

# Use of Big data – Metagenomic Analysis to Study Environmental Dissemination of ARGs

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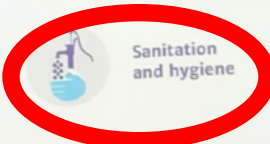
Southern University of Science and Technology, Shenzhen, China



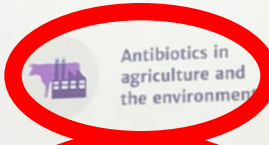
Regional Symposium on AMR  
Fighting AMR – Partnerships in Action  
抗菌素耐藥性區域研討會  
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Public awareness



Sanitation and hygiene



Antibiotics in agriculture and the environment



Vaccines and alternatives



Surveillance



Rapid diagnostics



Human capital



Drugs

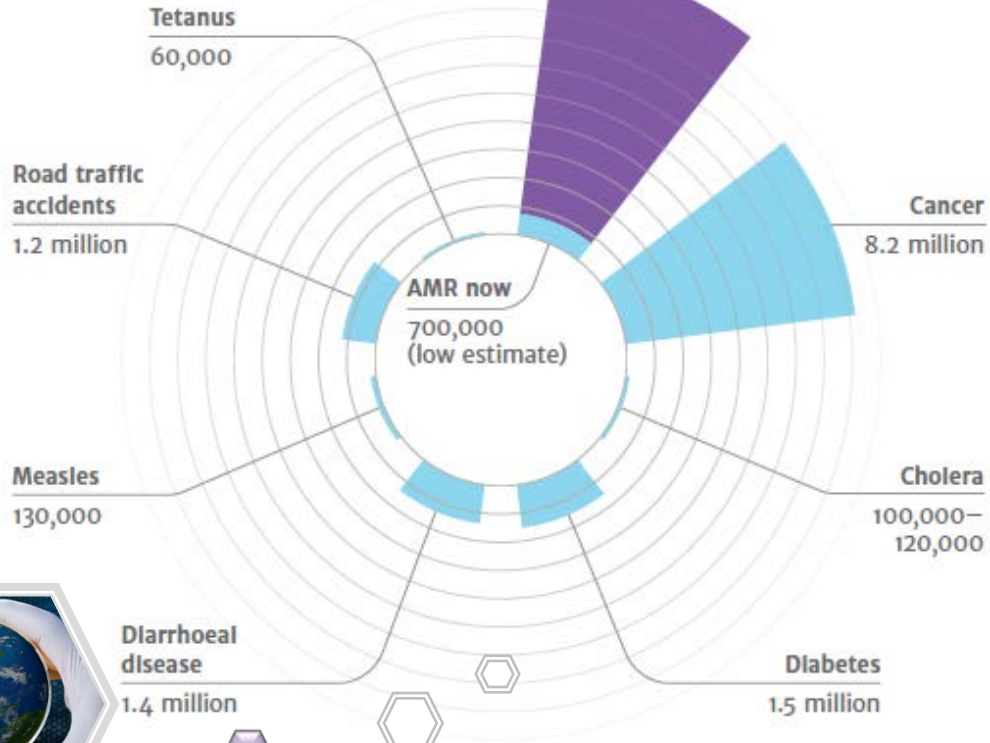


Global Innovation Fund



International coalition for action

AMR in 2050  
10 million



IF NOT TACKLED, RISING AMR COULD HAVE A DEVASTATING IMPACT



By 2050, the death toll could be a staggering **one person every three seconds** if AMR is not tackled now.

Source: Review's best analysis

Review on Antimicrobial Resistance

- *The Review on Antimicrobial Resistance (2016).*



# Antibiotics and Antibiotics Resistance Genes in Environment

## (Frontiers Report 2017, UNEP)

The environment is key to antibiotic resistance. Bacteria in soil, rivers and seawater can develop resistance through contact with resistant bacteria, antibiotics, and disinfectant agents released by human activity. People and livestock can then be exposed to more resistant bacteria through food, water, and air.

**Human antibiotic use** jumped 36% in the 2000s



Up to **75% of antibiotics** used in aquaculture may be lost into the surrounding environment

**70% of antibiotics** are used by animals

Manure fertilizers cause antibiotic contamination in surface runoff, groundwater and drainage networks

**Antimicrobial use** for livestock will jump **67% by 2030**

Antibiotics are increasingly used to boost animal growth in intensive farming, especially in developing countries



Antibiotics can be absorbed by plants and crops



**Major waste flows** including wastewater, manures and agricultural run-off contain antibiotic residues and antibiotic-resistant bacteria

Wastewater treatment plants **cannot remove** all antibiotics and resistant bacteria

Up to **80% of consumed antibiotics** are excreted through urine and faeces

**30% of antibiotics** are used by humans

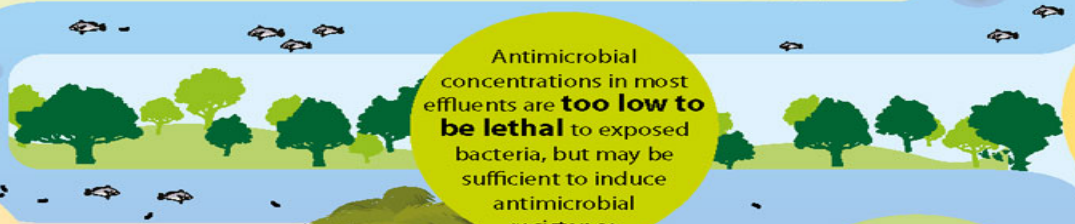
Antibiotic resistant bacteria may be present in **raw source water and treated drinking water**



**More than 50% of municipal solid waste** ends up in landfills and open dumps. This can include unused or expired drugs.

