中國科學院 CHINESE ACADEMY OF SCIENCES

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Animal microbiome: A reservoir of antimicrobial resistance in intensive animal farming

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June 20th, 2019, Thailand

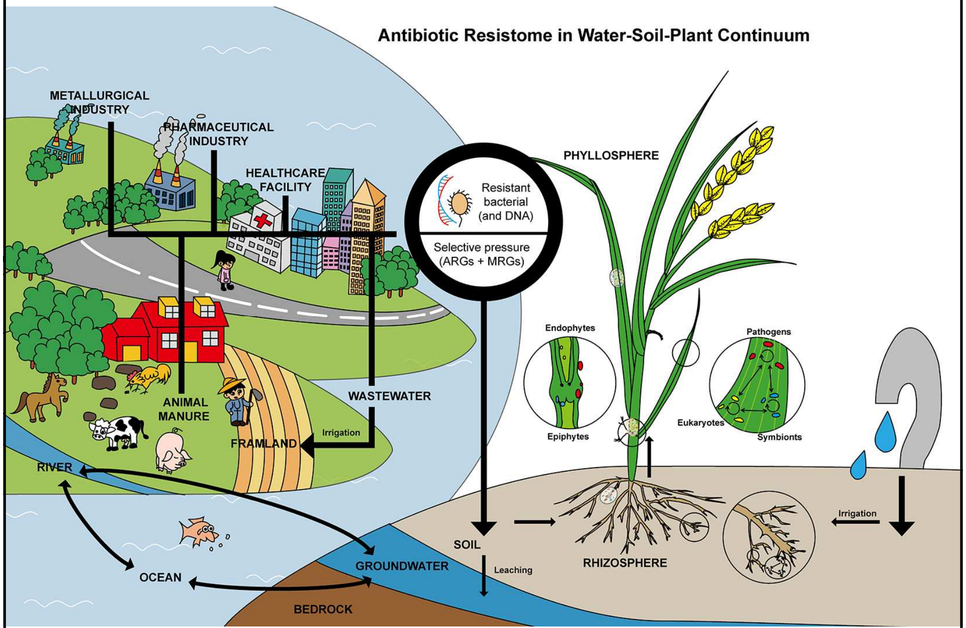
Intensive and overuse of antibiotic to increase meat production

Industrial livestock farms rely on antibiotics as a growth additive for production

(Akoury, 2015)

- Therapeutic use for animal: disease treatment and disease prevention.
- Non-therapeutic use: supplemented in feed at low concentration to boost the animal growth (as growth promoter)

One health concept of antimicrobial resistance



The start of wide use of antibiotics in animal industry since 1940s

NATURE January 2, 1954 Vol. 173

and infra-red spectra may be of importance in addition to X-ray data.

Dr. K. W. Andrews said … convenient at times to … known patterns than to … for identification. In Mr. … the use of lithium fluoride … valuable in identification … frequent use of X-ray … J. D. Bernal suggested th… could be simplified by breaking up the complete A.S.T.M. index into groups of related substances; the weakest lines, the longest spacings, or 'lonely lines' of a pattern are often more characteristic of a substance than the strongest line. Prof. E. G. Cox announced that he is investigating the best method of indexing single-crystal data for i…

A valuable feature of the … unusually extensive exhibition … stimulating evening discourse … R. W. G. Wyckoff, whose subj… X-ray Diffraction in the Unite… conference of the Group will b… during April 1–2, 1954, and … modern structure analysis.

Taken as a whole, the results of tests carried out under the ægis of the Agricultural Research Council showed the usefulness of antibiotics in British pig-farming practice. They made it clear, however, that

Be that as it may, the trials showed that antibiotics have a place in British pig farming, and the findings of the report no doubt influenced the recent official decision about their use.

ANTIBIOTICS IN PIG FOOD

UNTIL recently the use of antibiotics in animal feeds was prohibited in Great Britain; but the Penicillin Act, 1947, has now been amended by the

Conclusion of this study

After about 20 years, concerns over antibiotics use in animal industry emerged

Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man

THE natural ecology of *Escherichia coli* and its infectious plasmids is not understood, although there is suggestive evidence that animals may serve as reservoirs for *E. coli* found in humans[1,2]. Investigation in this area becomes additionally important in view of the practice of introducing plasmids with pieces of foreign DNA into *E. coli*[3,4]. During an examination of the effects of antibiotic-supplemented animal feed on flora of farm animals and human personnel (ref. 5 and S.B.L., G.B.F. and A.B.M., unpublished), a study was initiated to determine if *R* plasmids and their *E. coli* hosts were naturally transferred among chickens and from chickens to human handlers. Our results illustrate the spread of antibiotic-resistant organisms from chicken to chicken and from chicken to man

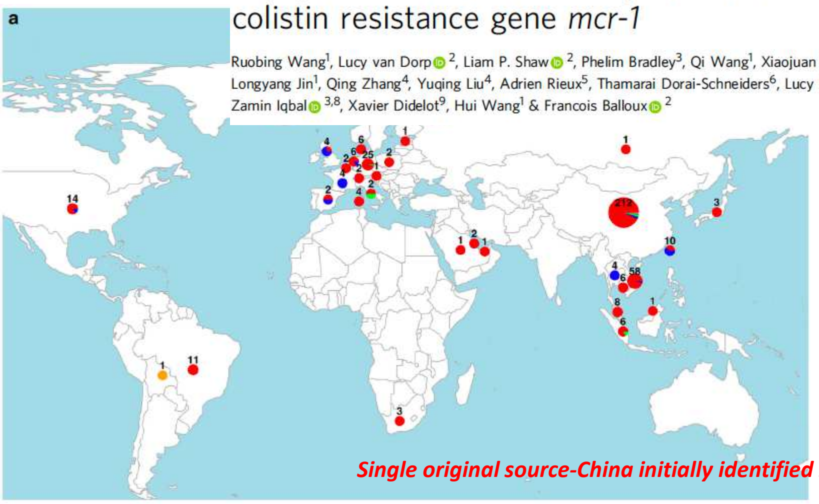
Nature Vol. 260 March 4 1976

ARTICLE

DOI: 10.1038/s41467-018-03205-z OPEN

The global distribution and spread of the mobilized colistin resistance gene *mcr-1*

