Mobile Antibiotic Resistome In Human And Animal Gut Microbiota

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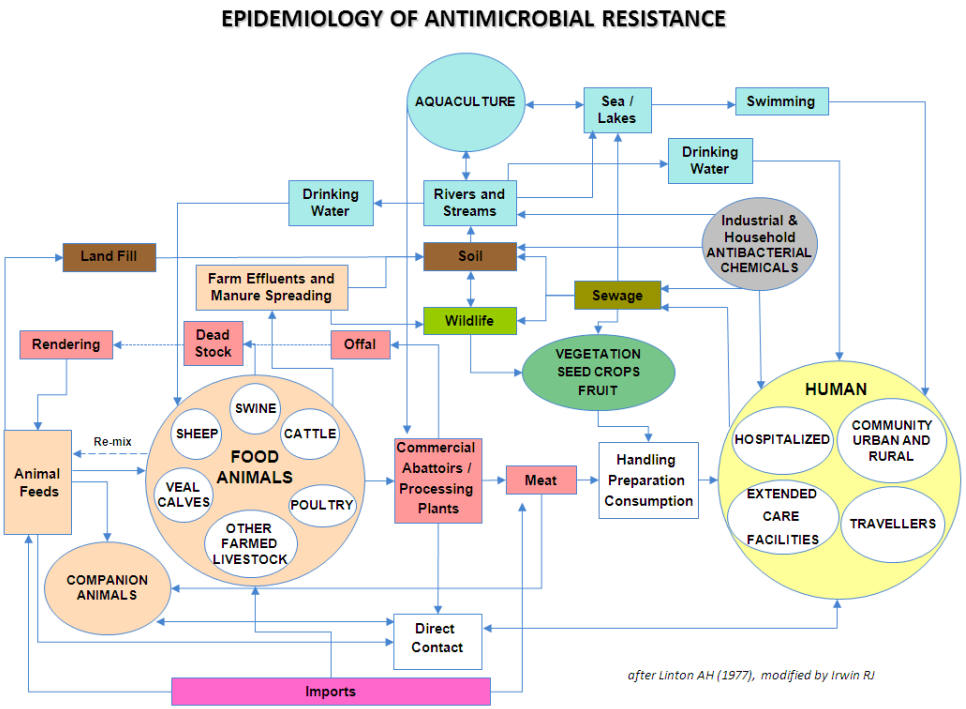
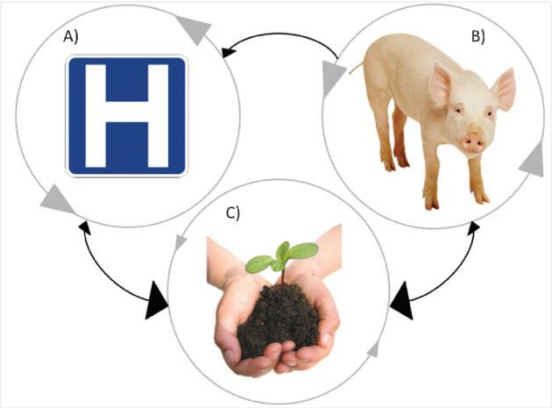
Euro-China·2016年10月28日

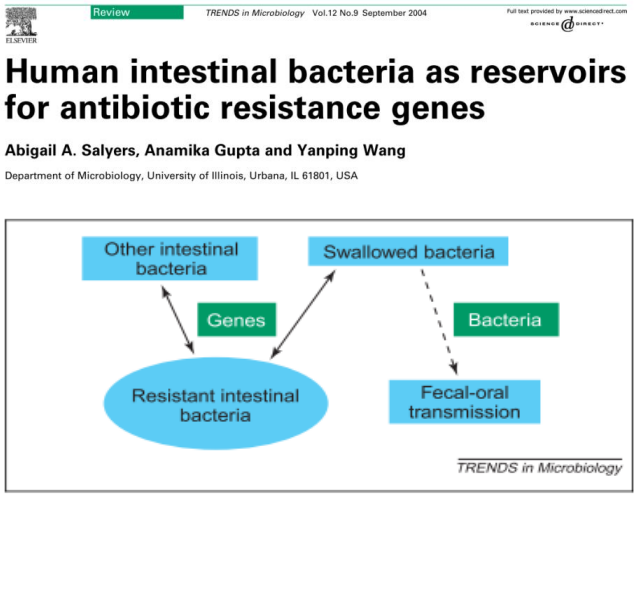
Dissemination of Antibiotic Resistance Genes



The circles represent potential anthropogenic antimicrobial selection pressure. Some pathways are well described, while others are plausible but lack substantive evidence. Not all plausible pathways are depicted.