Antimicrobial concentrations in most effluents are **too low to be lethal** to exposed bacteria, but may be sufficient to induce antimicrobial resistance

A vast array of **contaminants in municipal and industrial wastewater** increases pressure on bacteria to become resistant



**Multi-drug resistant bacteria** are prevalent in marine waters and sediments in close proximity to aquaculture, industrial and municipal discharges



# AMR from Environmental Pollution

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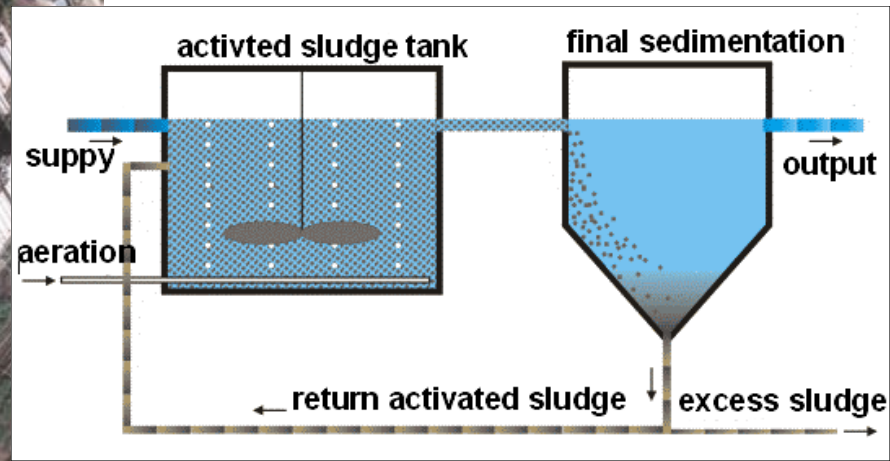
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05 DEC 2017 | PRESS RELEASE | ENVIRONMENTAL GOVERNANCE

## Antimicrobial resistance from environmental pollution among biggest emerging health threats, says UN Environment

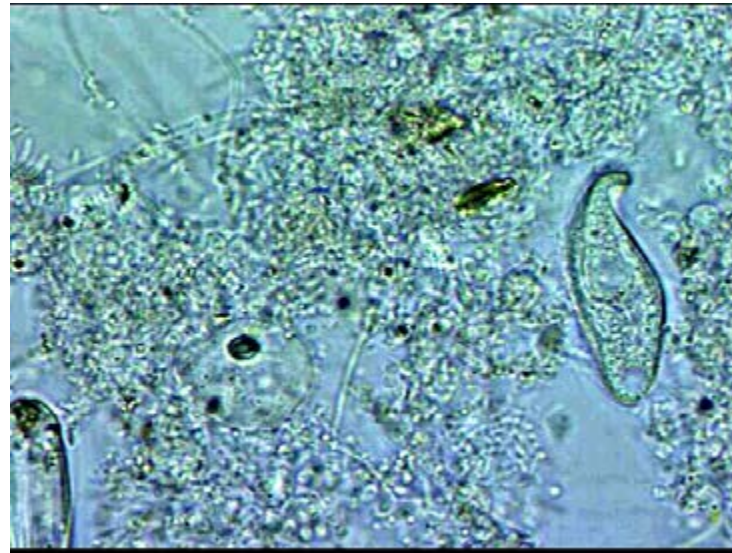
- Yang, Y., Li, B., Ju, F. and Zhang, T. (2013). Exploring variation of antibiotic resistance genes in activated sludge over a four-year period through a metagenomic approach. *Environmental Science & Technology*, 47(18), 10197-10205.
- Zhang, T. (2016). Antibiotics and resistance genes in wastewater treatment plants. *AMR Control*, 9 July 2016.



**Activated sludge: an old process with >100 years history.**

[http://cgi.tu-harburg.de/~awwwweb/wbt/emwater/lessons/lesson\\_c1/lm\\_pg\\_1425.html](http://cgi.tu-harburg.de/~awwwweb/wbt/emwater/lessons/lesson_c1/lm_pg_1425.html)

