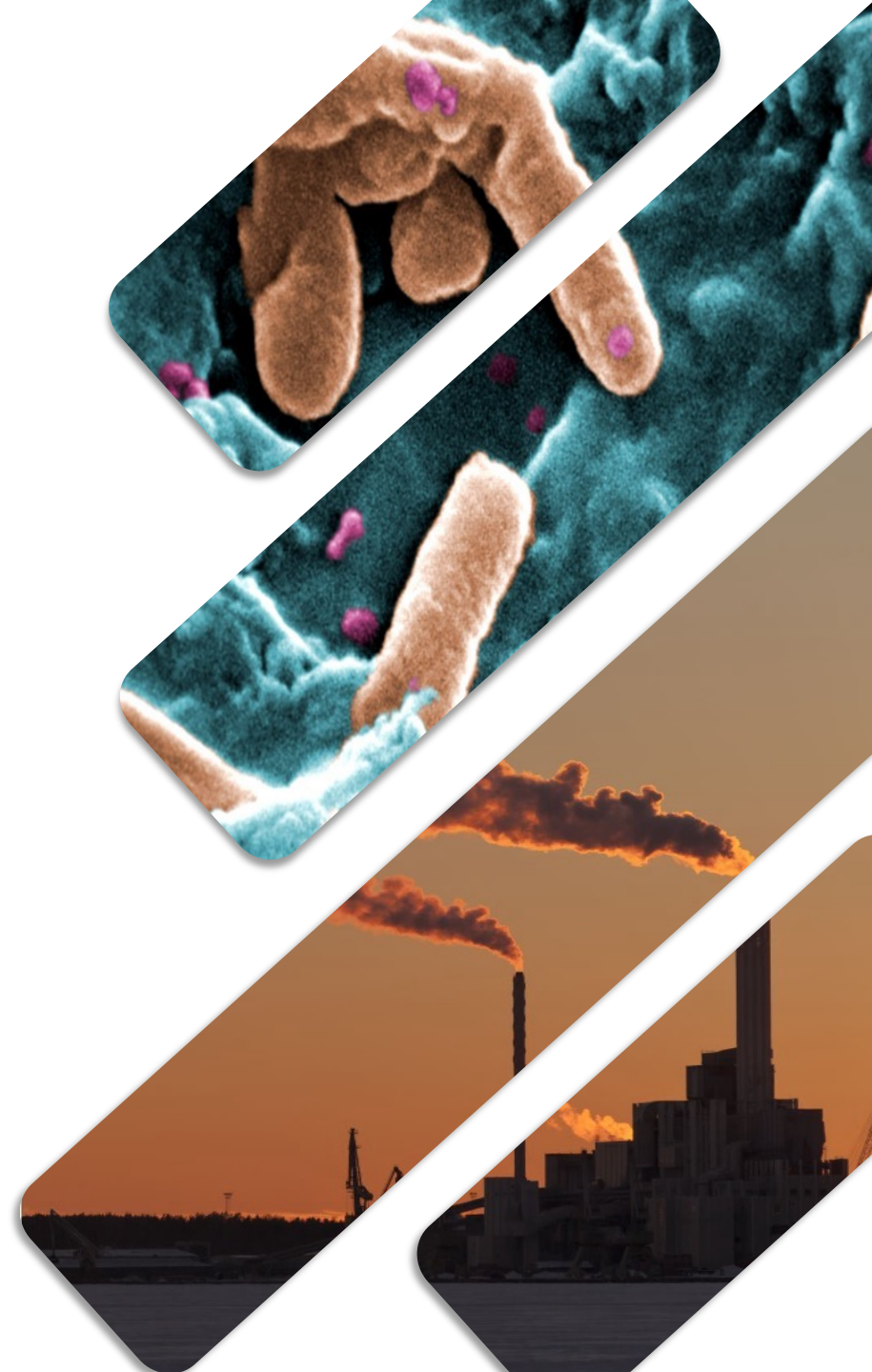Joint Graduate Student Seminar

# Unveiling the Nexus between Antimicrobial Resistance and Air Pollution

Presented by: Andrew CH KAN (Year 1 PhD Student)
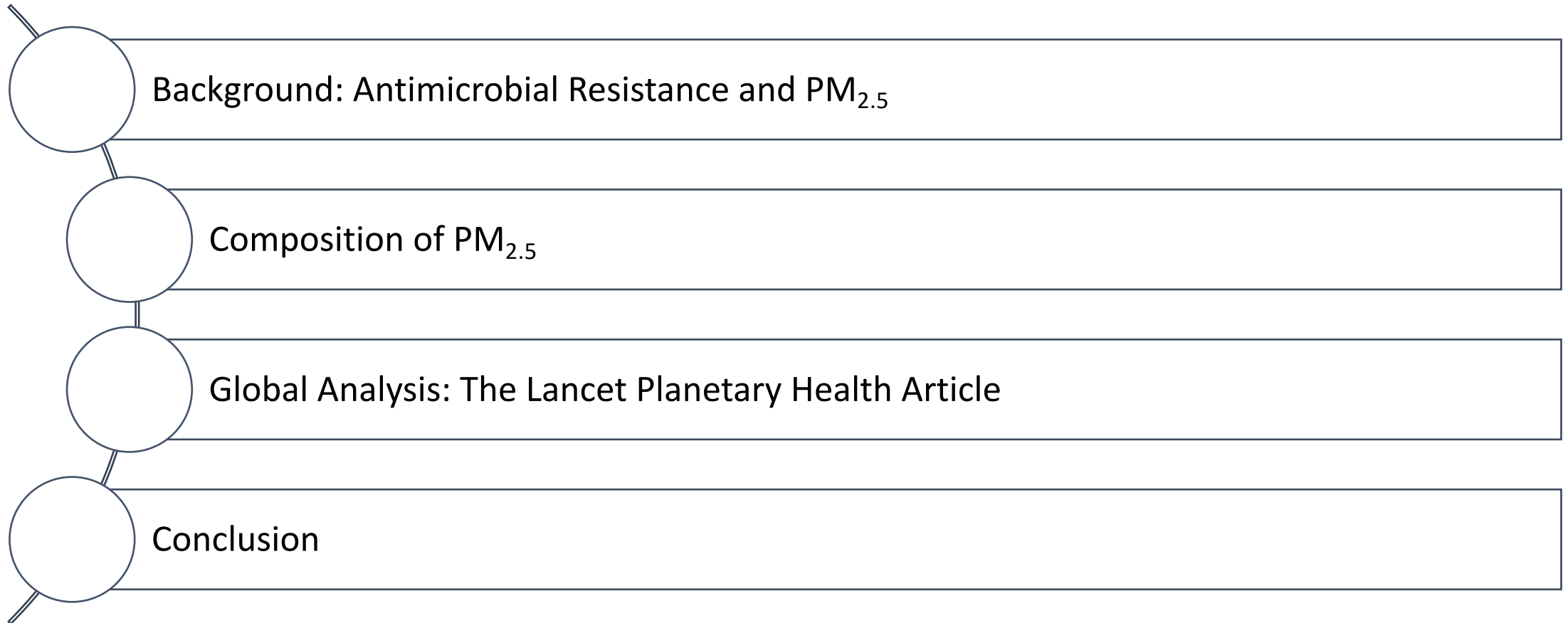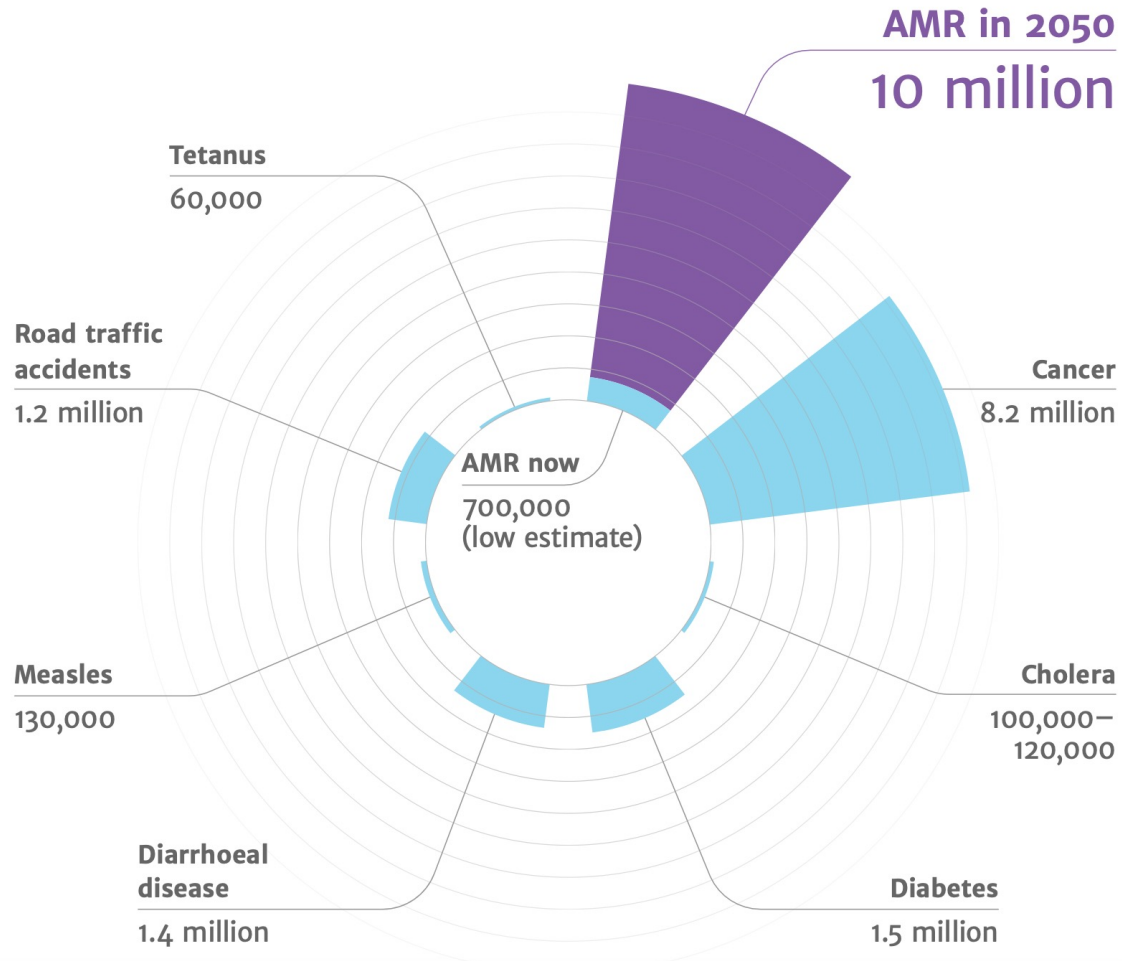
Supervisor: Professor Xiao YANG

24 November 2023

Department of Microbiology, CUHK

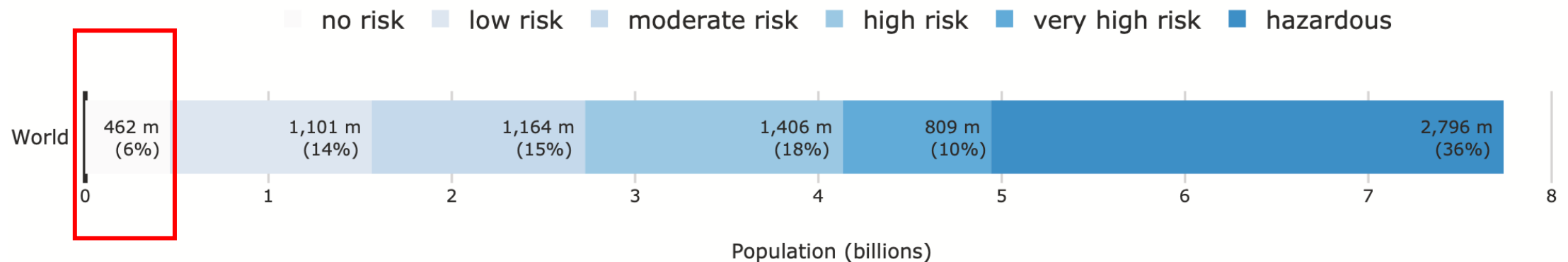# Outline

Background: Antimicrobial Resistance and PM$_{2.5}$

Composition of PM$_{2.5}$

Global Analysis: The Lancet Planetary Health Article

Conclusion

# Antimicrobial Resistance (AMR)

**AMR in 2050**
**10 million**

Tetanus
60,000

Road traffic
accidents
1.2 million

AMR now
700,000
(low estimate)

Cancer
8.2 million

Measles
130,000

Cholera
100,000−
120,000

Diarrhoeal
disease
1.4 million

Diabetes
1.5 million

00:00:03

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

Figures: Review on Antimicrobial Resistance, 2014. Attribution 4.0 International (CC BY 4.0).

# Air Pollution – Particulate Matter (PM$_{2.5}$)

*PM$_{2.5}$: Fine particles with a size ≤ 2.5 µm*

- [WHO Global Air Quality Guidelines (2021)](#)

- Annual average concentrations of PM$_{2.5}$ < 5 µg/m³ (i.e., no risk)

- 24-hour average exposures < 15 µg/m³ (limiting to 3~4 times a year)



Figure: Rentschler & Leonova, Nat. Commun. **2023**, *14*, 4432. Attribution 4.0 International (CC BY 4.0 DEED).

# Relationship between Antimicrobial Resistance and PM$_{2.5}$?
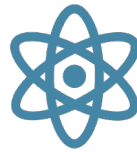
What can be found in PM$_{2.5}$?