Julie A. Perry 2014. *Bioessays.*



Review TRENDS in Microbiology Vol.12 No.9 September 2004

Human intestinal bacteria as reservoirs for antibiotic resistance genes

Abigail A. Salyers, Anamika Gupta and Yanping Wang

Department of Microbiology, University of Illinois, Urbana, IL 61801, USA

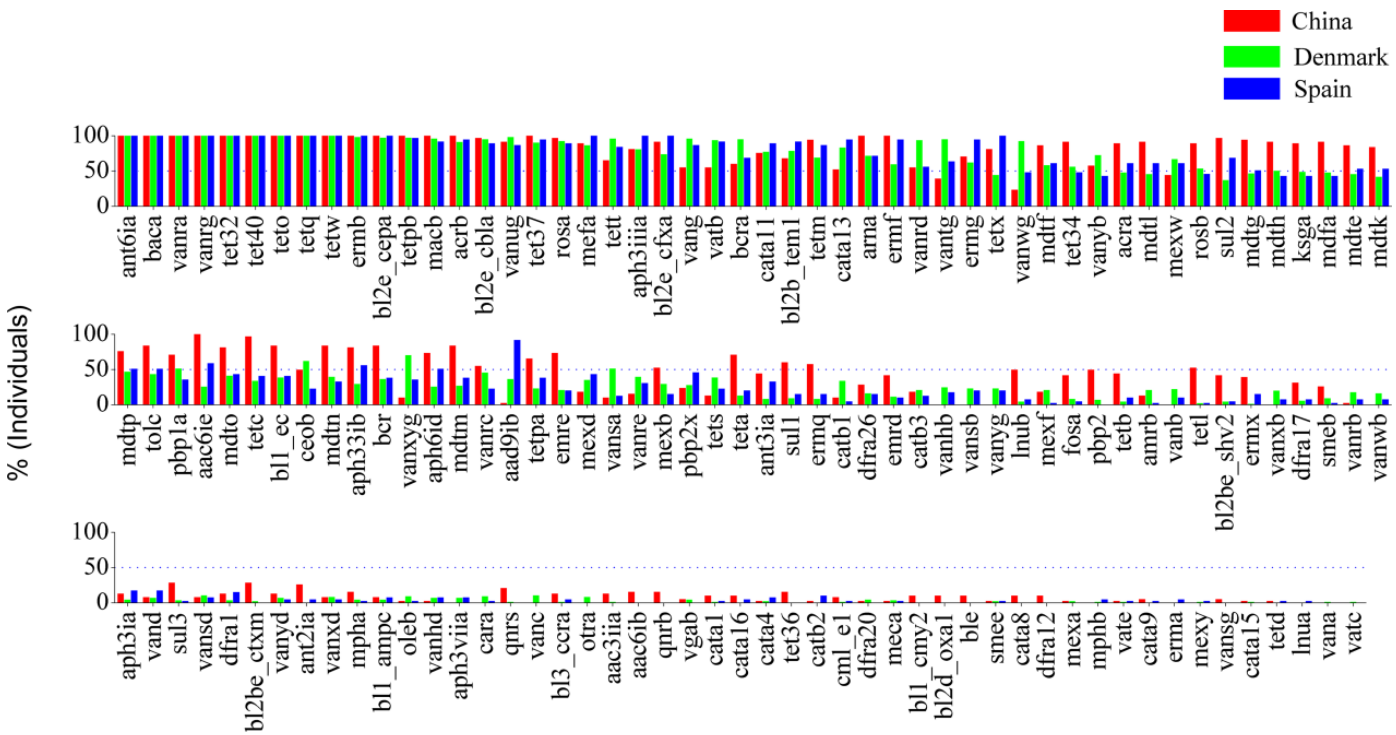
Large scale metagenomic analysis

Datasets Used in Initial Analysis on Human Gut Microbiota

- The 4.1 million human gut gene catalog created BGI **(from 85 Danish, 39 Spanish and 38 Chinese gut microbiota).**
- the Antibiotic Resistance Genes Database (ARDB) : **a core set of 7,825 antibiotic resistance genes**, which belong to **380** gene types that correspond to **249** antibiotics.

Different countries have different AR gene types

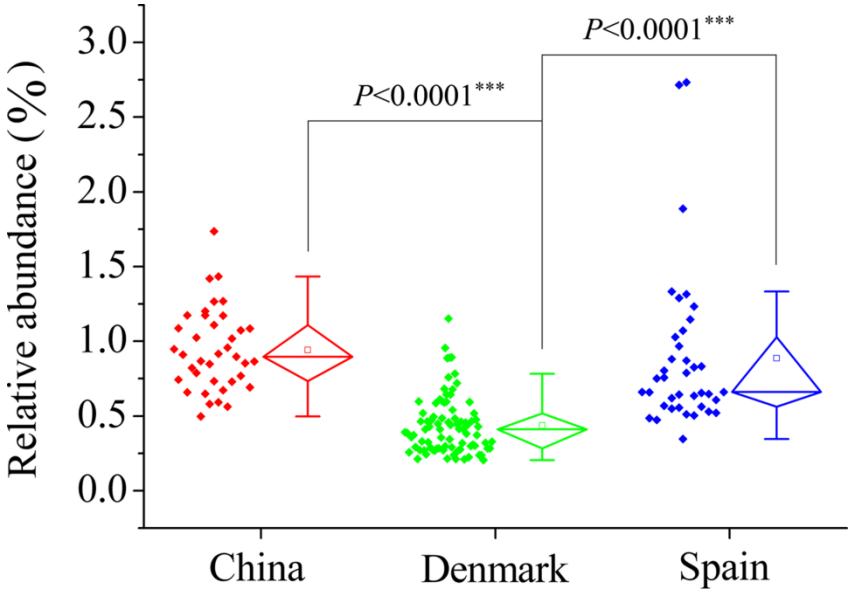
- 1,093 ARGs in the human gut， belonging to 149 AR gene types



More than 50% individuals: China 70 types; Denmark 45 types: Spain 49 types.

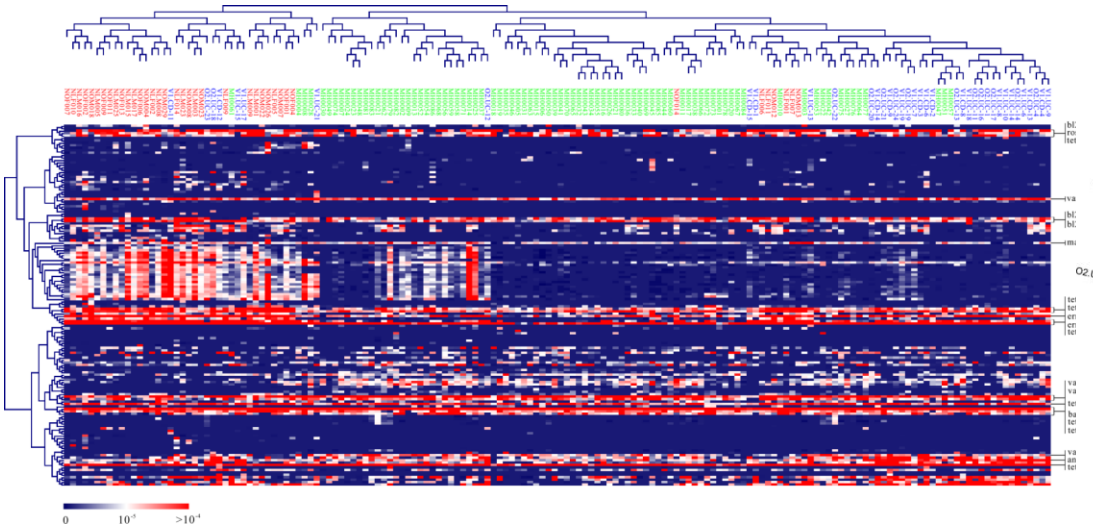
Abundance of the resistance genes in China is the highest

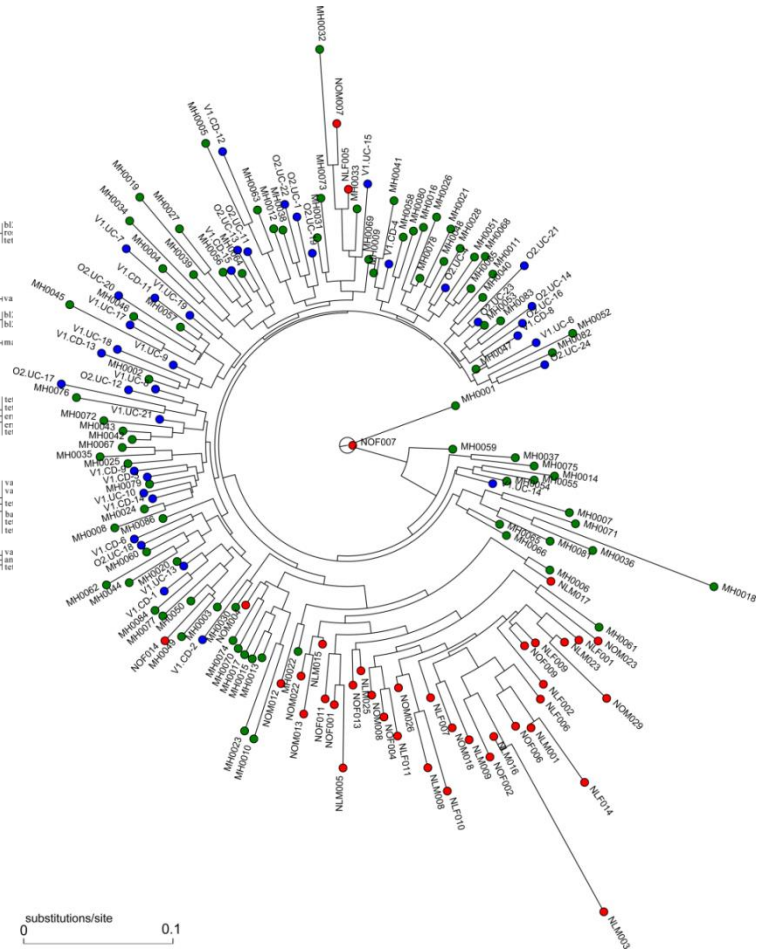
- To compare the abundances of antibiotic resistance genes in the Chinese, Danish and Spanish populations, we computed the relative enrichment of each of the genes based on sequencing coverage by using the original Illumina GA short reads.