Ruobing Wang[1], Lucy van Dorp[2], Liam P. Shaw[2], Phelim Bradley[3], Qi Wang[1], Xiaojuan Wang[1], Longyang Jin[1], Qing Zhang[4], Yuqing Liu[4], Adrien Rieux[5], Thamarai Dorai-Schneiders[6], Lucy Anne Weinert[7], Zamin Iqbal[3,8], Xavier Didelot[9], Hui Wang[1] & Francois Balloux[2]



Global map of mcr-1-positive isolates included colored by genus with the number and size of pies providing the sample size per location

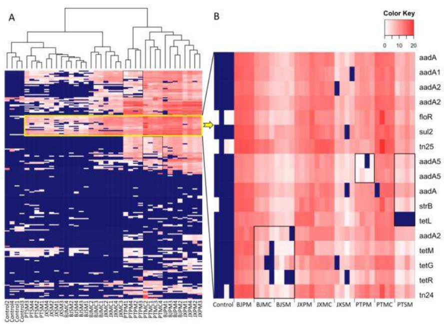
Wang et al., 2018 Nature Communications

Intensive animal farming: major point sources of antibiotics and antibiotic resistance genes

We used:
Metagenomics and high throughput qPCR **to characterize the resistome**

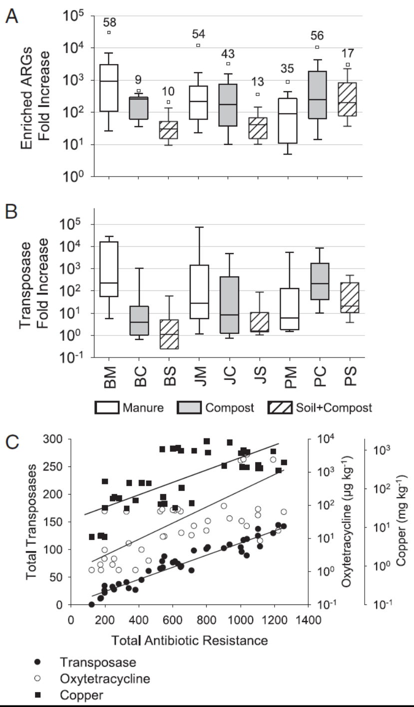
（5184 pores in one Chip）

Intensive livestock in peri-urban and antibiotic resistance



Diverse and abundant antibiotic resistance genes in farm environment, posing health risk to humans-urban and peri-urban

Zhu YG et al., PNAS, 2013



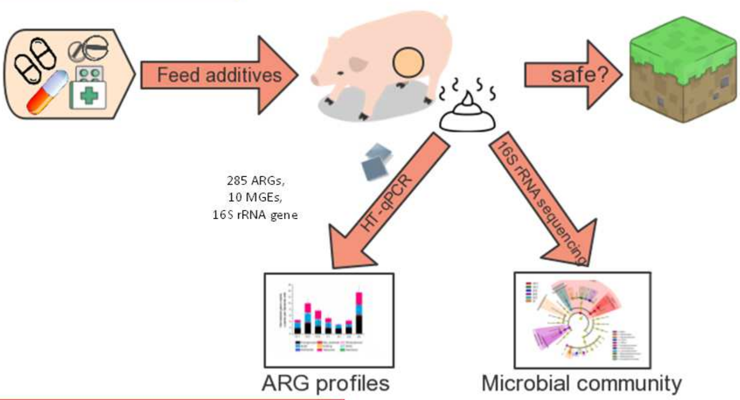
- A total of 63 unique ARGs were significantly enriched in at least one sample compared to controls
- Overall median enrichment of 192-fold for all samples
- Nearly 10,000-fold when considering only the manure.
- The maximum enrichment of a single ARG was over 28,000-fold
- Transposase all highly enriched, and correlated well with ARGs

Zhu YG et al., PNAS, 2013

Effect of feed additives on ARGs in animal gut?