# Inside PM$_{2.5}$

Combustion by-products

Dust and soil particles

Biological particles

Industrial emissions
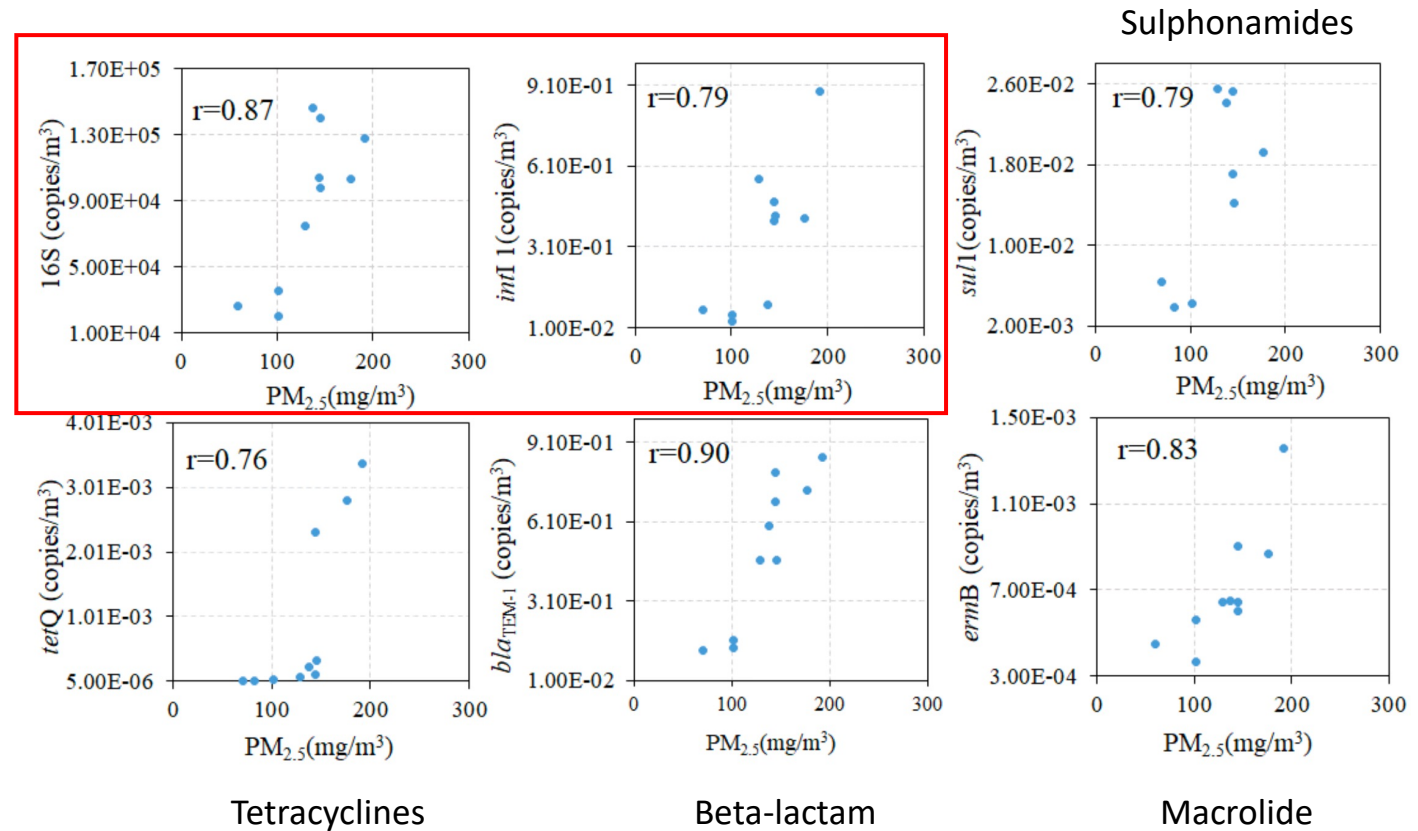
Vehicle emissions

Viruses

Bacteria

Airborne antimicrobial resistance genes

Mould spores

# (1) Increased Amounts of Bacteria

- Method: qPCR

- Higher [$PM_{2.5}$] → Higher [16S rRNA]

  - R = 0.87

  - **Dead vs. live cells??**

- Meanwhile higher [*intI 1* gene + ARGs]

  - R = 0.79

  - Class 1 integron-integrase gene

- → Antimicrobial resistant bacteria ↑



Sulphonamides

Tetracyclines          Beta-lactam          Macrolide

# (2) Antimicrobial Resistance Genes (ARGs)

- Detected in all environments

- Anthropogenic activities are the main drivers of the dissemination of ARGs

- Can spread from one place to another in the environment

- Smog increases $PM_{2.5}$ and ARGs (Hu et al., Sci. Total Environ. **2018**, *615*, 1332)

Mobile Genetic Elements

Self-inheritance

Horizontal gene transfer (HGT)
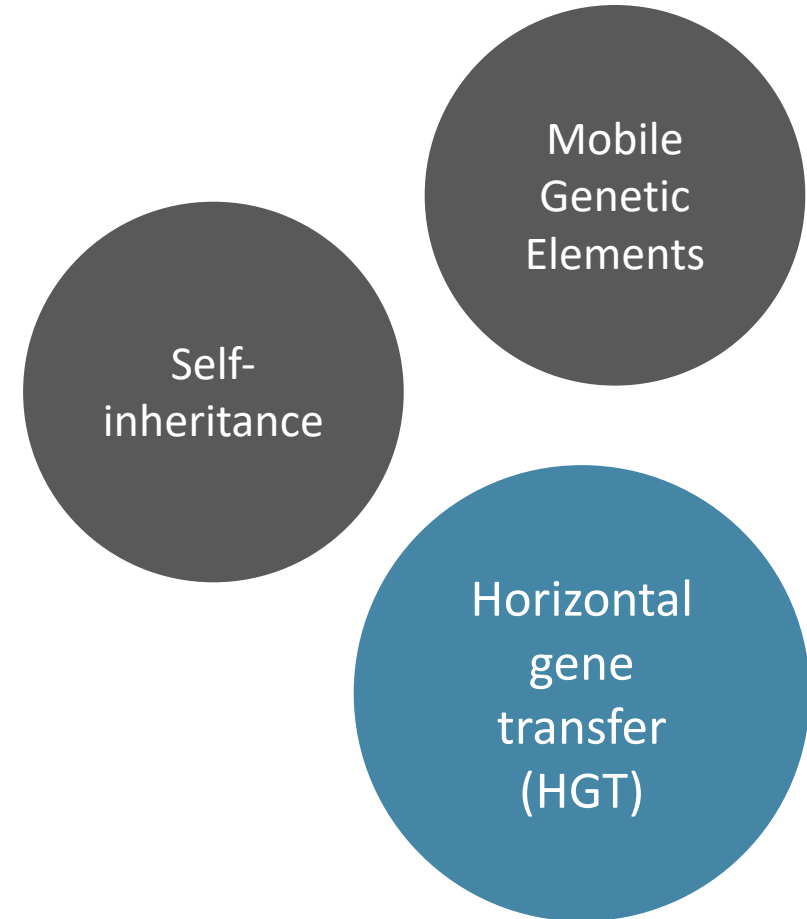
# PM$_{2.5}$ – Driver of Horizontal Gene Transfer (HGT)

- Control: intact and smooth with clear border

- Exposed group:

  - ↑ Pilus or pores on the surface

  - Cell membranes damaged

  - Cells membrane filled with many spherical and highly dense particles → Pores

- ↑ *ompA* expression with increasing [PM$_{2.5}$]