Comparison of relative abundance of total AR genes in each individual. The diamond boxes on the right denote the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively) and the line and the square inside denote the median and mean, respectively.China: n=38; Denmark: n=85; Spain: n=39

Resistance gene abundance and SNPs are regionally different

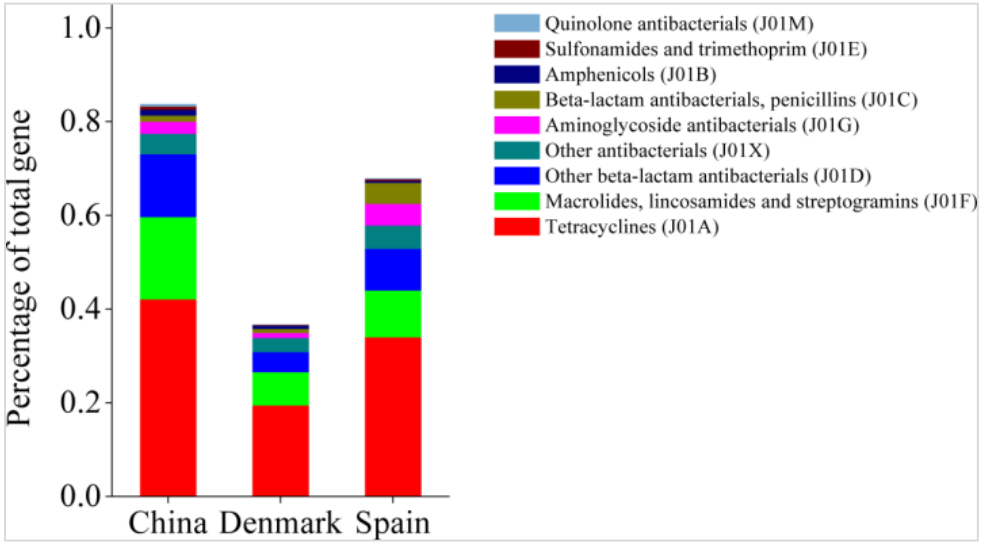


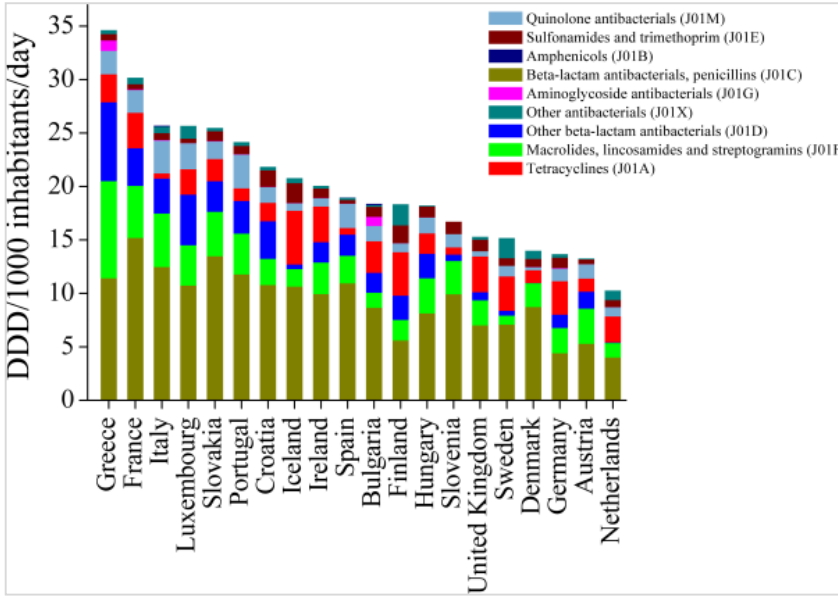


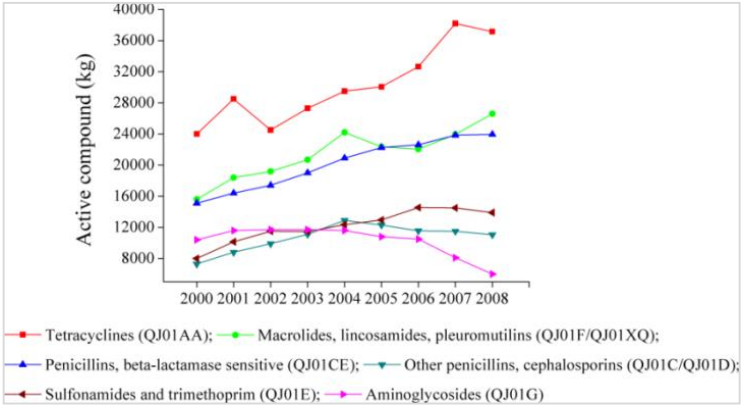
Hit map of relative abundance of each genotype in each individual and hierarchical clustering. The sample names of Chinese, Danish and Spanish individuals were colored by red, green and blue, respectively.