Antibiotic and metal in feed: growth promoter

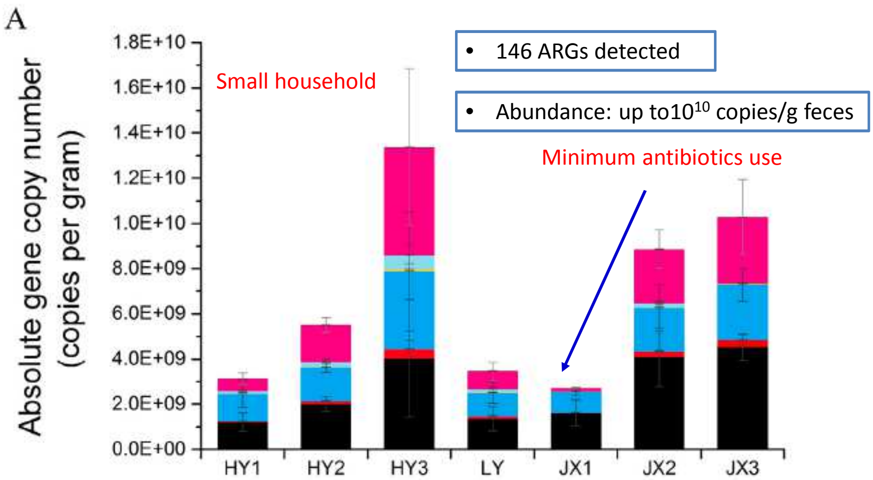
ARGs in pig gut



- 3 large-scale Chinese pig farms
- 7 feed samples
- 28 fresh fecal samples

Zhao Y et al. Sci. Total Environ., 2018

Highly abundant ARGs detected in Chinese pig gut



Sampling pigs farms with different scales and management regimes

Zhao Y et al. Sci. Total Environ., 2018

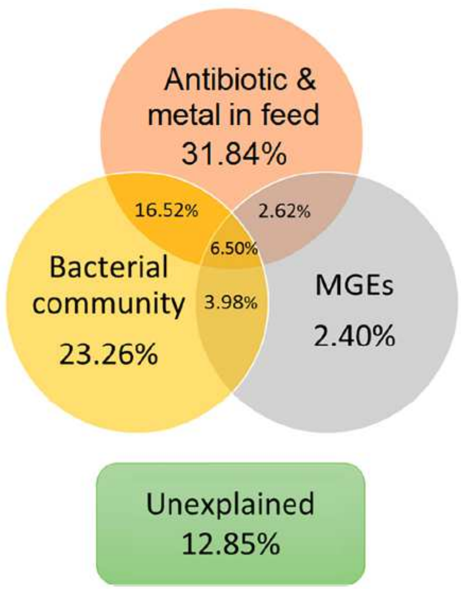


Fig. 5. Partial redundancy analysis differentiating the effect of antibiotic and metal concentrations in swine feed, swine gut microbial community composition and mobile gene elements on the overall ARG profiles.

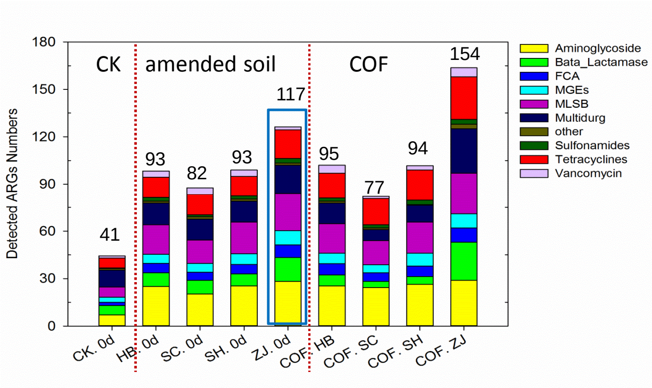
Driving factors of ARGs in swine gut

Pearson's r (*P* < 0.05)	ARGs
In-feed antibiotic	0.868
In-feed metal	0.871
Microbial abundance	0.65

Zhao Y et al. Sci. Total Environ., 2018

ARGs from animals to environments

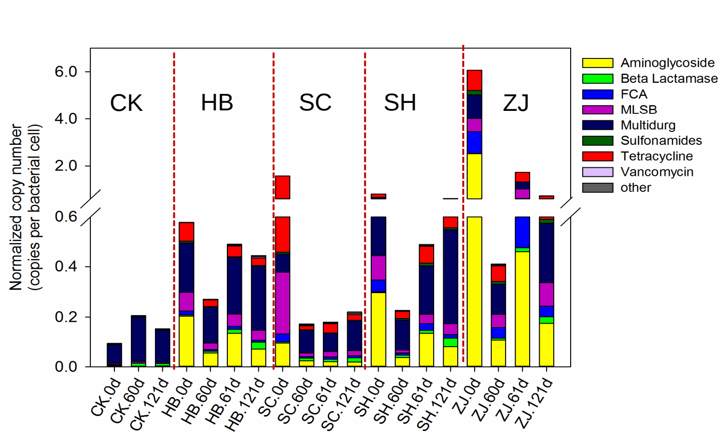
Commercial organic fertilizers
Diversity of ARGs in soils after COFs application



- Use of COFs increased ARGs numbers in soil.

Zhou X et al. Environ Sci. & Pollution Res., 2017

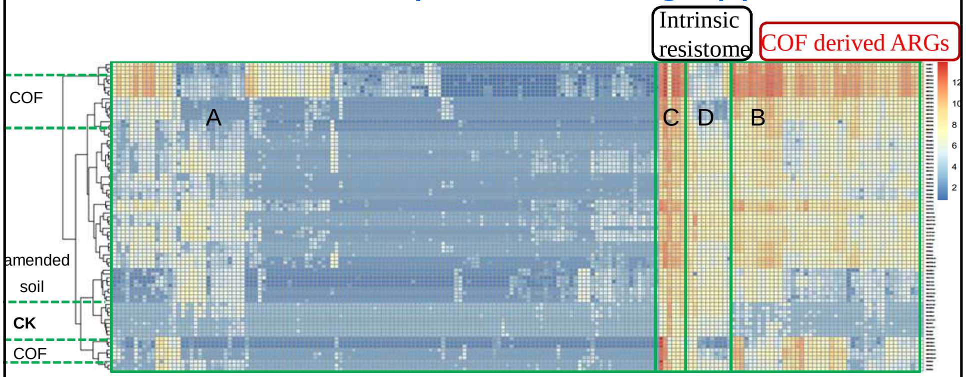
Abundance of ARGs in soils after application of COFs



- COF application universally increased the abundance of ARGs in soils. CK.0d: 0.1 copy cell^{-1}, ZJ.0d: 6.1 copy cell^{-1}.
- The normalized copy number of ARGs decreased during the first application, but the abundance of ARGs still remained at a high level at the end of experiment. ZJ, 5 times.