- Facilitate cell permeability and plasmid transfer
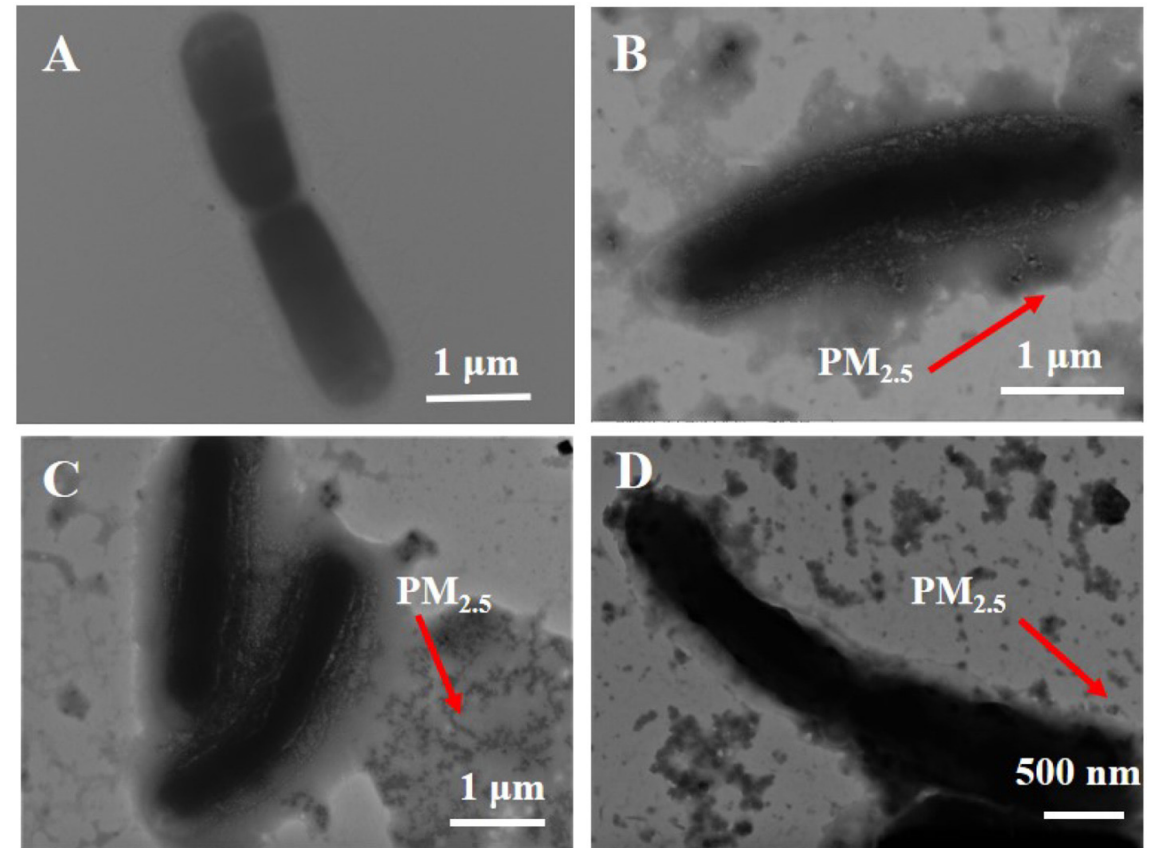


(A): Control. (B)-(D): *E. coli* S17-1 and K12 exposed to 125 µg/mL PM$_{2.5}$ from different regions. [During conjugative transfer]

9

# Gene Expression and Conjugation Rate

- Effects of different PM$_{2.5}$ samples to conjugation rate showed

- Top: outer membrane protein gene (*ompA*)

- Bottom: oxidative stress regulatory gene (*rpoS*)

  - Induced by intracellular ROS

- Positive correlation between gene expression and conjugative transfer
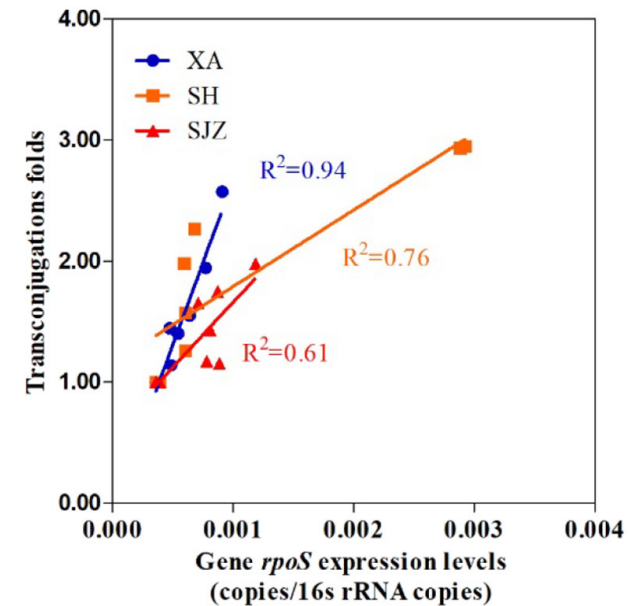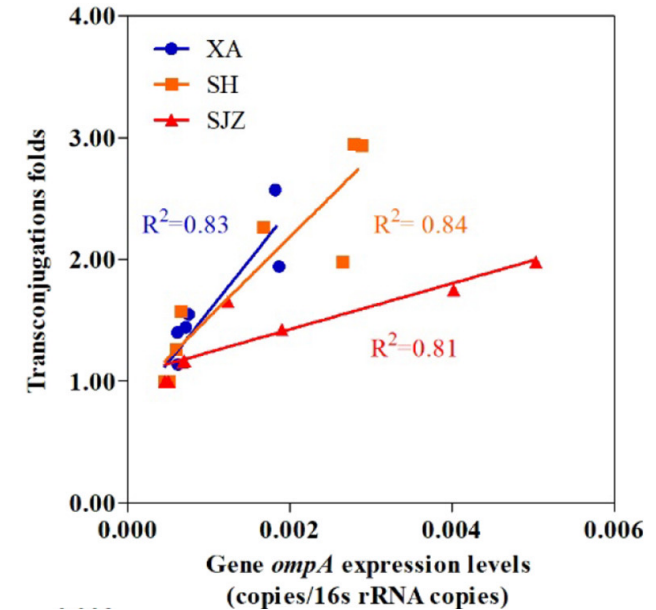
# ARGs in PM$_{2.5}$

- Target: airborne ARGs (from automobile air conditioning (AC) filter)