中国科学院
Institute o
of Sciences

The Abundance of AR genes in human gut and the agricultural use of Antibiotics



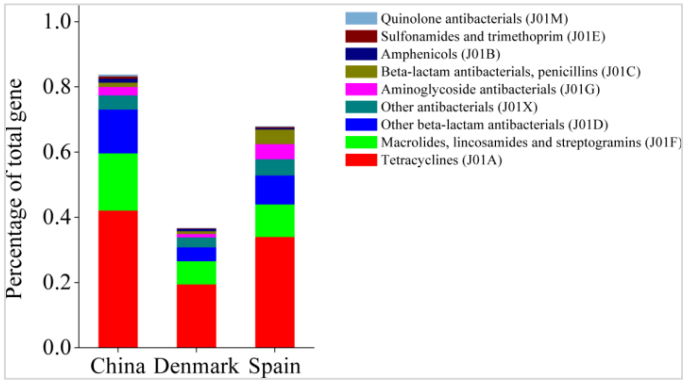


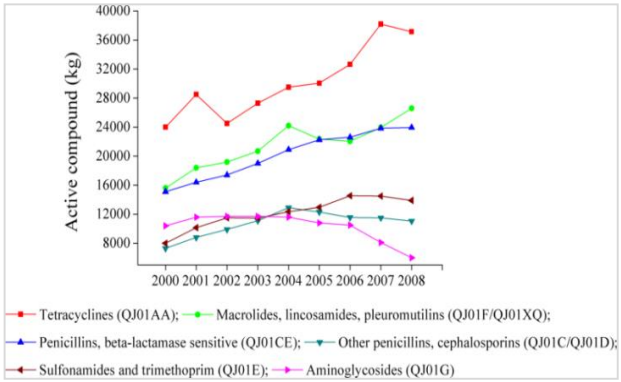


Hu*et al*. *Gut Microbes* 2014

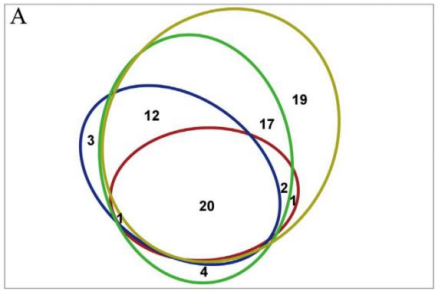
人体肠道耐药基因的多样性

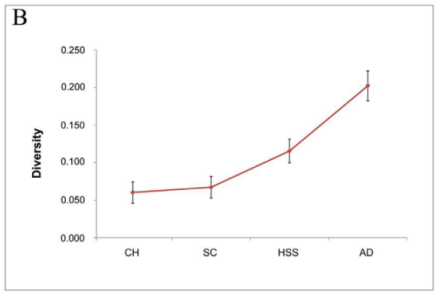
- 人体肠道细菌耐药基因与兽用抗生素使用相关

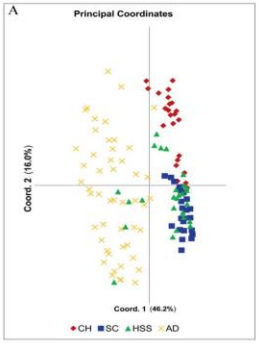


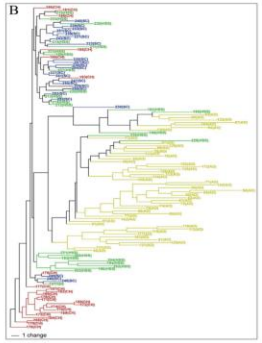


- 人体肠道细菌耐药基因随个体年龄增长变得复杂和多样









Lu N & Hu Y *et al*. ***Scientific Reports* 2014;** Hu Y, *et al*. ***Gut Microbes* 2014**

可移动的耐药基因组(Mobile Resistome)

- Forces governing the transfer of antibiotic resistance genes (ARGs)

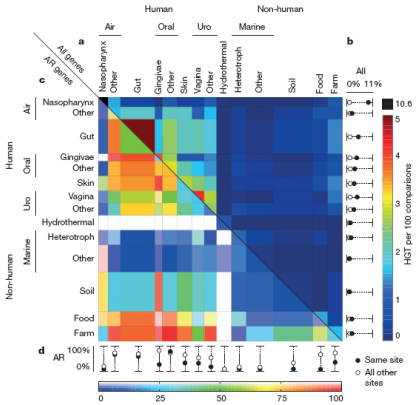
LETTER

doi:10.1038/nature10571

Ecology drives a global network of gene exchange connecting the human microbiome

Chris S. Smillie[1]*, Mark B. Smith[2]*, Jonathan Friedman[1], Otto X. Cordero[3], Lawrence A. David[4] & Eric J. Alm[3,5,6]

- Recently transferred gene (>99% nucleotide identity).
- 2,235 bacterial genomes



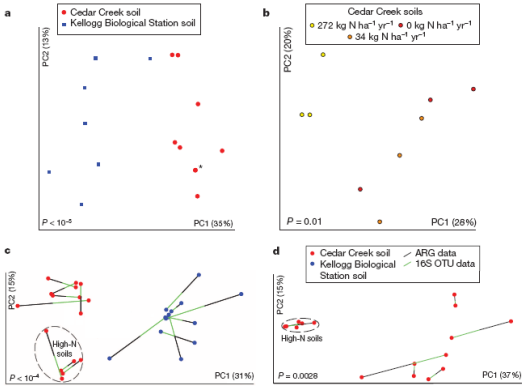
LETTER

doi:10.1038/nature13377

Bacterial phylogeny structures soil resistomes across habitats

Kevin J. Forsberg[1]*, Sanket Patel[1,2]*, Molly K. Gibson[1], Christian L. Lauber[3], Rob Knight[4,5], Noah Fierer[3,6] & Gautam Dantas[1,2,7]

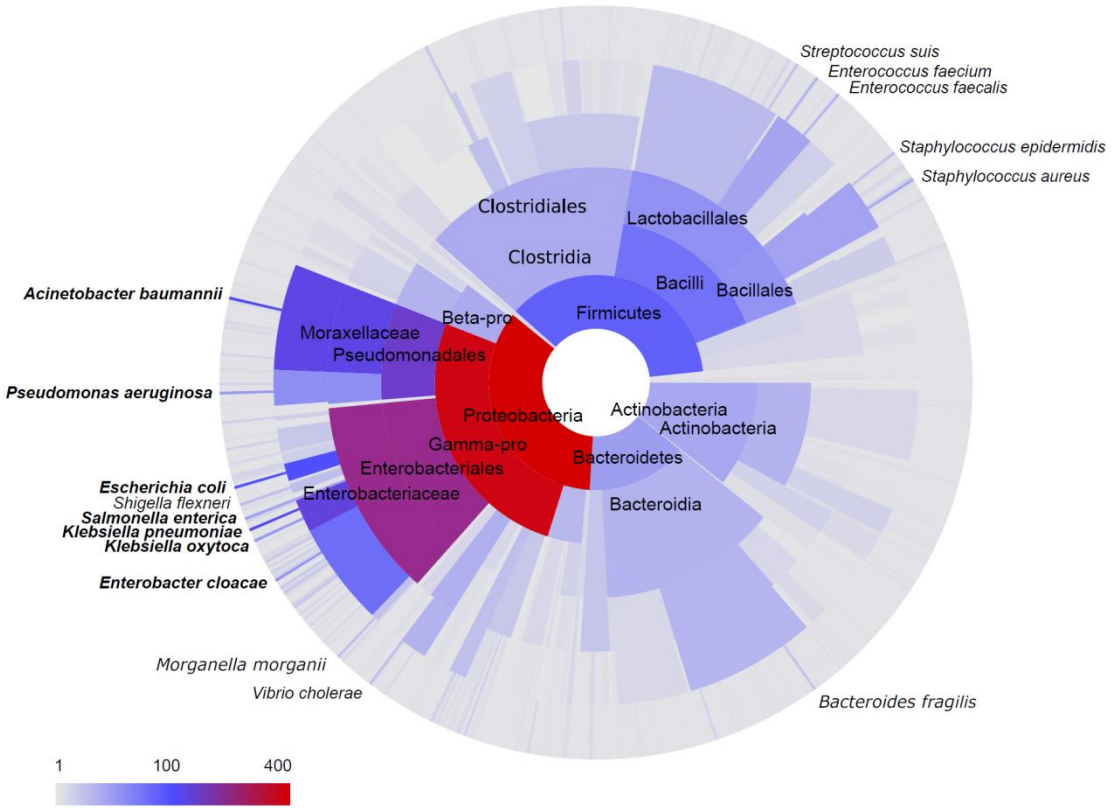
-18 agricultural and grassland soils
- 2,895 ARGs
- ≥99% nucleotide identity



Contents

- 23,425 genomes (2,768 complete + 20,657 draft)
- Chicken/pig/cattle
- human gut microbiome: 9,879,896 bacterial genes
- Mobile antibiotic resistance genes (ARGs)