A typical STP (sewage treatment plant) in Hong Kong



**AS floc: aggregate of billions of microbial cells.**

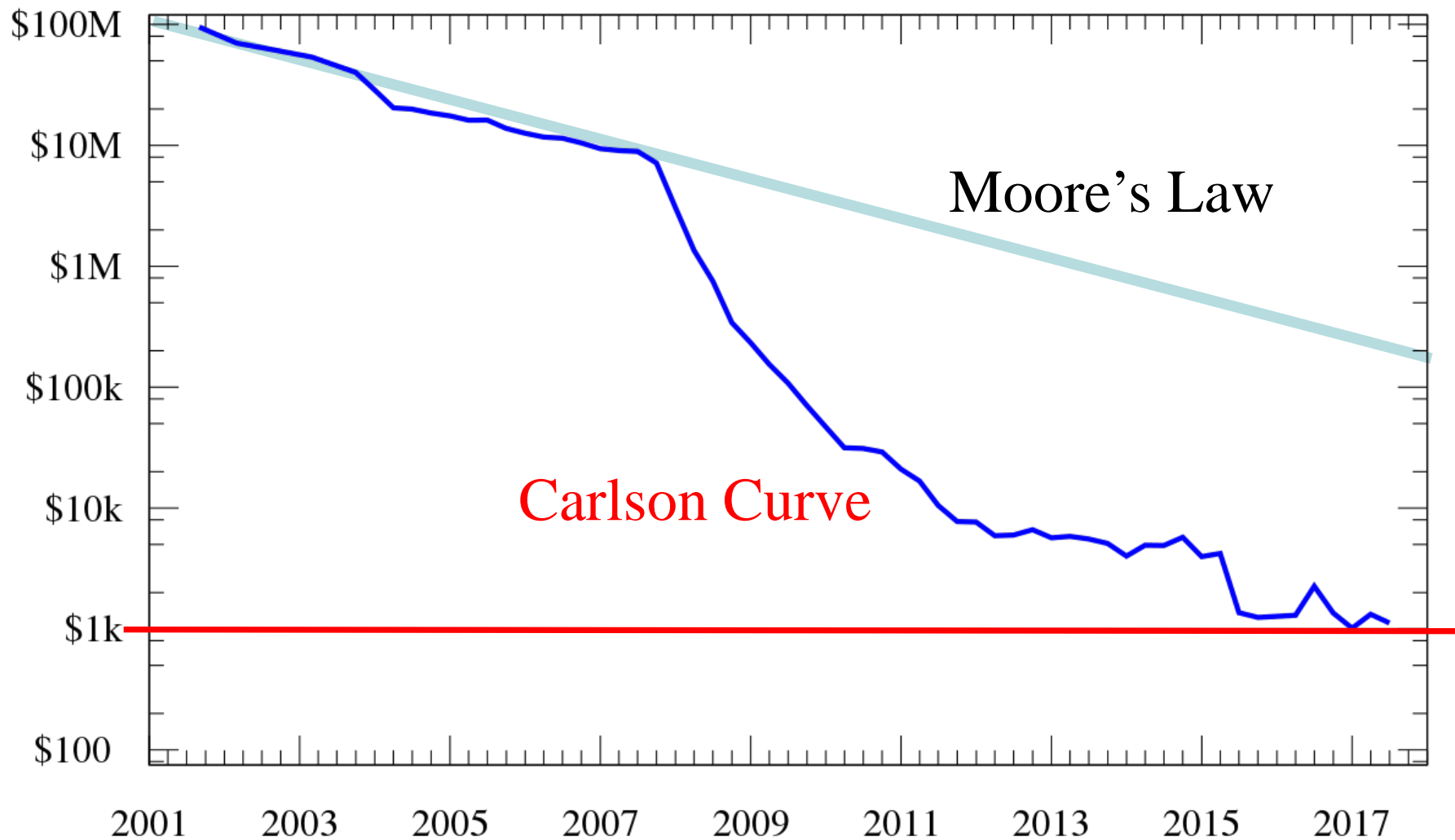
# Microbiome in WWTPs as Hot Spots of ARGs

- Created by environmental engineers, which not exist naturally.
- High diversity : thousands species.
- High biomass density : 2 ~ 50 g/L ( ~  $10^{13}$  cells/L, assuming dry weight of a bacteria cell is ~  $2 \times 10^{-13}$  g).
- Bacteria are close to each other in the flocs of activated sludge, granule sludge or biofilm, making HGT easier.
- SRT (sludge retention time, average generation time) of activated sludge: 6~12 days, 30~60 generations in a year
- Bacteria from fecal waste of thousands ( $10^3$ - $10^6$ ) peoples.
- Selective pressure: almost all the antibiotics, heavy metal, etc.
- Concentrated antibiotics in the micro-environment formed by EPS (extracellular polymeric substances)
- Bacteria discharged in effluent or sludge.

# Methods Used to Study ARGs in the Environment

- Isolation of resistant bacteria based on their phenotypes using selective media with specific antibiotic(s).
- PCR/sequencing to investigate genotypes of ARGs.
- qPCR to quantify ARGs in relative/absolute ways.
- High-throughput qPCR and microarray for wide spectrum of ARGs if those primers/probes are available.
- .....
- Metagenomic tools for a complete list of known and unknown/novel ARGs (*via* functional metagenomics)