- Method: qPCR

- Normalised by 16S rRNA gene

- 30 ARG subtypes conferring resistance to 7 types of common antibiotics

Figure: Li et al., Environ. Sci. Technol. **2018**, *52*, 10975. Attribution Standard *ACS AuthorChoice/Editors' Ch*oice usage agreement.
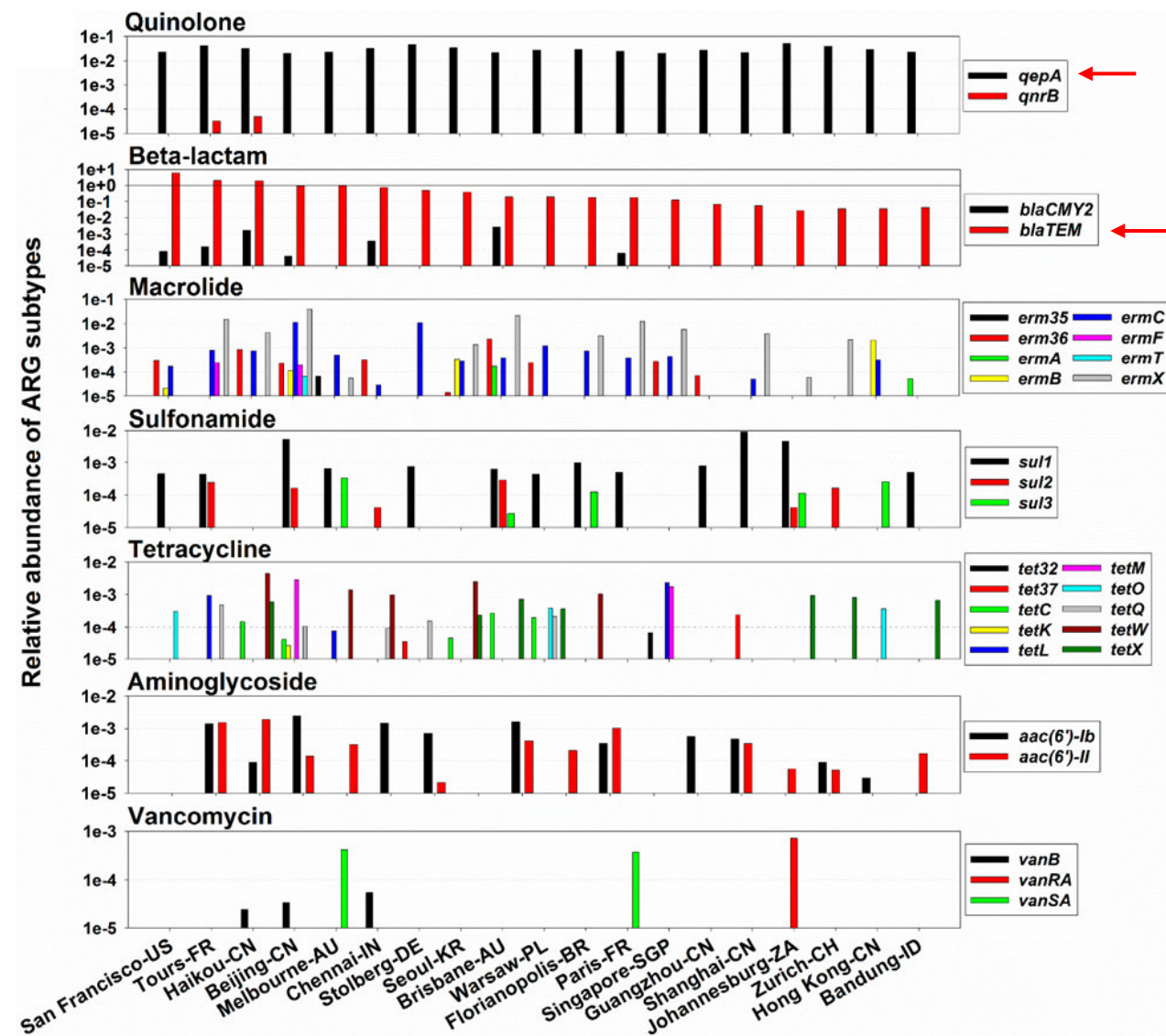
# Global Analysis

Lancet Planetary Health

# Association between particulate matter (PM)$_{2.5}$ air pollution and clinical antibiotic resistance: a global analysis

*Zhenchao Zhou, Xinyi Shuai, Zejun Lin, Xi Yu, Xiaoliang Ba, Mark A Holmes, Yonghong Xiao, Baojing Gu, Hong Chen*

## Summary

**Background** Antibiotic resistance is an increasing global issue, causing millions of deaths worldwide every year. Particulate matter (PM)$_{2.5}$ has diverse elements of antibiotic resistance that increase its spread after inhalation. However, understanding of the contribution of PM$_{2.5}$ to global antibiotic resistance is poor. Through univariate and multivariable analysis, we aimed to present the first global estimates of antibiotic resistance and burden of premature deaths attributable to antibiotic resistance resulting from PM$_{2.5}$ pollution.

- Data on multiple potential predictors (i.e., air pollution, antibiotic use, sanitation services, economics, health expenditure, population, education, climate, year, and region) were collected in 116 countries from year 2000 to 2018

- Univariate and multivariable analysis were conducted

- Future scenarios were predicted until 2050

# Positive Correlation between PM$_{2.5}$ and AMR

- Estimated 0.48 million premature deaths and 18.2 millions of life lost in 2018 worldwide due to PM$_{2.5}$-associated AMR

- Corresponding to an annual welfare loss of US$395 billion due to premature deaths

- R² = 0.47

- p < 0.0001



Region
- African
- Eastern Mediterranean
- European
- Americas
- Southeast Asia
- Western Pacific

$y = 0.9971 + 0.8116x;\ R^2 = 0.47,\ p = 6.869e^{-128}$

Tested isolates
- 1000
- 5000
- 10000
- 20000
- 40000
- 80000
- 120000

LN aggregate resistance (%)

Ln PM$_{2.5}$(µg/m³)

14
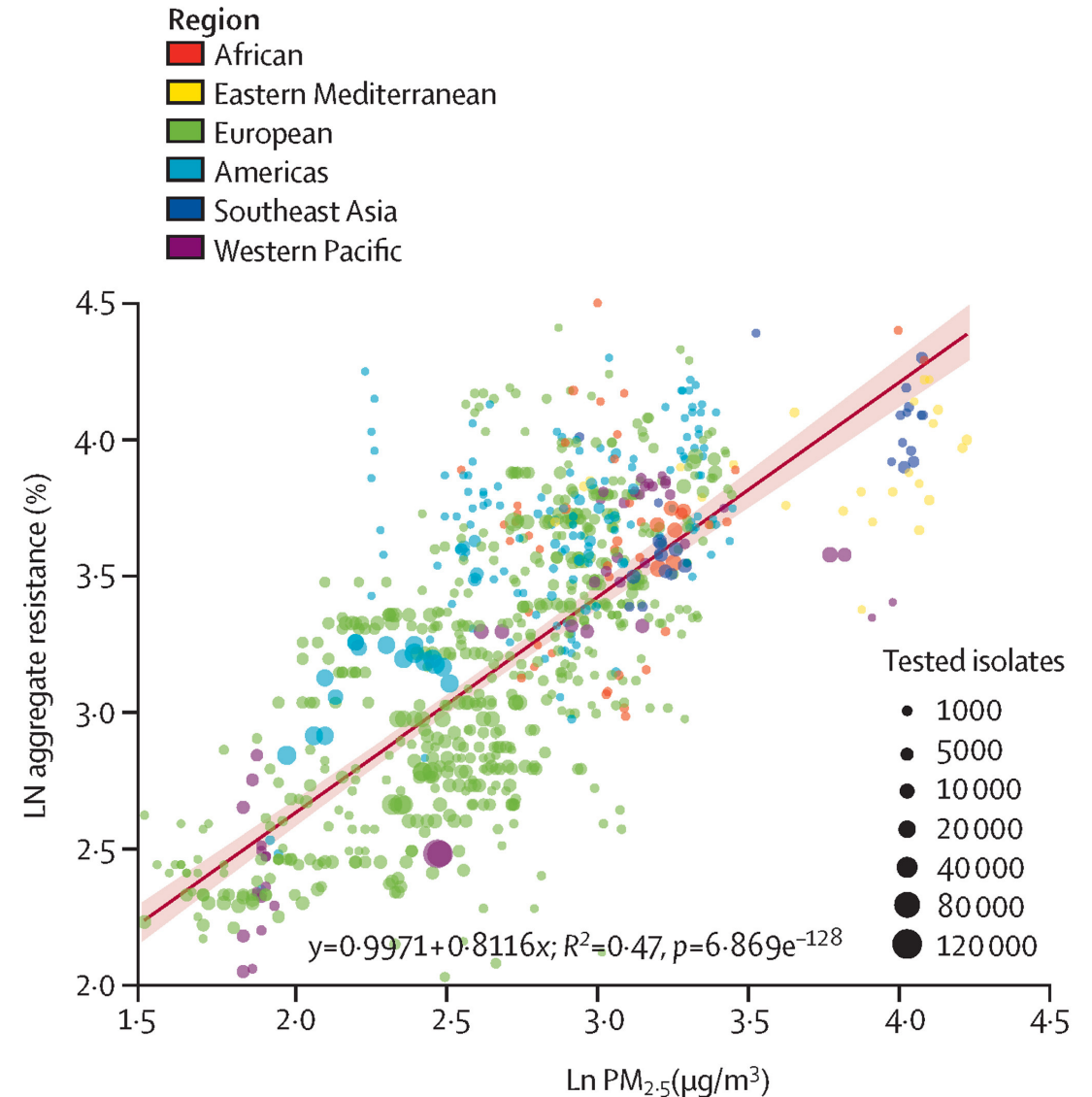
# Multivariable Analysis

- Inversely correlated:
  - Basic drinking-water services
  - Health expenditure
  - Rainfall