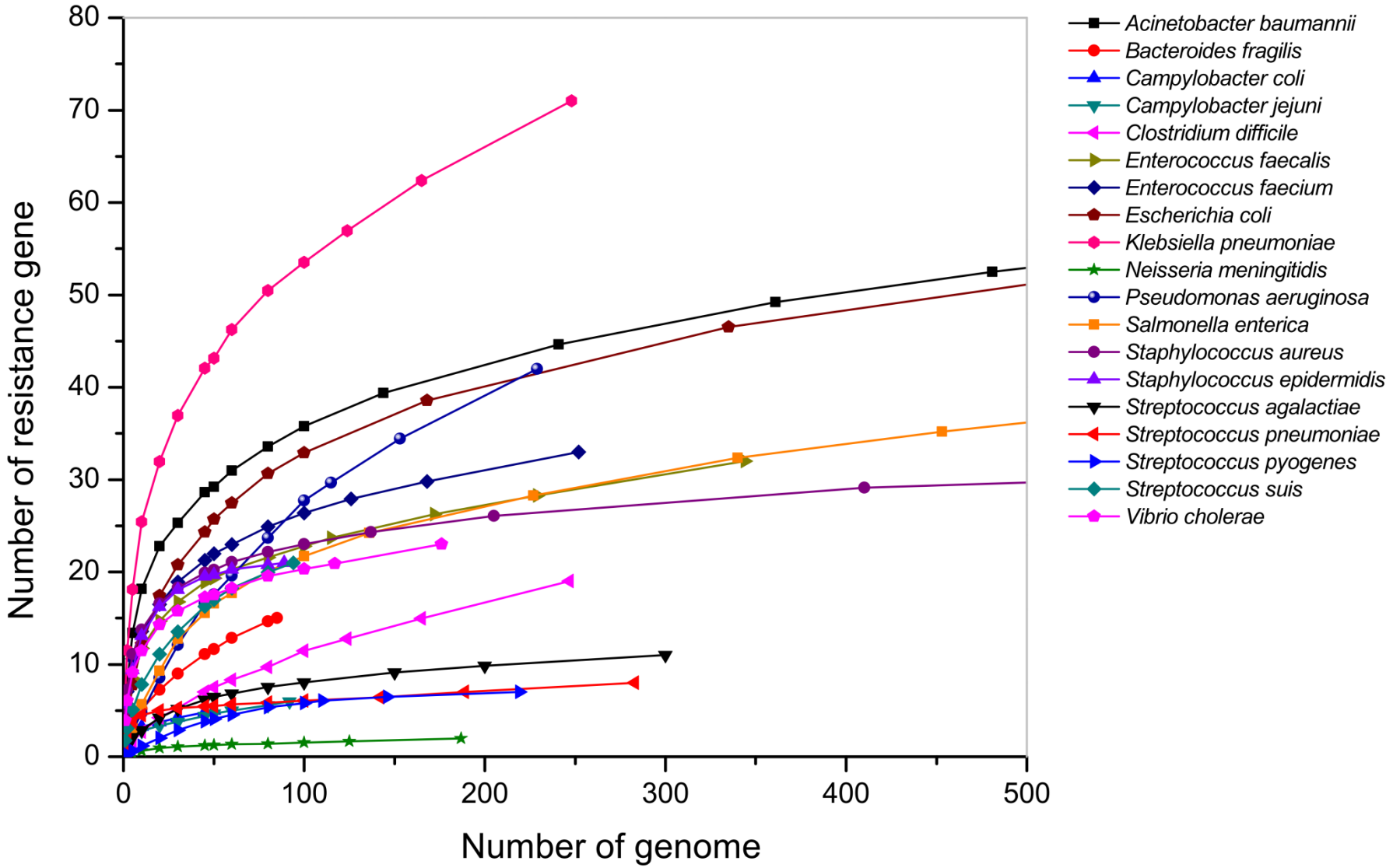
Mobile ARGs are enriched in Proteobacteria
(耐药基因在那些细菌中丰度高)



Proteobacteria	399
Firmicutes	86
Bacteroidetes	46
Actinobacteria	40

Klebsiella pneumoniae	**119**
Acinetobacter baumannii	**105**
Escherichia coli	**96**
Enterobacter cloacae	**51**
Klebsiella oxytoca	**50**
Pseudomonas aeruginosa	**49**
Salmonella enterica	**44**
Staphylococcus aureus	39
Enterococcus faecium	33
Enterococcus faecalis	31
Shigella flexneri	24
Vibrio cholerae	24
Morganella morganii	22
Staphylococcus epidermidis	22
Bacteroides fragilis	21
Streptococcus suis	21

$P=2.9\times10^{-4}$; Mann-Whitney *U*-test


Number of resistance gene
Number of genome
0
10
20
30
40
50
60
70
80
0
100
200
300
400
500
Acinetobacter baumannii
Bacteroides fragilis
Campylobacter coli
Campylobacter jejuni
Clostridium difficile
Enterococcus faecalis
Enterococcus faecium
Escherichia coli
Klebsiella pneumoniae
Neisseria meningitidis
Pseudomonas aeruginosa
Salmonella enterica
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus suis
Vibrio cholerae

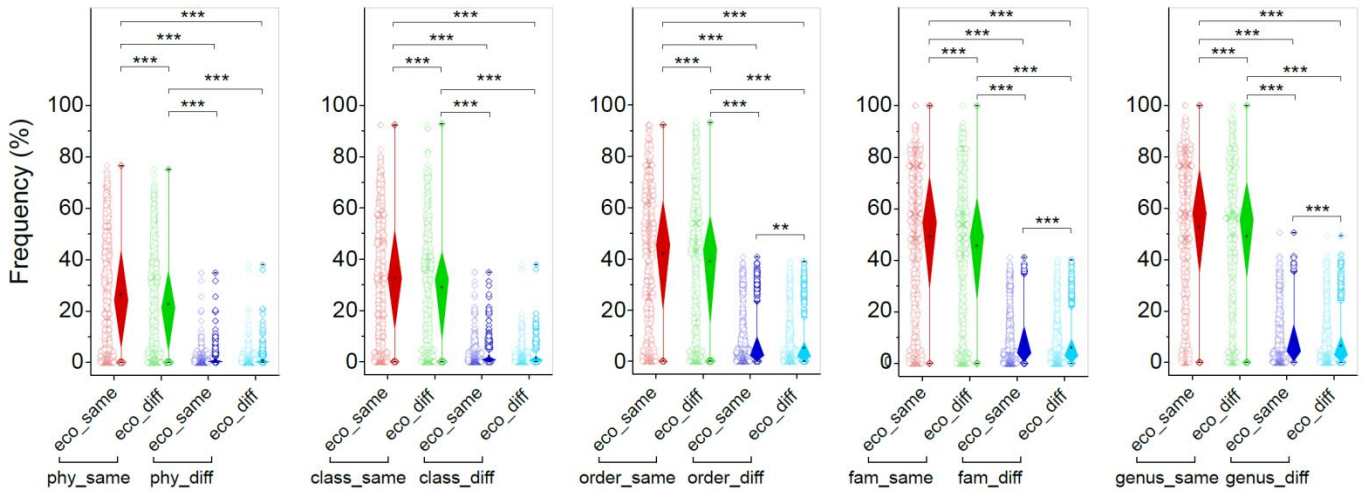
The AR genes shared by different phyla

39 AR genes are present in at least 2 phyla

Proteobacteria	369/399	92.5%
Firmicutes	57/86	66.3%
Bacteroidetes	32/46	69.6%
Actinobacteria	15/40	37.5%

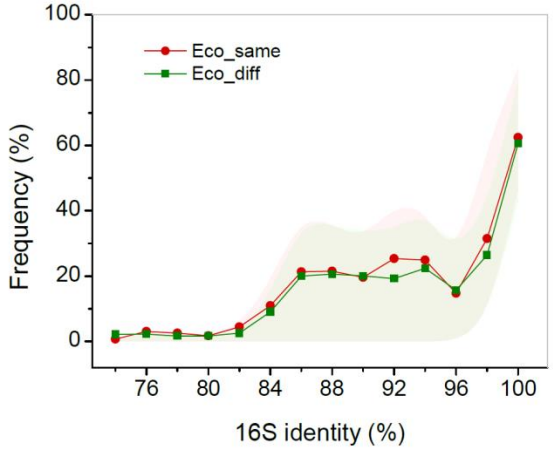
ARG transfer networks are shaped by bacterial phylogeny