# Cost to sequence a human genome (USD)



# Bioinformatics

*A “New Frontier” in environmental microbiology*

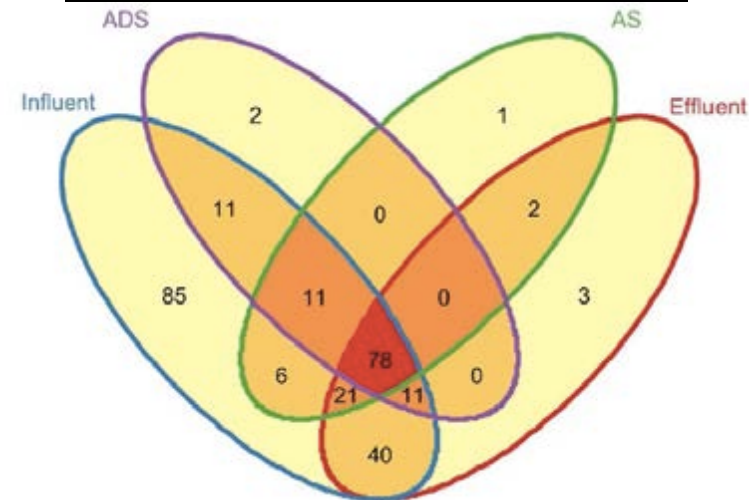
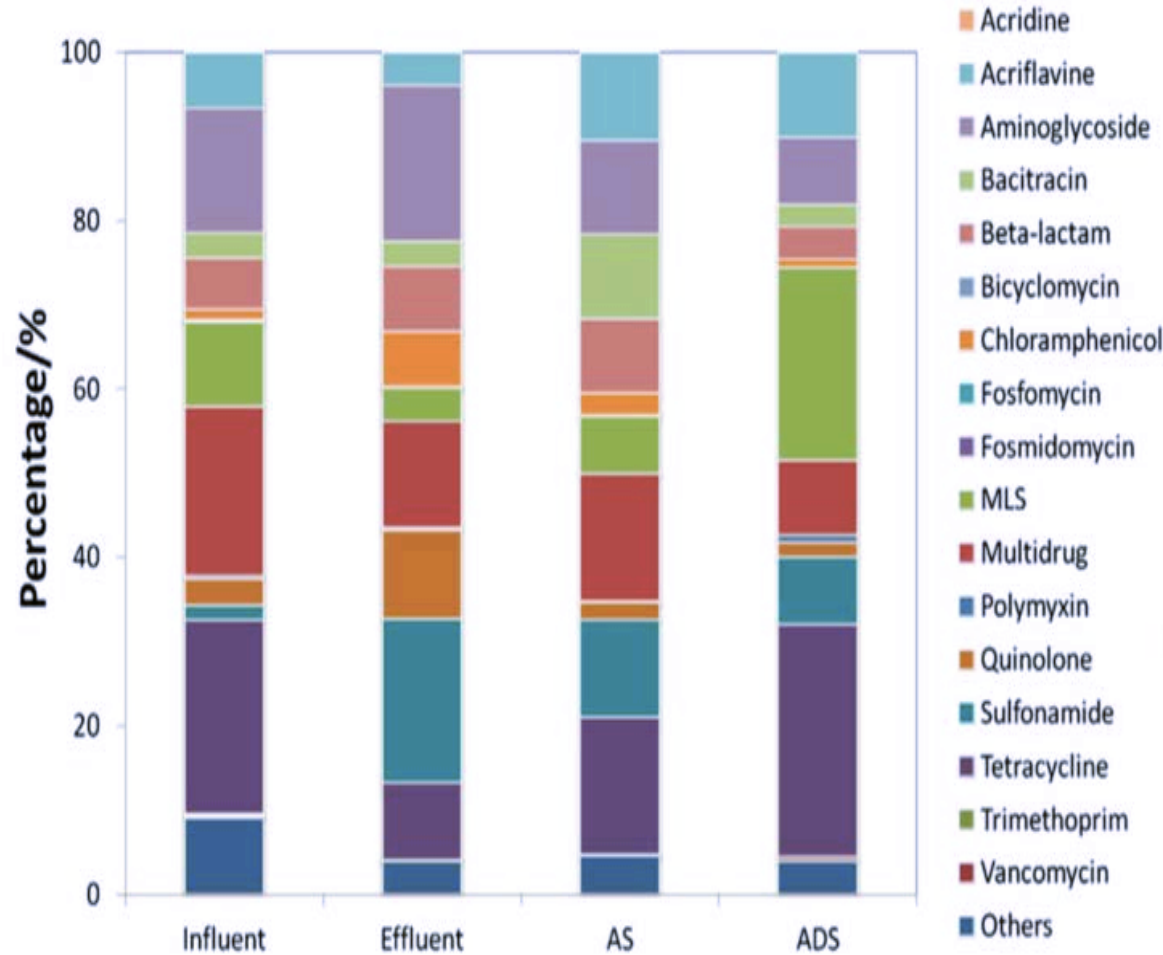
**Bioinformatics** : *translation* (from the different combinations of A, T, G and C to some biological terms, such as names of bacteria species and names of genes/enzymes) of **big data**, based on *databases* (like “*dictionaries*”).

**Bioinformatics** : another kind of the “*microscope*” to study microorganisms in wastewater reactors. It tells us the names and functions of different microbial populations.

# ARGs Profile in a full-scale WWTP at Hong Kong

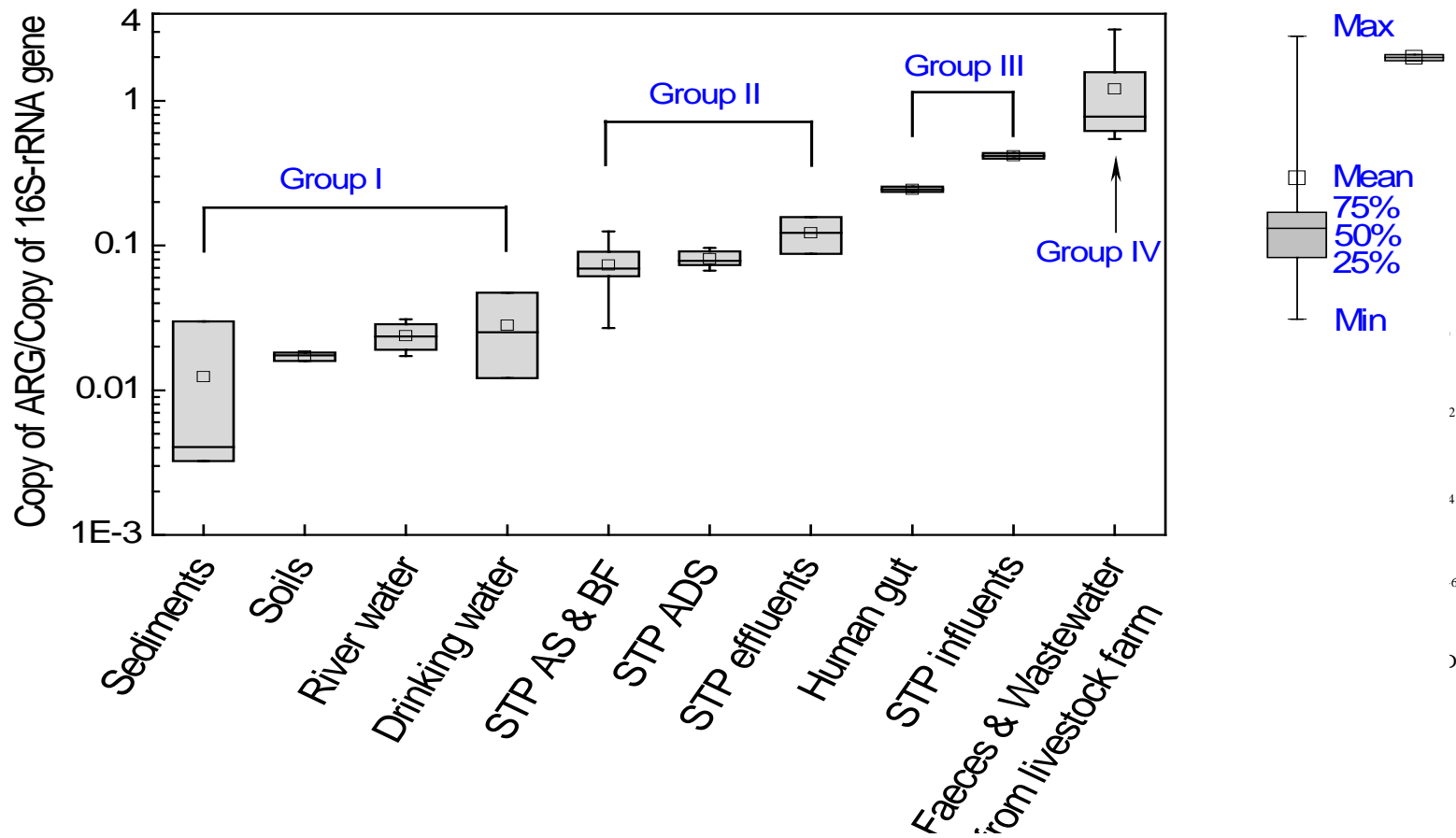
❖ ppm (part per million, one ARGs-like sequence per million sequences)