- Positively correlated:
  - Antibiotic use
  - $PM_{2.5}$

- **Infection control policies??**

- **Unrestricted use of antibiotics in animal farming??**

|  | Coefficient | Dominance (95% CI) | p value |
|---|---|---|---|
| $PM_{2.5}$ | 0·433 | 10·9% (4·3–17·4) | <0·0001 |
| CHE per capita | –0·00209 | 10·1% (2·8–17·3) | <0·0001 |
| People using at least basic drinking-water services | –0·582 | 2·7% (0·7–4·7) | <0·0001 |
| Antibiotic use | 0·479 | 2·4% (1·8–2·9) | <0·0001 |
| Rainfall | –0·00272 | 0·9% (0·7–1·1) | <0·0001 |
| Gross domestic product per capita | –0·0000423 | 9·5% (2·2–16·8) | 0·31 |
| Temperature | –0·0305 | 4·9% (0·0–9·7) | 0·67 |
| Governance | –0·354 | 4·1% (0·8–7·4) | 0·92 |
| Completion of primary education | 0·0675 | 1·2% (0·0–2·4) | 0·053 |
| Population density | 0·000586 | 0·2% (0·1–0·3) | 0·71 |
| Country income classification | Yes | 11·3% (3·7–18·9) | <0·0001 |
| Region | Yes | 7·5% (3·5–11·4) | <0·0001 |
| Year | Yes | 6·7% (4·6–8·8) | <0·0001 |
| $R^2$ | 0·72 | .. | .. |
| Total number of observations | 882 | .. | .. |

Income, Region, and Year are dummy predictors. Yes means that predictor has been considered in the analysis. CHE=current health expenditure. PM=particulate matter.

*Table*: Fixed-effects panel model of predictors associated with antibiotic resistance

# What If There is a 10% Increase of [PM$_{2.5}$]?

- Antibiotic resistance (top): Africa & Asia affected the most

  - Saudi Arabia: 3.0%

  - Niger: 2.9%

  - Nigeria & India: 2.5%

  - China: 2.1%

- Premature death (bottom): China & India (population size)

- **Lack of data in low- and middle-income countries**



Change in antibiotic resistance (%)

Change in premature deaths (%)
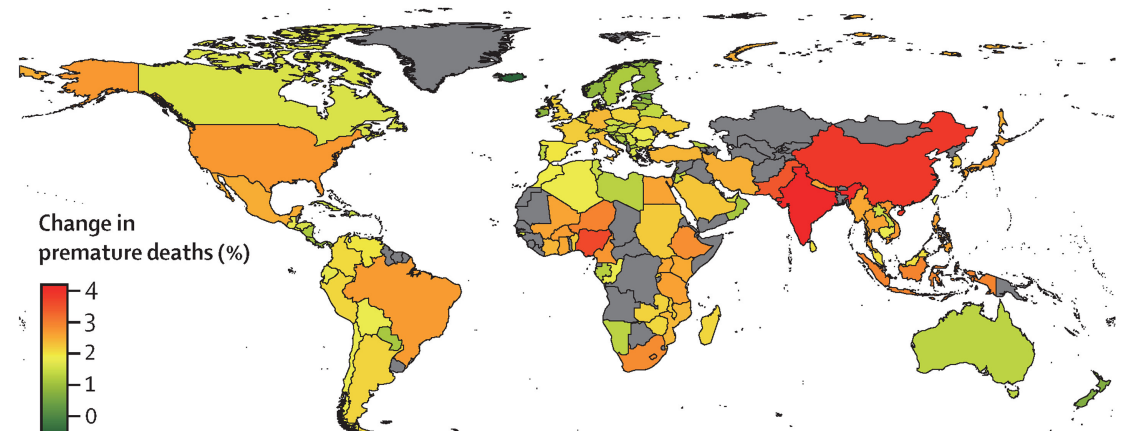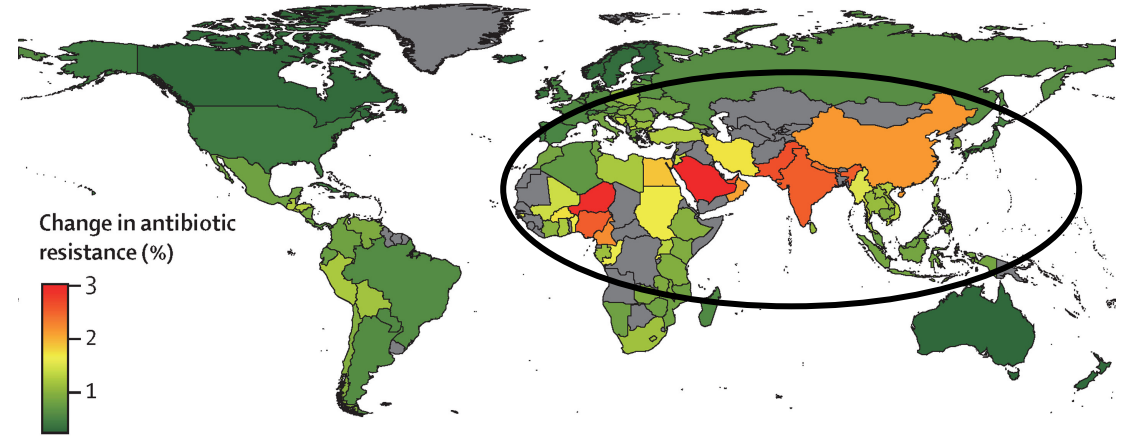
# WHO Global Priority Pathogens List
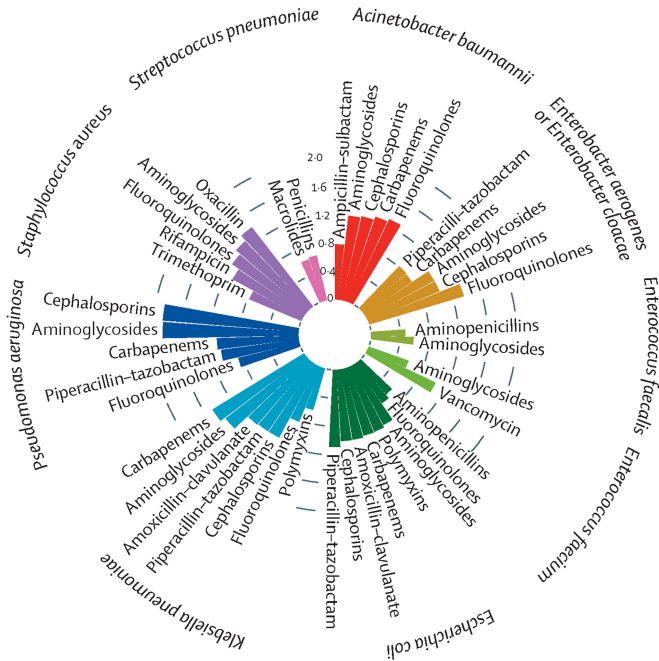