A

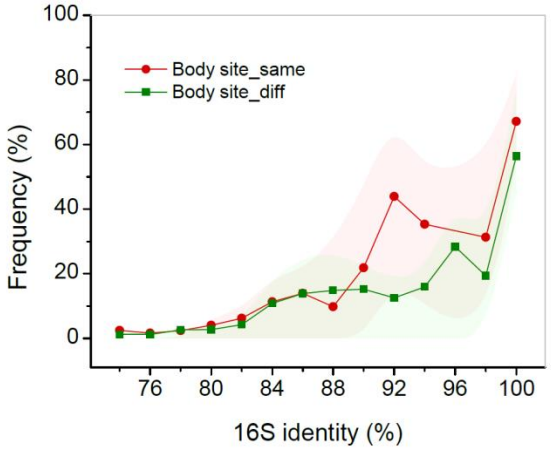


- human
- animal
- aquatic
- terrestrial

B



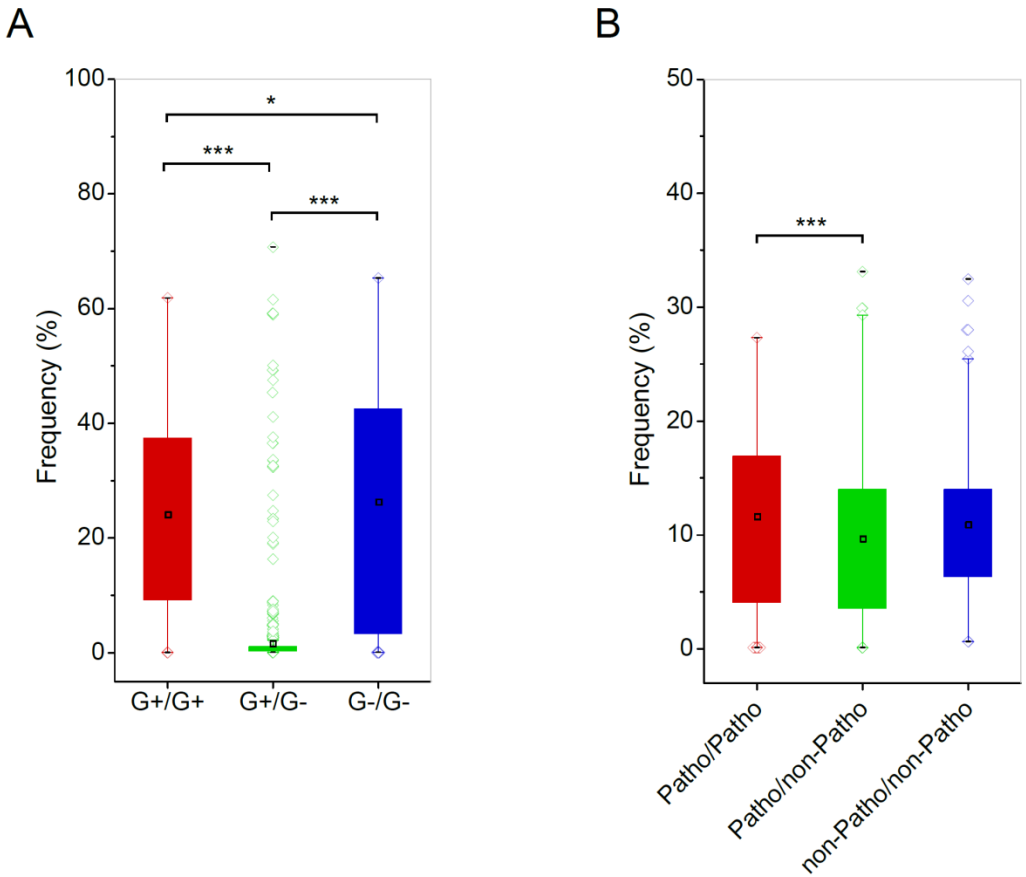
C



- Airways
- Blood
- gastrointestinal tract
- Oral
- Skin
- urogenital tract

There is almost no HGT between Gram(-) and (+) bacteria

G+ vs G-: *P*=0.04



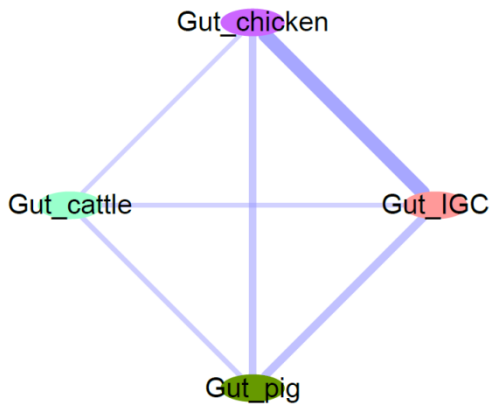
PathovsPatho/no-patho:
P=7.9×10^{-7}; Mann-Whitney *U*-test

Patho/no-pathovs non-Patho :
P=0.058; Mann-Whitney *U*-test

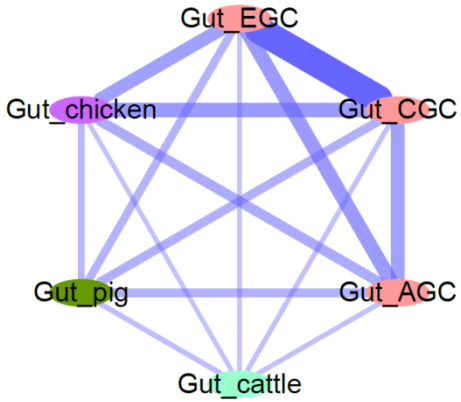
Pathovs non-Patho :
P=0.199; Mann-Whitney *U*-test

Shared mobile ARGs between human and animal gut microbiomes

A



B



IGC & chicken	36
IGC & pig	20
Pig & chicken	16
Pig & cattle	12
chicken & cattle	10
Cattle & IGC	10

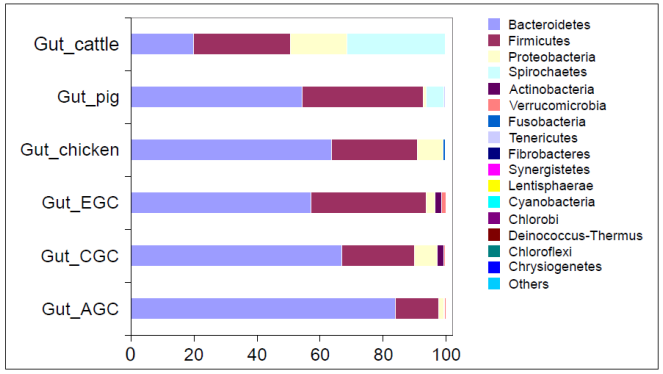
EGC & CGC	69
EGC & AGC	35
CGC & chicken	35
EGC & chicken	32
AGC & CGC	31
AGC & chicken	24
CGC & pig	21
AGC & pig	20
EGC & pig	19
Chicken & pig	16
Cattle & pig	12
CGC & cattle	11
AGC & cattle	11
Cattle & chicken	10
EGC & cattle	9