Abundance of ARGs / ppm		
IN_1	526	595
IN_2	664	
EF_1	84.0	82.6
EF_2	81.2	
AS_1	28.8	29.9
AS_2	31.0	
ADS_1	58.2	47.4
ADS_2	36.6	



Yang Y, Li B, Zou SC, Fang HHP, Zhang T\*. 2014. Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach. *Water Research*. 62, 97-106.

(B)



$$\text{Abundance} = \sum_1^n \frac{N_{\text{ARG-like sequence}} \times L_{\text{reads}} / L_{\text{ARG reference sequence}}}{N_{\text{16S sequence}} \times L_{\text{reads}} / L_{\text{16S sequence}}}$$

- The abundant ARGs were usually **associated with the extensively used antibiotics**.
- The abundance of ARGs increased with the influence of **anthropogenic activities**.

# Antibiotic Resistome in Drinking Water of China

## Abundance of ARGs

(copy of ARG per cell, capc)

- SXX Resistance level I (<0.1)
- SXX Resistance level II (0.1~0.2)
- SXX Resistance level III (>0.2)

## Pie Chart

(top 3 ARG types)

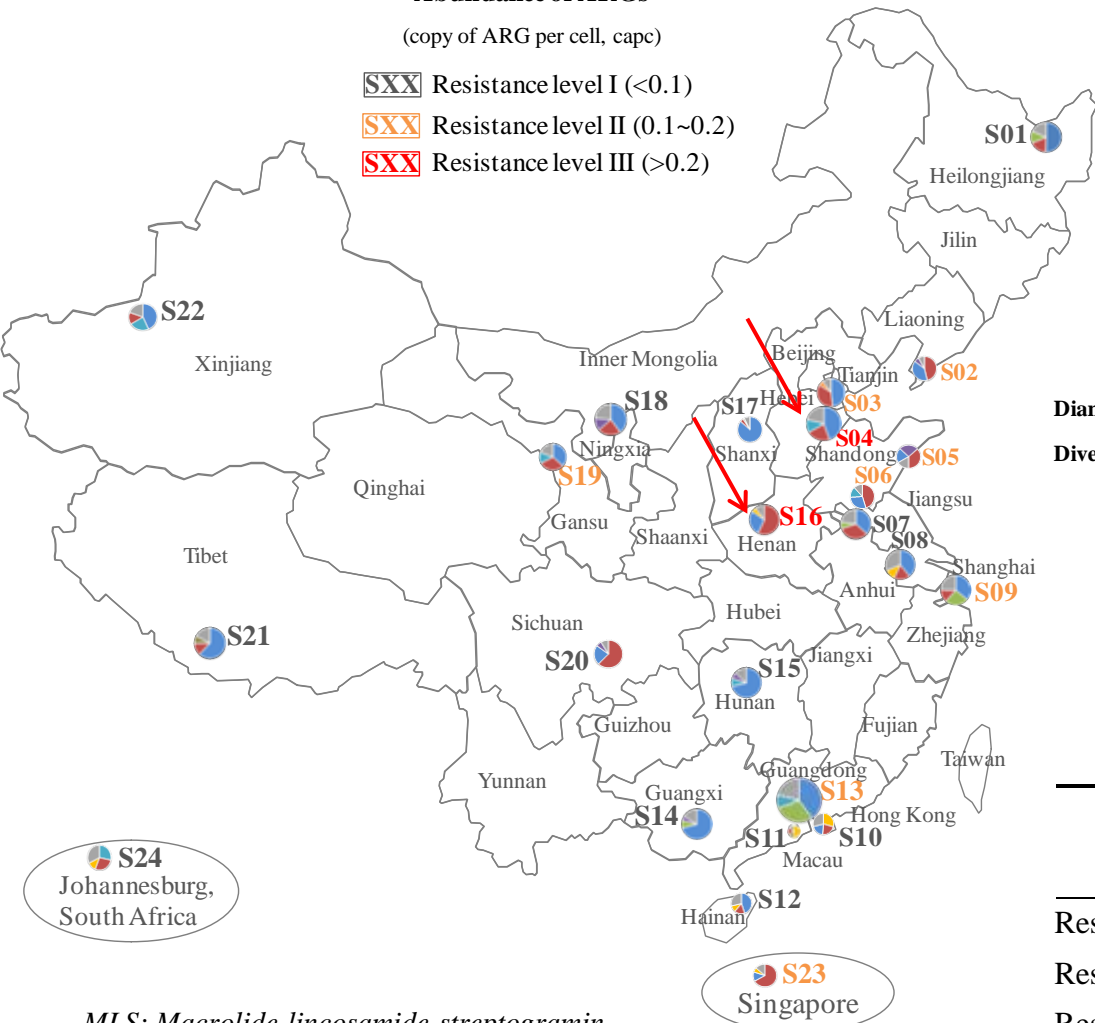


**16** ARG types

**181** ARG subtypes

**2.8E-2 ~ 4.2E-1** capc

**2~35** Cells



Diameter: 10 50 100  
Diversity

S25  
CA, USA

S24  
Johannesburg,  
South Africa

S23  
Singapore

MLS: Macrolide-lincosamide-streptogramin.