Slope of relationship between concentration of PM$_{2.5}$ and antibiotic resistance

Increase in 1% of PM$_{2.5}$ across regions was associated with increases in resistance ranging from of 0.5% to 1.9% in each of the nine pathogens ($p < 0.0001$)
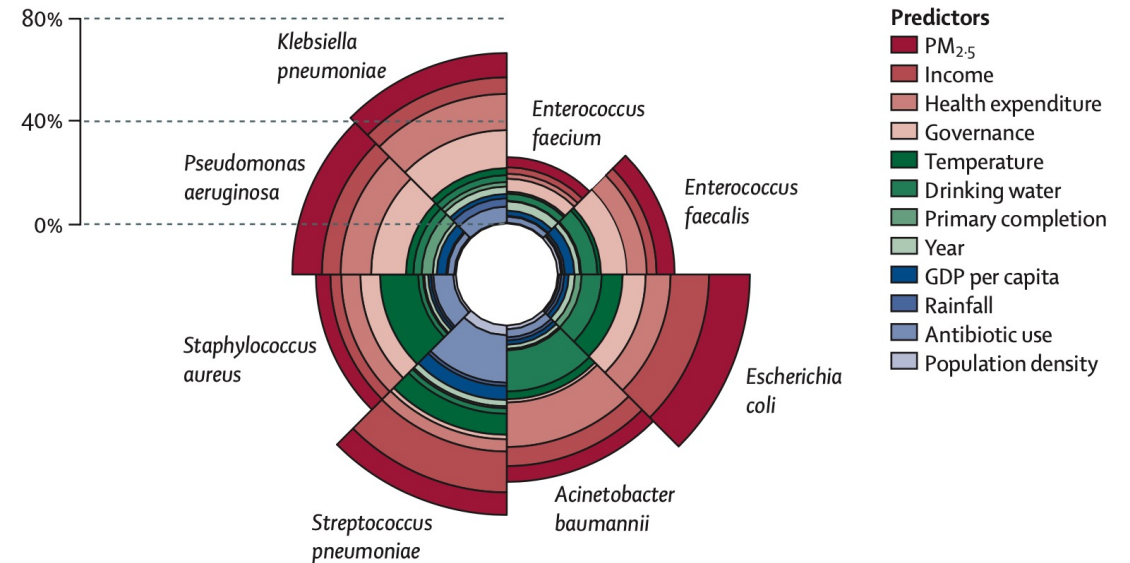
Contribution of predictors to antibiotic resistance for each pathogen

PM$_{2.5}$ consistently contributed to the increase of antibiotic resistance, ranging from 4.0% for *E. faecium* to 16.0% for *E. coli*

# Data Projection by 2050 (Assuming Causal-Relationship)

| | Without Intervention (Baseline) | PM$_{2.5}$ reduction to 5 µg/m$^3$ | Multivariate 3* |
|---|---|---|---|
| **Antibiotic resistance** | ↑ 17% | ↓ 16.8% | ↓ 50% |
| **Premature deaths attributable to resistant pathogens** | ↑ 56.4% | ↓ 23.4% | ↓ 54.5% |
| **Countries with the biggest impacts** | Sub-Saharan Africa | North Africa and West Asia | North Africa and West Asia |

*Multivariate 3: Combined control factors of current health expenditure (CHE) doubled, **50% reduced antibiotic use**, 100% basic drinking water services (BDWS), and PM$_{2.5}$ (5 µg/m$^3$)

## Antibiotic Resistance



Baseline
PM$_{2.5}$ (controlled to 5 µ/m$^3$)
Multivariate 3
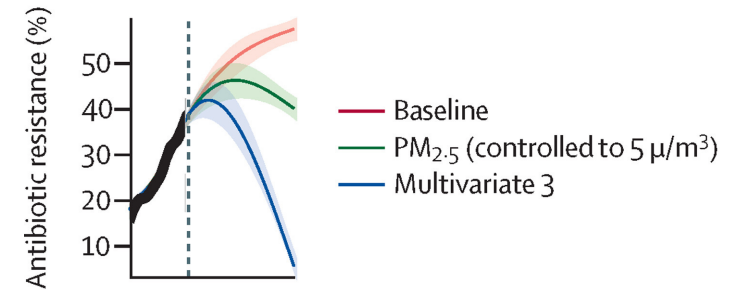
## Mortality Difference (%) Attributable to AMR



Baseline
PM$_{2.5}$ (controlled to 5 µg/m$^3$)
Multivariate 3

18
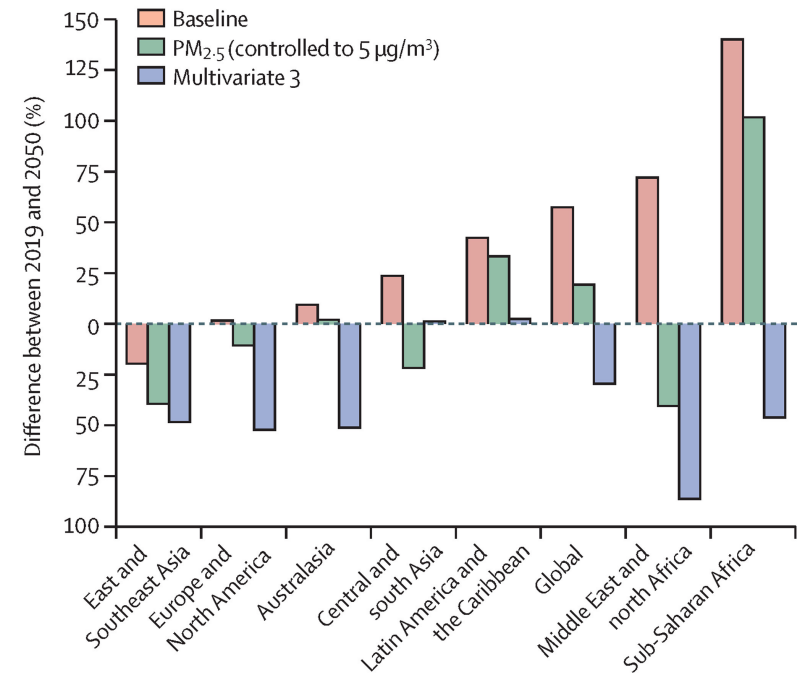
# Limitations of the Study

- Observational study

  - Experimental data needed to validate $PM_{2.5}$–AMR relationship

- Limitations of aggregate average data

  - Failure to capture regional variations within large countries may overlook important details

- Study did not consider other confounding factors (environmental, social, economic)

  - Differences between countries with different $PM_{2.5}$ levels, and between different years, apart from the $PM_{2.5}$ levels themselves

  - Statistically adjusted some factors to include in their statistical models

# Conclusion

- Preliminary data showed today suggested there is a potential relationship between $PM_{2.5}$ and AMR

- The exact mechanisms between $PM_{2.5}$ and AMR remains to be elucidated (transfer of ARGs?)

- The main driver of AMR is still misuse and overuse of antibiotics

- "One-Health" framework and global actions are needed

# Thank You!

Do you have any questions?

