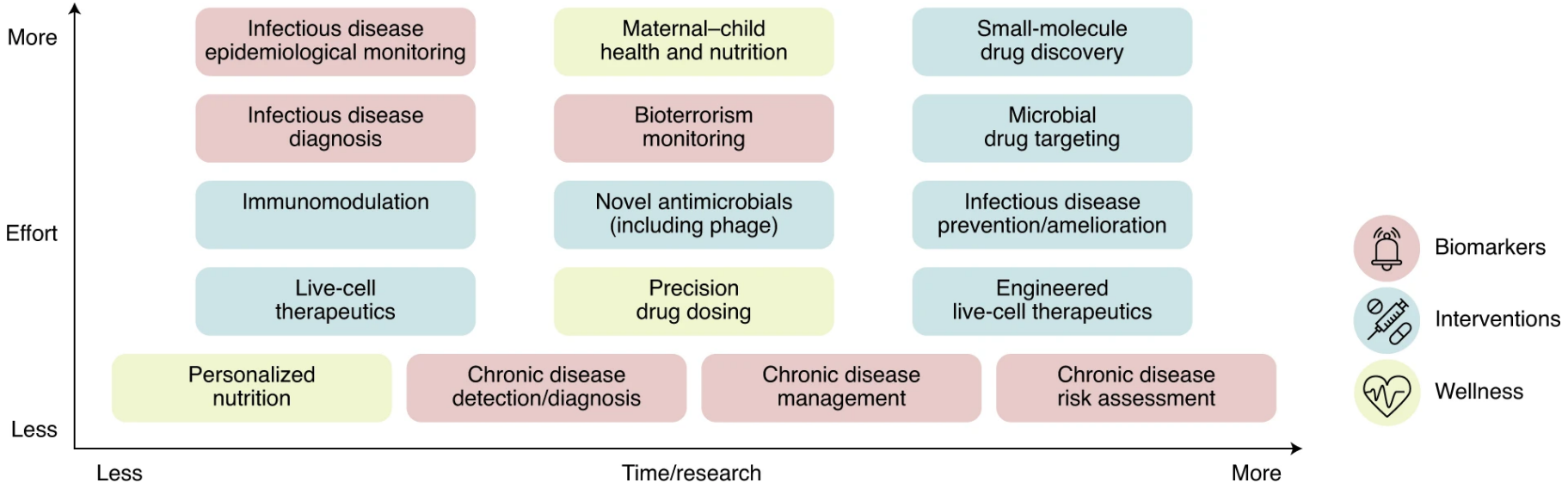
Baseline levels of three taxa were associated with AAD

- *B. fragilis* [OR 0.35; 95% CI (0.11, 0.79)]
- *Bacteroides sp.* [OR 0.59; 95% CI (0.24, 0.90)]
- Lachnospiraceae [OR 3.76 95% CI (1.06, 12.28)]

Taxa identified as differentially abundant at enrollment by LefSe



Opportunities

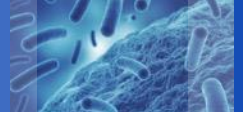


Financial support

•Research reported in this presentation was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Numbers HHSN27200018, HHSN27200022, HHSN27200011, HHSN27200006, and UM1AI104681

•The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health

ARLG
Antibacterial Resistance Leadership Group



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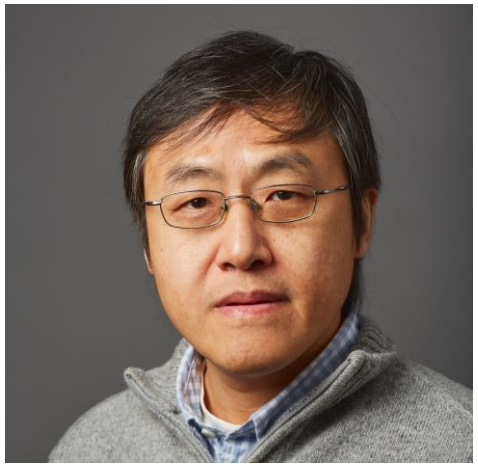

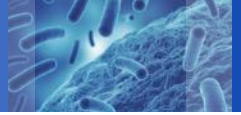

Thank you!