- Color of sample ID showed the resistance level of ARGs (I, II and III).
- Pie chart presented the profiles of ARG abundance (top 3 ARG types).
- Diameter of pie chart indicated the ARG diversity (number of ARG subtypes).

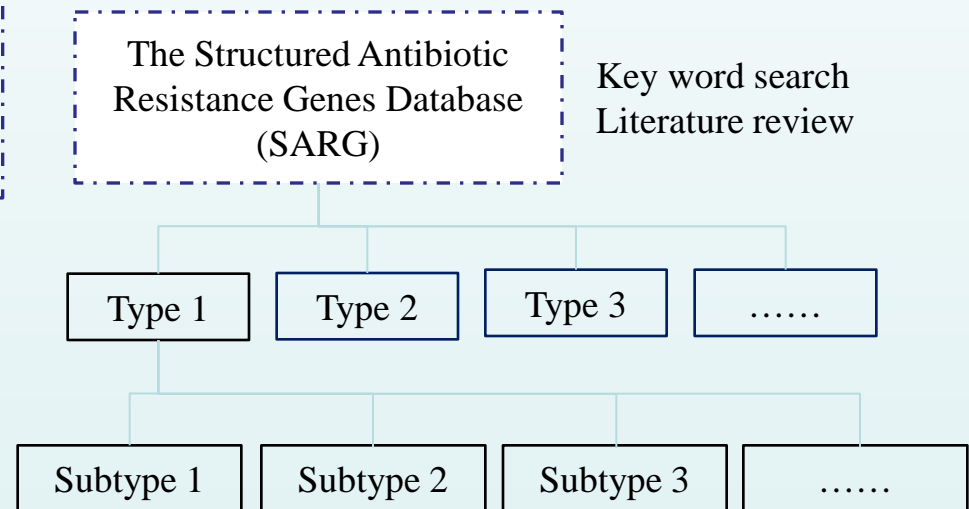
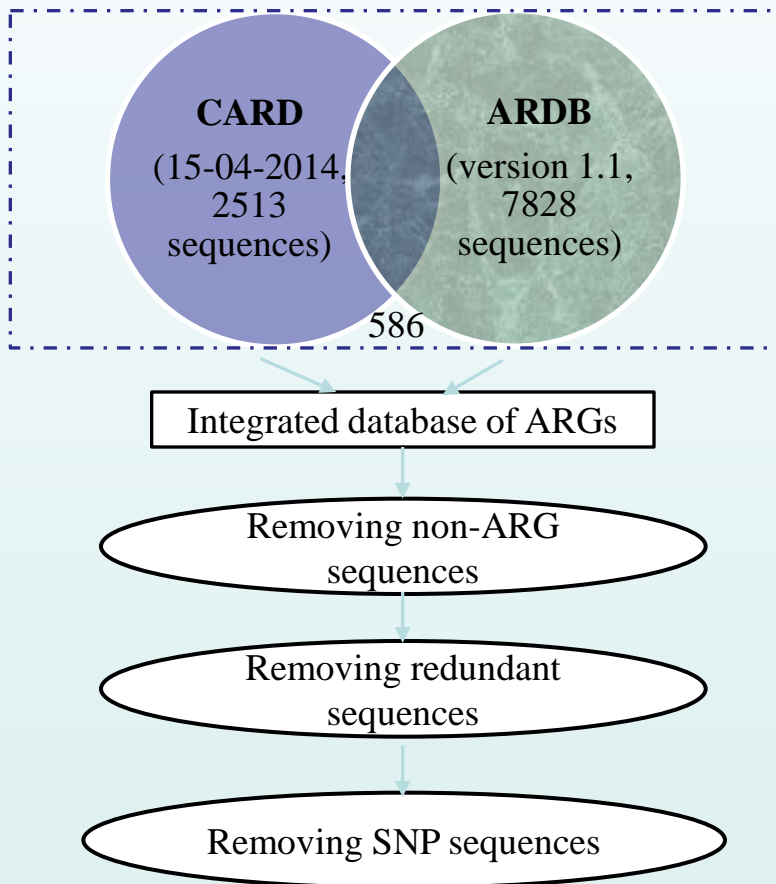
	Number of samples	Percentage of samples
Resistance level I (<0.1)	14	56%
Resistance level II (0.1~0.2)	9	36%
Resistance level II (>0.2)	2	4%

Ma LP, Li B, Jiang XT, Wang YL, Xia Y, Li, AD Zhang T\*. 2017. Catalogue of Antibiotic Resistome and Host-tracking in Drinking Water Deciphered by a Large Scale Survey. *Microbiome*, 5:154.

# **SARG Database and Analysis Pipeline**

**SARG v1.0 & SARG v2.0**

# Database - SARG v1.0



Yang Y, Jiang XT, Chai BL, Ma LP, Li B, Cole J, Tiedje MJ\*, Zhang T\*. 2016. ARGs-OAP: Online Analysis Pipeline for Antibiotic Resistance Genes Detection from Meta-genomic Data Using an Integrated Structured ARG-database. *Bioinformatics*. 32(15):2346-51



# Analysis pipeline - ARGs-OAP v2.0

You may select online or offline mode

## Annotation

Align metagenomic sequences against SARG database  
Annotate the sequences based on a cut-off (80% similarity and 75% hit length) strategy.

## Classification and quantification

ARGs are classified and quantified in both type and subtype levels.