Zhou X et al. Environ Sci. & Pollution Res., 2017

ARGs distribution profiles during application



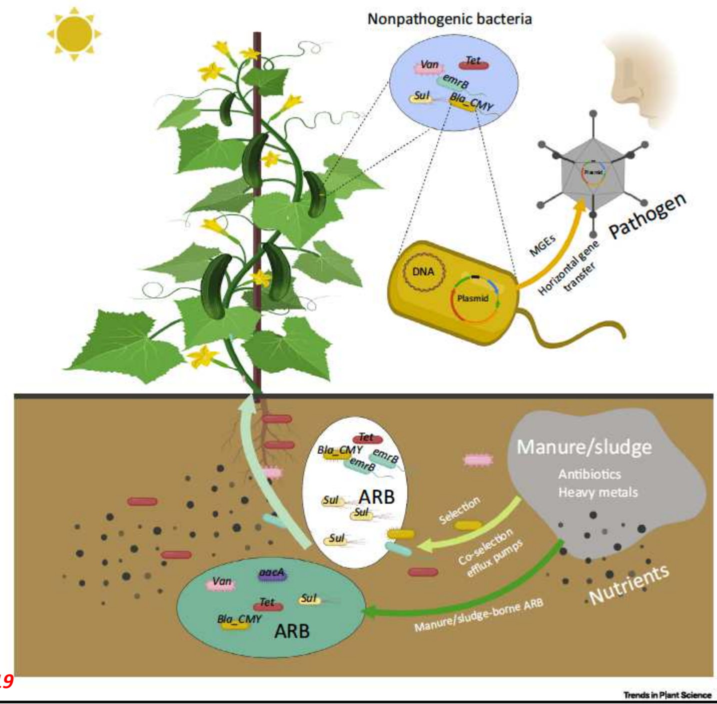
A: ARGs detected in all samples with low abundance.
B: ARGs detected in control soils with low abundance, enriched after applications of COFs.
C: ARGs detected in control soils with high abundance, enriched after applications of COFs
D: ARGs detected in control soils with high abundance, persisted after applications.

- Three ARGs (*tet*L, *mex*F, *qacE*delta1) and three MGEs (*tnp*A, *cIn*l1, *Int*l1) were shown as the most abundant ones.

Zhou X et al. Environ Sci. & Pollution Res., 2017

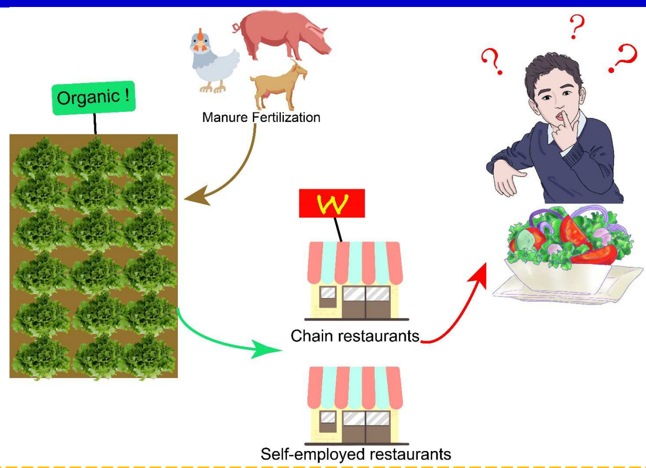
Transport of ARGs from soil to plant

- Direct human risks: plant food, particularly leafy vegetables for salad (both endophytes and phyllosphere microbial communities);
- Human contact with plant (leaves) and exposure to ARGs and resistant bacteria (*e.g.* in urban environment-*green space*)
- Major pathway for the spread of ARGs (*via air and trading etc.*)

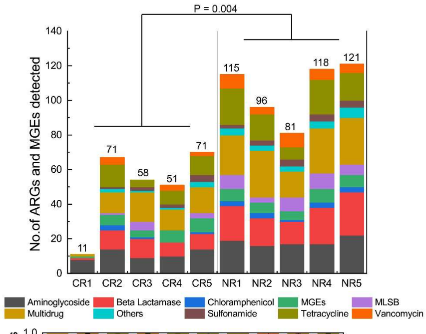


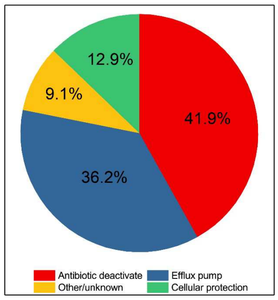
Chen at al., TiPS, 2019

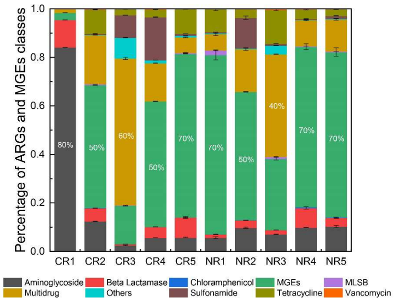
The Prevalence of Antibiotic Resistome in Ready-to-eat Produce.



City scale sampling: 10 sample sites,
3 boxes of serving mixed salad in each site, 3 replicates,
2 restaurant types (Chain and Self-employed Restaurants).
In total, 90 samples, (45 Chain and 45 Self-employed Restaurants)