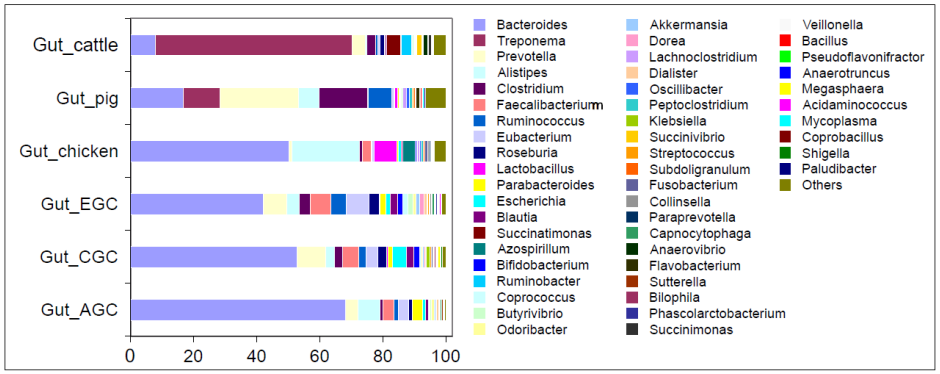
- chicken
- pig
- cattle

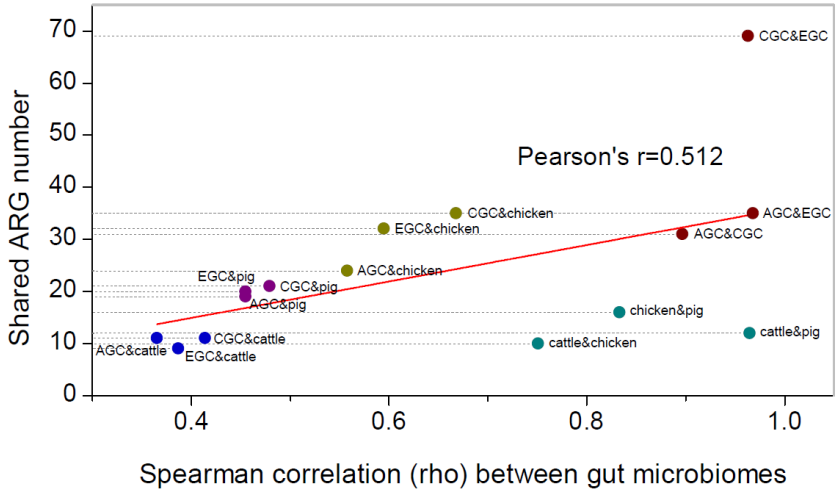
5G/sample×5

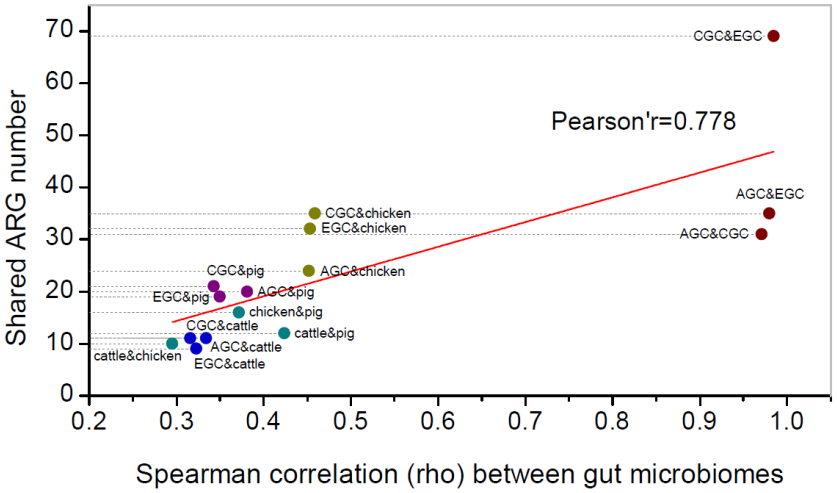
- human gut microbiome: integrated gene catalog (IGC) comprising 9,879,896 genes

The transfer of mobile ARG is correlated with bacterial community structures









Conclusions

- The transfer of mobile ARGs in bacteria has preference; Proteobacteria harbors the most number and the highest transfer frequency of Mobile ARGs.
- The recent horizontal gene transfer network of ARGs is shaped by bacterial phylogeny, while ecology barrier also exists.
- Mobile ARGs are enriched in human and animal associated bacteria, and are frequently exchanged between human and animal.
- Compared with other food animal, the chicken gut microbiomes share more mobile ARGs with human, probably due to the relative similar composition in bacterial community.
- Human and animal gut mobile ARGs are also harbored by human pathogens.

Background

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*

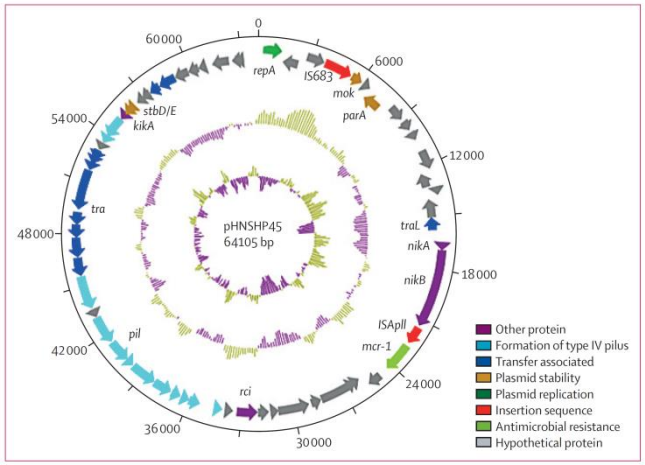


Figure 2: Structure of plasmid pHNSHP45 carrying *mcr-1* from *Escherichia coli* strain SHP45

	Year	Positive isolates (%)/number of isolates
Escherichia coli		
Pigs at slaughter	All	166 (20·6%)/804
Pigs at slaughter	2012	31 (14·4%)/216
Pigs at slaughter	2013	68 (25·4%)/268
Pigs at slaughter	2014	67 (20·9%)/320
Retail meat	All	78 (14·9%)/523
Chicken	2011	10 (4·9%)/206
Pork	2011	3 (6·3%)/48
Chicken	2013	4 (25·0%)/16
Pork	2013	11 (22·9%)/48
Chicken	2014	21 (28·0%)/75
Pork	2014	29 (22·3%)/130
Inpatient	2014	13 (1·4%)/902
Klebsiella pneumoniae		
Inpatient	2014	3 (0·7%)/420

Table 2: Prevalence of colistin resistance gene *mcr-1* by origin

Significance:

The last-resort antibiotic;

The first plasmid-mediated colistin resistance

THE LANCET Infectious Diseases

Idea

JGI IMG INTEGRATED MICROBIAL GENOMES

IMG Content

	Datasets
Bacteria	31444
Archaea	610
Eukarya	220
Plasmids	1194
Viruses	3905
Genome Fragments	1192
Metagenome	4534
Total Datasets	43099

Last Datasets Added On:

Genome	2015-10-30
Metagenome	2015-11-23

All available genome and metagenome data to date…

NIH HUMAN MICROBIOME PROJECT

NCBI

RESOURCE

nature biotechnology

An integrated catalog of reference genes in the human gut microbiome

Junhua Li[1–3,19], Huijue Jia[1,19], Xianghang Cai[1,19], Huanzi Zhong[1,19], Qiang Feng[1,4,19], Shinichi Sunagawa[5], Manimozhiyan Arumugam[1,5,6], Jens Roat Kultima[5], Edi Prifti[7], Trine Nielsen[6], Agnieszka Sierakowska Juncker[8], Chaysavanh Manichanh[9], Bing Chen[1], Wenwei Zhang[1], Florence Levenez[7], Juan Wang[1], Xun Xu[1], Liang Xiao[1], Suisha Liang[1], Dongya Zhang[1], Zhaoxi Zhang[1], Weineng Chen[1], Hailong Zhao[1], Jumana Yousuf Al-Aama[10,11], Sherif Edris[11,12], Huanming Yang[1,11,13], Jian Wang[1,13], Torben Hansen[6], Henrik Bjørn Nielsen[8], Søren Brunak[8], Karsten Kristiansen[4], Francisco Guarner[9], Oluf Pedersen[6], Joel Doré[7,14], S Dusko Ehrlich[7,15], MetaHIT Consortium[16], Peer Bork[5,17] & Jun Wang[1,4,6,11,18]

1267 fecal samples; 9,879,896 genes

(GIGA)^n DB

Revolutionizing data dissemination, organization, and use

Data released on June 30, 2014

Supporting data for the paper: "An integrated catalog of reference genes in th human gut microbiome".