## Normalization

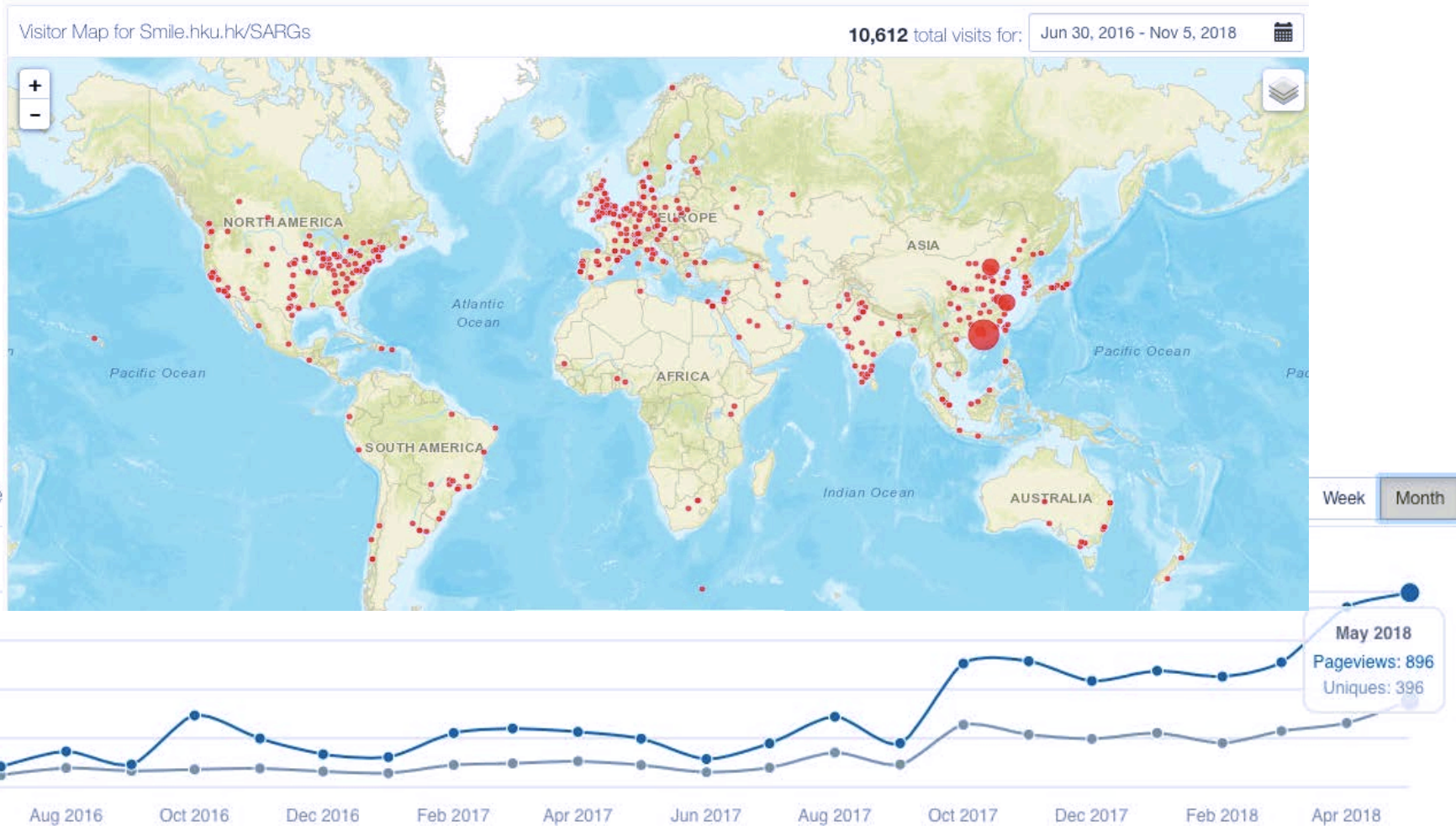
Normalize ARGs abundance in both type and subtype levels to three units:

- ❖ ppm (part per million, one ARGs-like sequence per million sequences)
- ❖ copies of ARGs/16S rRNA gene copies
- ❖ copies of ARGs/cell number



# Online Metagenomic Analysis of ARGs (ARGs-OAP)

Online metagenomic analysis became possible since the size of the metagenomic data set to be uploaded can be reduced significantly by UBALST pre-screening. Thus save the data uploading time by hundreds times. <http://smile.hku.hk/SARGs>



# Oxford Nanopore (MinION)



Sample

DNA / RNA Sequence

Real-time, actionable insights



MinION



GridION X 5



PromethION



SmidgION

- 3<sup>rd</sup> generation sequencing
- Fast sequencing (24h) with longer reads
- Long reads (>70kb) enable simultaneous host tracking