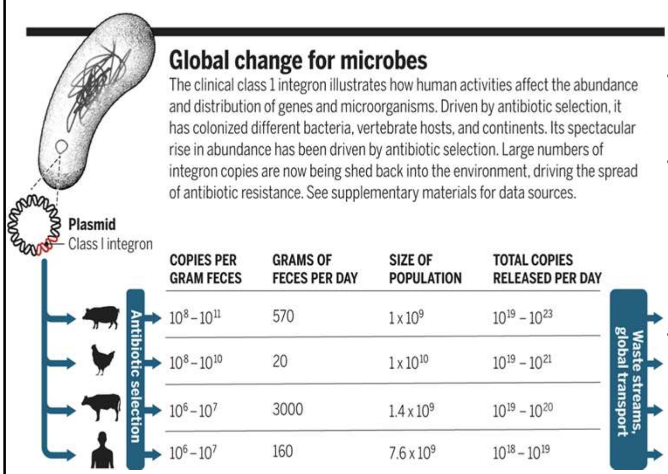




Sample sites	Integron genes	Transposase genes
CR1		*tnpA-01*
CR2	*intI-1LC, intI-1(clinic)*	*tnpA-01,tnpA-03, tnpA-04, tnpA-05*
CR3	*intI-1LC*	*tnpA-01, tnpA-02, tnpA-05*
CR4	*intI-1LC, intI-1(clinic), intI2*	*tnpA-01,tnpA-02, tnpA-03, tnpA-05*
CR5	*intI-1LC, intI-1(clinic), intI2*	*tnpA-01,tnpA-02, tnpA-03, tnpA-04, tnpA-05*
NR1	*intI-1LC, intI-1(clinic), intI2*	*tnpA-03, tnpA-04, tnpA-05, tnpA-07*
NR2	*intI-1LC, intI-1(clinic)*	*tnpA-01, tnpA-03, tnpA-05, tnpA-07*
NR3	*intI-1LC, intI-1(clinic)*	*tnpA-01, tnpA-03, tnpA-05*
NR4	*intI-1LC, intI-1(clinic), intI2*	*tnpA-01,tnpA-02, tnpA-03, tnpA-05, tnpA-07*
NR5	*intI-1LC, intI-1(clinic), intI2*	*tnpA-01,tnpA-02, tnpA-03, tnpA-05*

Prevalent existence of Resistome and MGEs in ready-to-eat produce were detected.

Bacteria shed by domestic animals contribute to the global spread of antibiotic resistance.



- Class 1 integron play the essential role of global spread of ARGs;
- Remarkable increase of class 1 integron in abundance and distribution largely attributed to antibiotic use for animals;
- Increased level of integron further aggravate the ARG dissemination via global transport.

Zhu YG et al. Science 2017
Zhu YG et al. Global Change Biology 2017

Elimination of manure-borne microorganisms maybe a potential mitigation option?

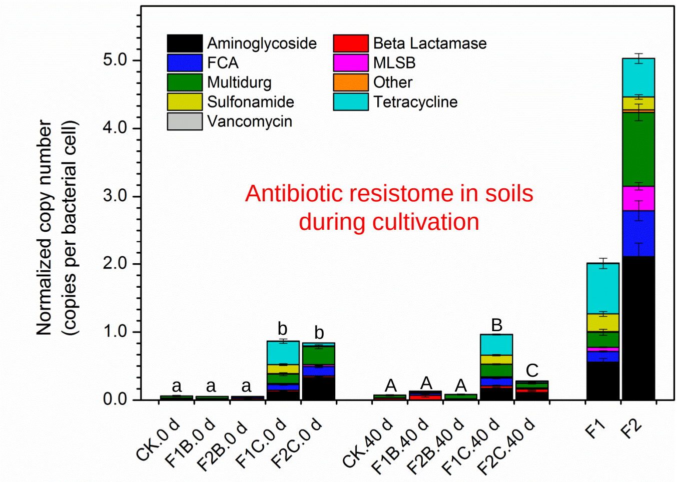
Prudent use of antibiotics in animal industry: a successful lesson from EU

Ban on antibiotics as growth promoters in animal feed enters into effect

An EU-wide ban on the use of antibiotics as growth promoters in animal feed enters into effect on January 1, 2006. The last 4 antibiotics which have been permitted as feed additives to help fatten livestock will no longer be allowed to be marketed or used from this date. The ban is the final step in the phasing out of antibiotics used for non-medicinal purposes. It is part of the Commission's overall strategy to tackle the emergence of bacteria and other microbes resistant to antibiotics, due to their overexploitation or misuse.

- Reduction of antimicrobial consumption in livestock systems: the most direct way to mitigate the animal-borne AMR (*Tang KL, Lancet 2017*);
- Learning from the success in EU, US banned antibiotics as growth promoter in 2017;
- China established "National Action Plan on Controlling ARB on anima origin (2016-2020)", aims to drop of antibiotic use as growth promoters with Exit Plan by 2020.

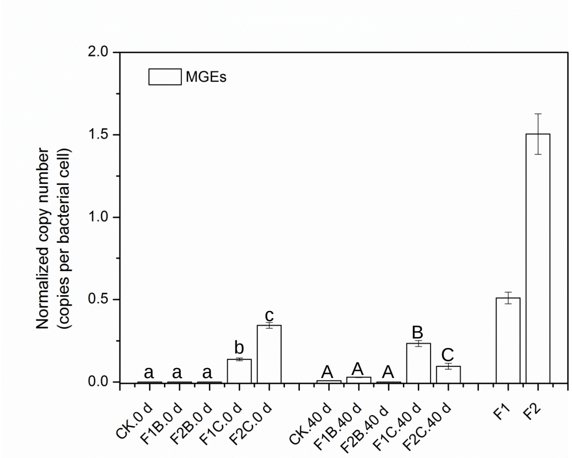
How to mitigate ARGs from animals? Turning manure to biochar



- The total ARGs abundance in the biochar-treated soils was significantly lower than those in the compost-amended soils, and similar to the control during cultivation.