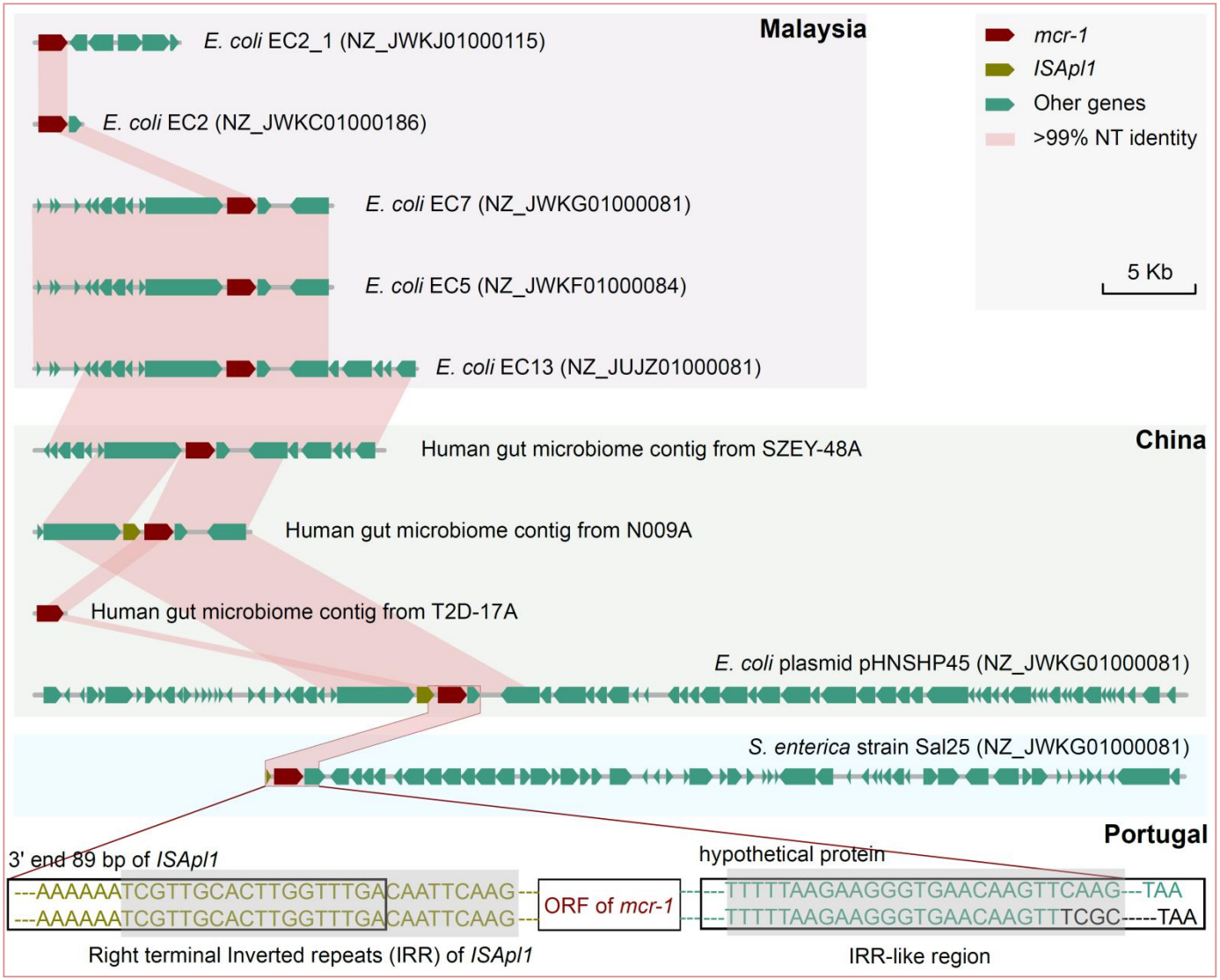
Li, J; Jia, H; Cai, X; Zhong, H; Feng, Q; Sunagawa, S; Arumugam, M; Kultima, J, R; Prifti, E; Nielsen, Juncker, A, S; Manichanh, C; Chen, B; Zhang, W; Levenez, F; Wang, J; Xu, X; Xiao, L; Liang, S; Zhan Zhang, Z; Chen, W; Zhao, H; Al-Aama, J, Y; Edris, S; Yang, H; Wang, J; Hansen, T; Nielsen, H, B; Bru S; Kristiansen, K; Guarner, F; Pedersen, O; Doré, J; Ehrlich, S; MetaHIT consortium, ; Bork, P; Wang (2014): Supporting data for the paper: "An integrated catalog of reference genes in the human gut microbiome". GigaScience Database. http://dx.doi.org/10.5524/100064 RIS BIBTEX TEXT

Result Summary

Sample/ Strain	Fragment Size (bp)	Nucleotide Identity (%)	Country	Sampling time	Sample Source
Plasmid pHNSHP45	*64,015*	*100*	*China*	*July-2013*	*Pig*
N009A	**11,314**	**100**	**China**	**Before 2011**	**Stool**
SZEY-48A	**18,522**	**100**	**China**	**Before 2011**	**Stool**
T2D-17A	**1,149**	**100**	**China**	**Before 2011**	**Stool**
***S. enterica* strain Sal25**	**50,891**	**100**	**Portugal**	**16-Jun-2011**	**Food**
E. coli EC2	2,648	100	Malaysia	15-Sep-2013	Pig Fecal
E. coli EC2_1	8,052	100	Malaysia	16-Sep-2013	Chicken Heart and Liver
E. coli EC5	16,531	100	Malaysia	15-Sep-2013	Chicken Liver
E. coli EC7	16,534	100	Malaysia	15-Sep-2013	Chicken Spleen
E. coli EC13	21,296	100	Malaysia	15-Sep-2013	Water

Query: *mcr-1* gene, 1,626 bp.

Comparison of genomic sequences harboring *mcr-1*



Conclusions

- The *mcr-1* gene may be longtime in China and had already spread to the Chinese healthy human gut several years ago.

- The *mcr-1* gene showed up before or around 2011 in Europe as evidenced by the presence of *mcr-1* in the genome of a Portugal *Salmonella enterica* isolate from food sample.

- Not only the plasmid itself or bacterial clonal expansion, but also the *ISApl1-clrA* (*mcr-1*) mobile element alone can be transferred to or captured by other mobile genetic elements.

- The wide distribution of *mcr-1* gene in meat and food samples, and its presence in human microbiome strongly suggest a potential food chain dissemination pathway.

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