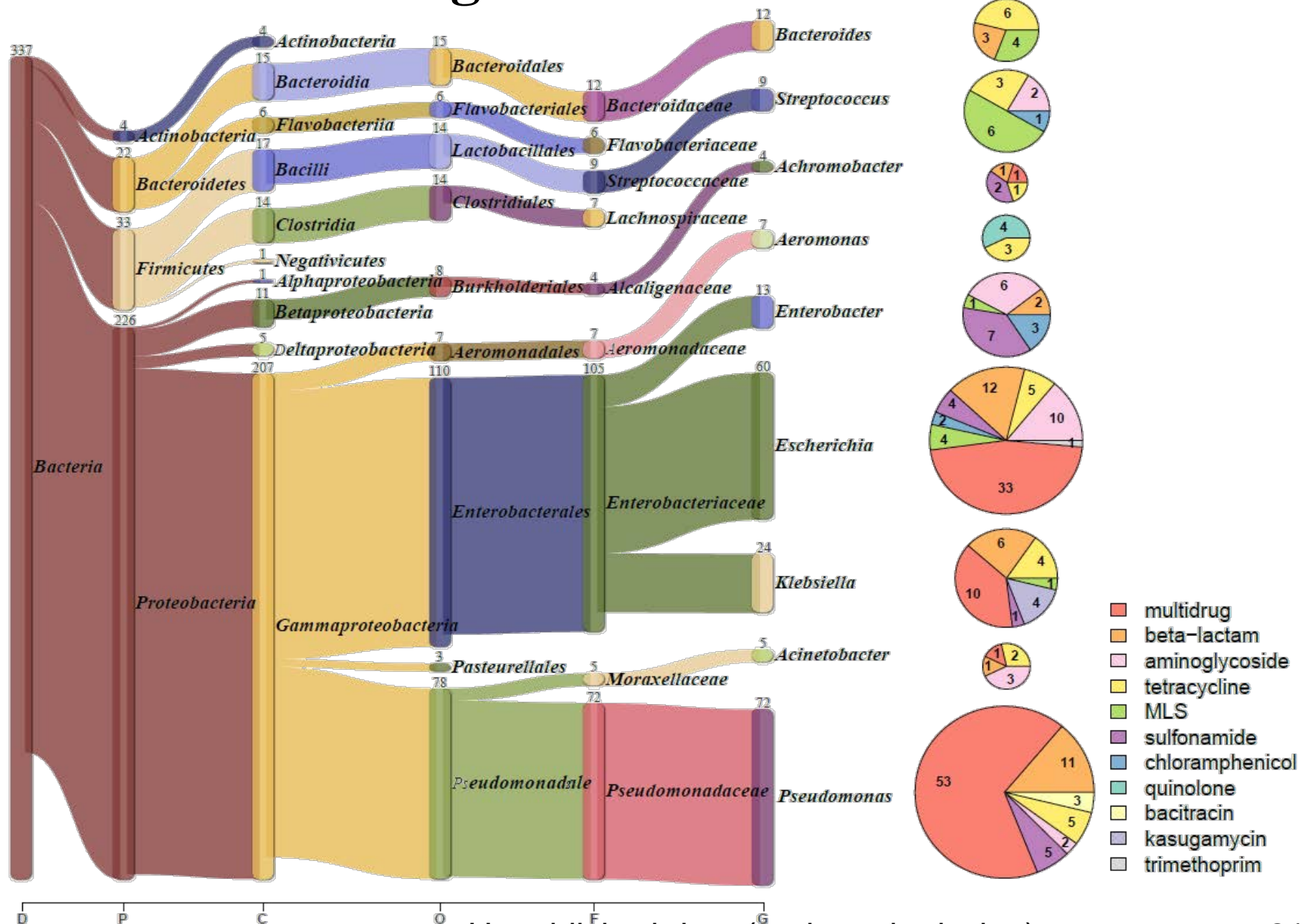
<https://nanoporetech.com>

# Metagenomics sequencing using Oxford Nanopore (MinION)

Sample	Number_reads	Meanlen	Maxlen	N50	Size G(fq)
ST-IN	627701	3921	31490	5872	4.7
ST-AS	494724	5059	21559	7029	4.8
ST-EFF	720148	4667	54416	7340	6.4
SWH-IN	586169	4846	33567	7833	5.4
SWH-AS	522782	5656	45452	9044	5.6
SWH-EFF	<b>839725</b>	5054	52163	7899	8.1
STL-IN	469061	6302	<b>73530</b>	10183	5.6
STL-AS	739597	<b>7329</b>	48719	<b>10674</b>	<b>11</b>
STL-EFF	703599	4620	46566	7167	6.2

Unpublished data (under submission)

# Host tracking of ARGs in WWTPs



Unpublished data (under submission)



Individually, bacteria/ARGs will beat us one by one eventually.  
However, they can never beat our human being as a team.

# Acknowledgements

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Fegn Ju	Lin Ye
You Che	Yuanqing Chao
Lei Liu	Yulin Wang



# Welcome to Hong Kong! Welcome to EDAR 2019!

## 5<sup>th</sup> International Symposium on the Environmental Dimension of Antibiotic Resistance

9-14 June 2019 • Hong Kong

CONGRESS DETAILS



**EDAR 2019**  
5<sup>th</sup> INTERNATIONAL SYMPOSIUM ON THE  
ENVIRONMENTAL DIMENSION OF ANTIBIOTIC RESISTANCE

9-14 JUNE 2019



### Welcome Note

Dear friends and colleagues,

Antibiotic resistance (AMR) is an emerging global challenge of fundamental importance that is threatening human health, agriculture, economies and the ecosystem. It increasingly has been recognized across society, including by scientists, doctors, engineers, governments and the general public in the recent years. Effective policies and actions to combat AMR depend on understanding this problem including the factors driving the development of AMR, such as current practices in the different use sectors, assessment of the global scope and nature of the problem, and what are the most effective mitigation and stewardship practices. The role of the environment in AMR has been listed by UNEP Frontiers 2017 as the first of six emerging issues of environmental concern.

EDAR1, held in Canada in 2012, was a catalyzing international effort to address the environmental aspect of this problem. This momentum continued with EDAR2, 3, and 4 in China, Germany and United States, respectively. Over these four conferences, the scope of discussion has grown in size and become more comprehensive. The focus of concerns now encompasses all critical environmental aspects with an emphasis on considering issues holistically, and complementary to discussions in health and other arenas.

Research programs have continued to provide new insights making another gathering, EDAR5, a very timely opportunity to share findings and to identify key issues for advancing protection of public health and the environment. EDAR5 will broadly cover environmental AMR but will especially emphasize:

- fundamental scientific aspects of environmental AMR including sources and drivers
- approaches to effective mitigation in different use sectors
- connections between environmental hotspots and point sources such as medical settings, day-care and long-term care facilities



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Keiji Fukuda