Zhou X. et al., Sci. Total Environ, 2019

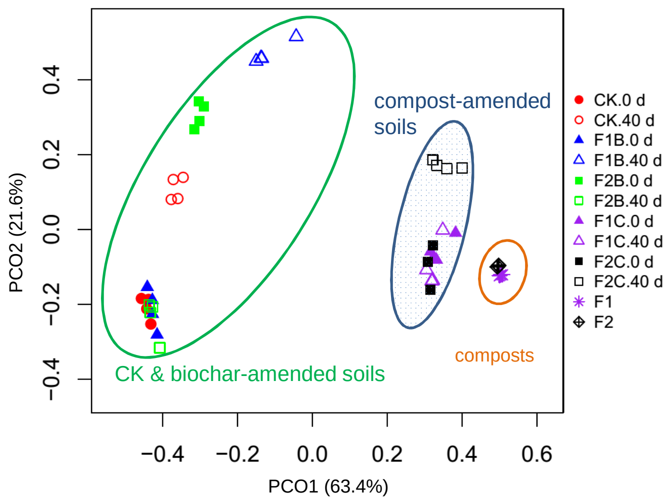
Abundance of mobile genetic elements (MGEs) in soils



- Compared to the compost, turning composted pig manure into biochar, significantly reduced the MGEs abundance.

Zhou X. et al., Sci. Total Environ, 2019

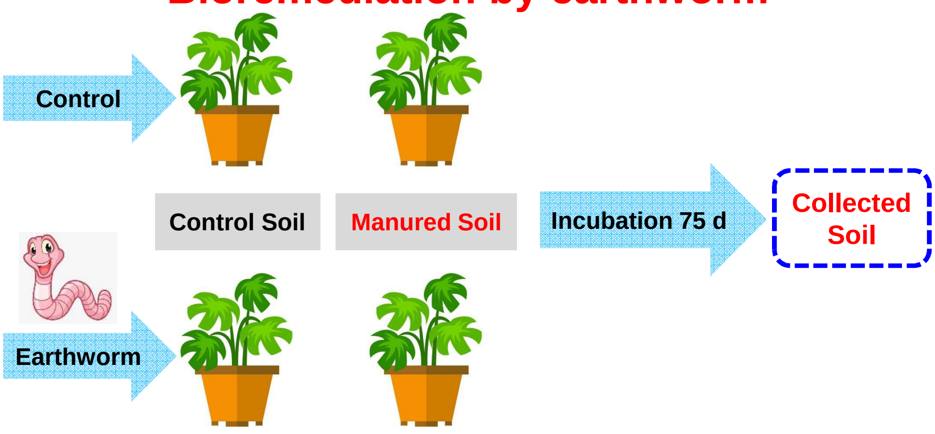
ARGs distribution profiles



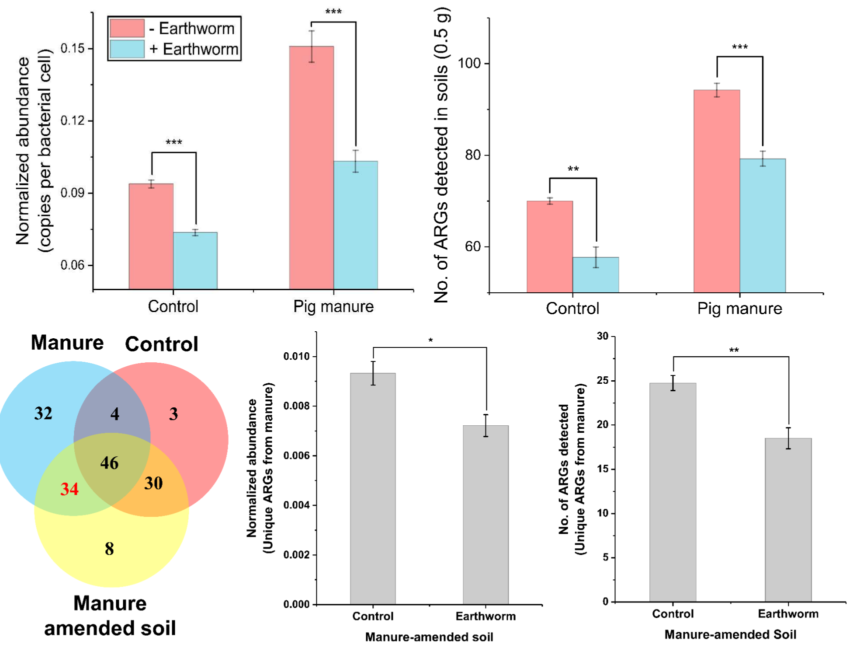
- PCoA analysis of the ARGs profiles showed that compost-amended soils clustered together, and were separated from the control and biochar-amended soils.

Zhou X. et al., Sci. Total Environ, 2019

How to mitigate ARGs in animal manure? Bioremediation by earthworm



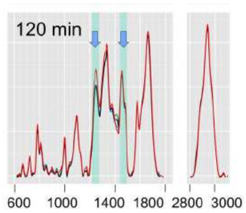
Lactuca sativa; Manure (0.5%); Earthworm 15/ Pot; 4 Replicates.

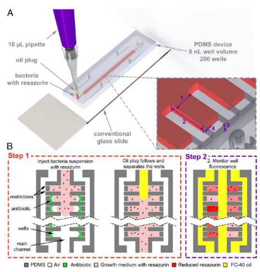


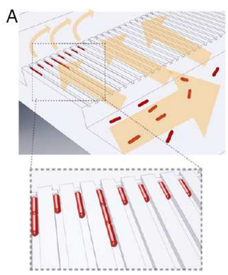
Earthworms reduced total ARGs and ARGs associated with manures in soils.

Recent progress:

Rapid Antibiotic Susceptibility Testing of Pathogenic Bacteria using D_2O-Labeled Single-cell Raman Spectroscopy





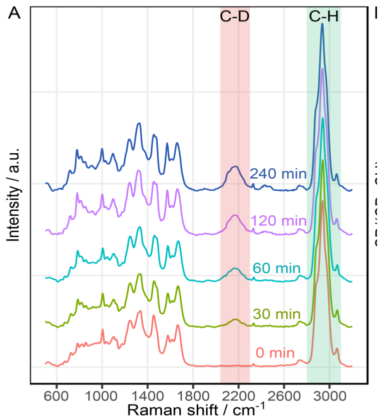


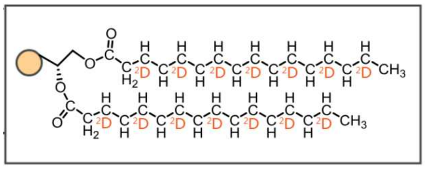
Yang K et al., Analytical Analysis, 2019

Rapid AST of pathogenic bacteria in urine using D_2O-labeled single-cell Raman spectroscopy

Aim: rapid, accurate, simple workflow, towards clinical applications, urinary tract infections

C-D: as early as 30 min

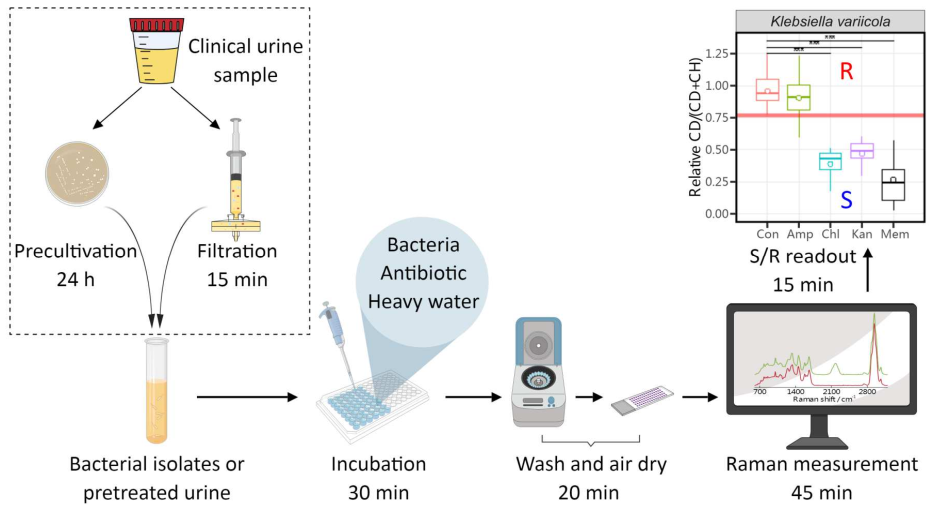




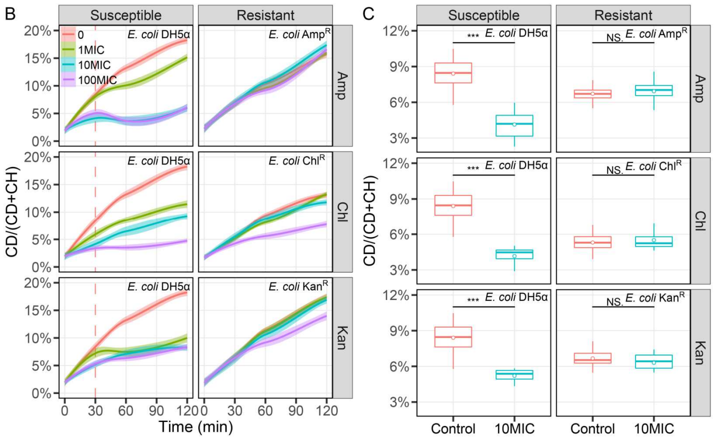
Metabolic activity-based rapid AST

- Assimilation of D_2O-derived D forms a new C-D Raman band.
- Governed by metabolic activity
- C-D indicate activity responses to antibiotics
- 30 min, rapid

Workflow



Time and dose-dependent discrimination of R and S based on C-D ratios



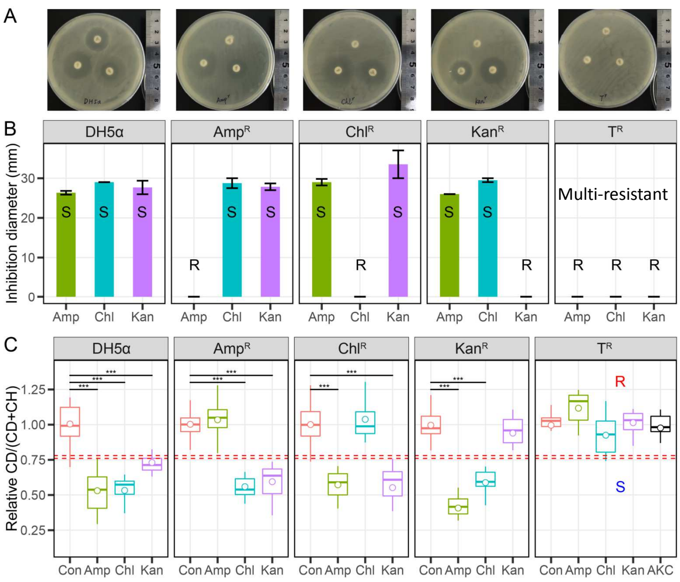
Ampicillin (Amp)
Chloramphenicol (Chl)
Kanamycin (Kan)
R: resistance
S: susceptibility

- 1xMIC, 30 min, no differentiation
- Growth-arrested cell can still be metabolic active. Asynchronous activity and growth responses
- 10x MIC, 30 min

Raman+D_2O: rapid antimicrobial resistance testing (AST)

5 *E.coli* strains x 3 antibiotics

- 10x MIC, 30 min



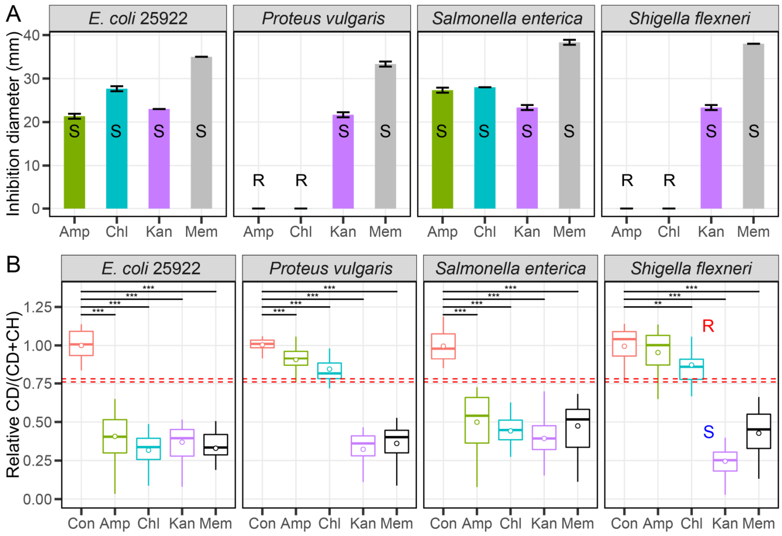
Gold standard
Disk diffusion
inhibition zone

24 h

Raman-D_2O assay

30 min

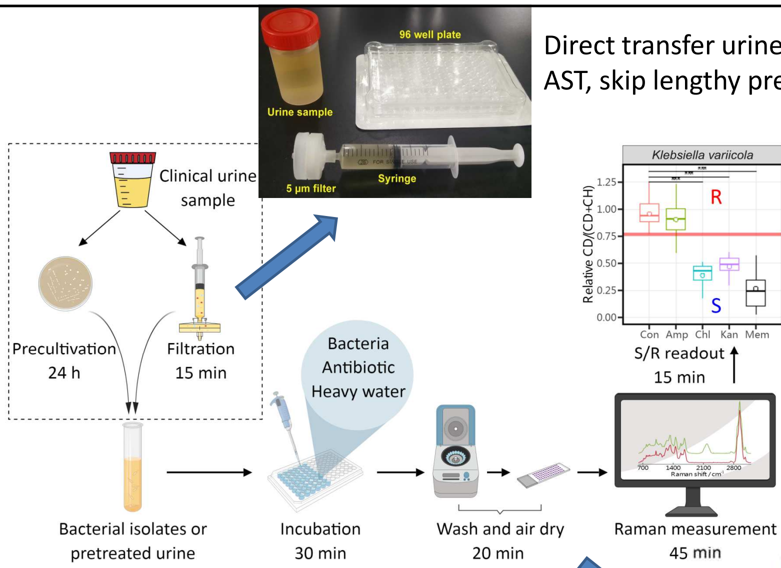
WHO ‘priority pathogen’ x 4 antibiotics

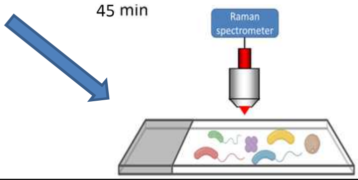


32 bacteria-antibiotic combinations

Criterion for Raman-D_2O based AST, i.e., S: ≤0.76, R: ≥ 0.78

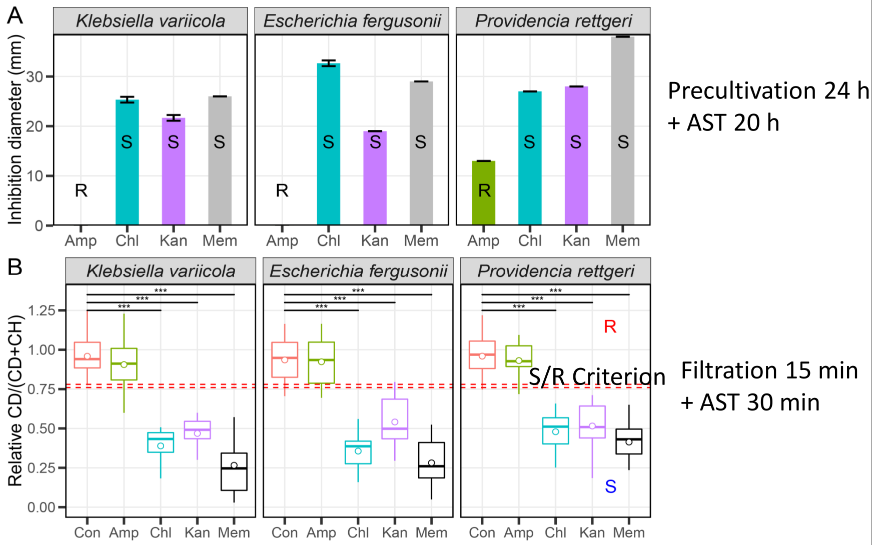
Direct transfer urine filtrate for AST, skip lengthy precultivation





Single-cell level detection, no limit from low number of bacteria in urine. 20 cell are enough to deliver S/R readout

Three anonymous clinical urine confirmed with positive urinary tract infections.



- 44 combinations, 100% agreement with disk diffusion assay
- The whole diagnosis time: <2.5 h

Concluding remarks

- Intensive animal farming is a significant source of AMR pollutant (resistome);
- Animal manure derived organic fertilizers can spread AMR to the soil, then to the general environment;
- Reducing general use of antibiotics in intensive farming system can mitigate the burden of AMR;
- Waste management represents the key to contain AMR from animal farms, and biochar production from manure appears to be a good option;
- Global surveillance (rapid and robust), national regulation and intervention following One Health concept are urgently needed.


Thanks！