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# Appendix 1: PM$_{2.5}$ Levels in Hong Kong 2022

Pollutant:   Fine Suspended Particulates  (PM$_{2.5}$)   (Annual limit value = 25 μg/m$^3$)

| Station | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Annual |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Central/Western | 25 | 13 | 18 | 15 | 10 | 6 | 8 | 8 | 23 | 15 | 13 | 17 | 14 |
| Southern | 19 | 10 | 13 | 13 | 9 | 6 | 8 | 7 | 21 | 13 | 11 | 16 | 12 |
| Eastern | 22 | 12 | 16 | 14 | 10 | 5 | 8 | 7 | 21 | 14 | 13 | 17 | 13 |
| Kwun Tong | 23 | 12 | 18 | 16 | 12 | 9 | 10 | 8 | 20 | 13 | 12 | 17 | 14 |
| Sham Shui Po | 21 | 12 | 15 | 13 | 9 | 6 | 9 | 8 | 18 | 12 | 10 | 16 | 12 |
| Kwai Chung | 25 | 14 | 18 | 15 | 11 | 8 | 10 | 9 | 24 | 15 | 13 | 19 | 15 |
| Tsuen Wan | 22 | 14 | 14 | 10 | 12 | 8 | 7 | 9 | 25 | 17 | 15 | 20 | 14 |
| Tseung Kwan O | 19 | 10 | 15 | 14 | 9 | 6 | 9 | 8 | 23 | 17 | 14 | 18 | 13 |
| Yuen Long | 28 | 16 | 17 | 16 | 12 | 6 | 9 | 9 | 24 | 15 | 15 | 23 | 16 |
| Tuen Mun | 30 | 19 | 20 | 18 | 13 | 8 | 11 | 9 | 27 | 19 | 15 | 25 | 18 |
| Tung Chung | 30 | 15 | 15 | 14 | 10 | 5 | 9 | 9 | 23 | 15 | 12 | 19 | 14 |
| Tai Po | 23 | 13 | 15 | 14 | 10 | 6 | 10 | 8 | 22 | 15 | 13 | 20 | 14 |
| Sha Tin | 22 | 12 | 15 | 14 | 10 | 6 | 9 | 7 | 22 | 14 | 10 | 17 | 13 |
| North | 21 | 12 | 14 | 10 | 9 | 6 | 9 | 7 | 22 | 17 | 15 | 21 | 14 |
| Tap Mun | 20 | 10 | 13 | 12 | 7 | 4 | 7 | 6 | 20 | 14 | 11 | 17 | 12 |
| Causeway Bay | 30 | 18 | 24 | 19 | 16 | 13 | 15 | 16 | 31 | 22 | 20 | 25 | 21 |
| Central | 27 | 15 | 20 | 18 | 13 | 9 | 12 | 12 | 26 | 18 | 16 | 21 | 18 |
| Mong Kok | 26 | 13 | 20 | 17 | 13 | 8 | 10 | 10 | 25 | 18 | 17 | 20 | 16 |

*Unit: microgram per cubic metre (μg/m$^3$)

# Appendix 2: PM$_{2.5}$-Borne Bacterial Community in Xi'an
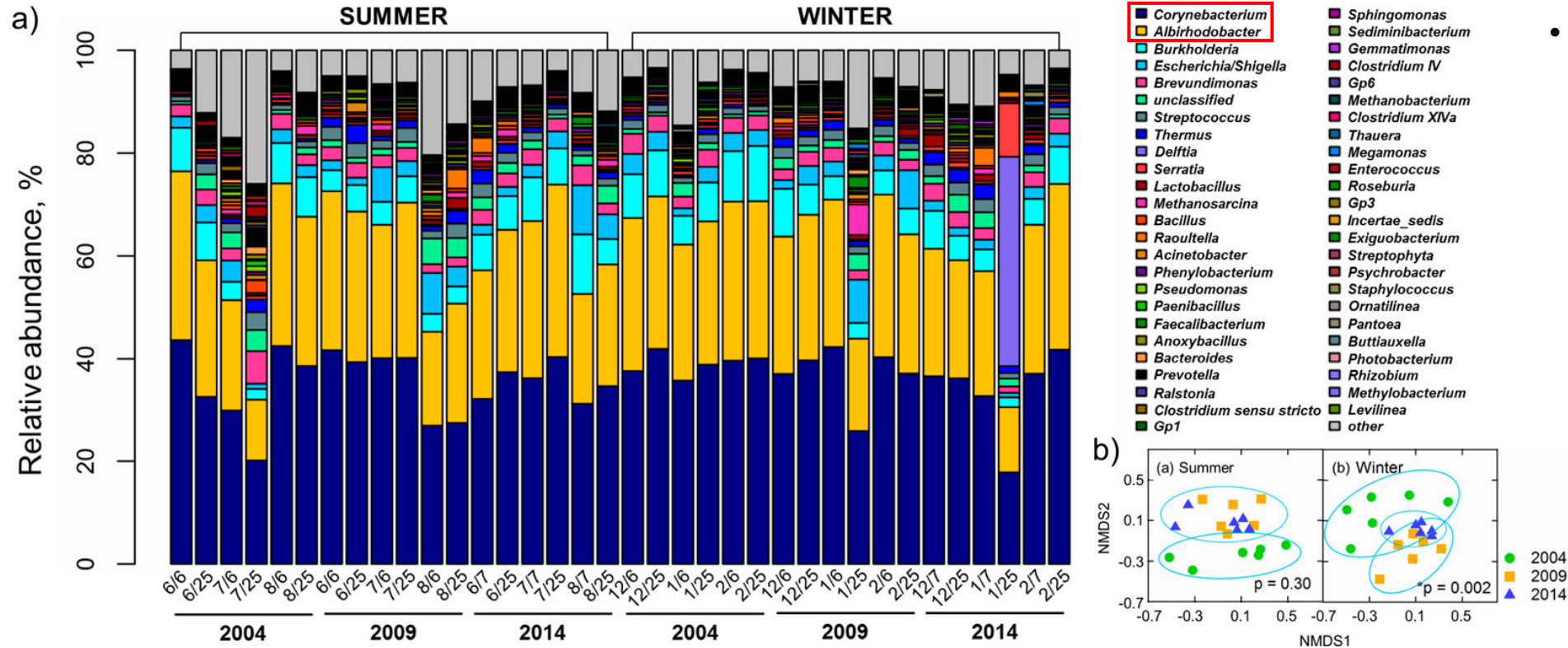


(a) Distribution bar plot of PM$_{2.5}$-borne bacterial community compositions in PM$_{2.5}$ samples collected in summer and winter in **Xi'an** on the genus level and the corresponding illustration of each genus, with sampling dates displayed in *X*-axis; and (b) NMDS analyses for the relative abundances of PM$_{2.5}$-borne bacterial genera in respective summer and winter among the years of 2004, 2009, and 2014 over a 10-year time span, where *p* is the statistical result of ANOSIM; \**p*-value < 0.05 indicates a statistical significance.

- *Faecalibacterium* and *Methanosarcina* in the year 2009, and *Delftia*, *Serratia*, and *Raoultella* in the year 2014 had significantly increased in winter

  - Facultatively anaerobic or strictly anaerobic
  - Enriched in anthropogenic sources

  ➔ The changes in winter might be explained by the increasing contribution from anthropogenic sources to airborne PM$_{2.5}$ that resulted from the increasing population density

# Appendix 3: Class 1 Integron-Integrase Gene (*intI 1*)

- Component of class 1 integrons

- Integrons are common in bacteria (~15% population contains integrons)

- Integrons allow microbial cells to capture and express exogenous gene cassettes

- In class 1 integrons, these cassettes are often antimicrobial resistance genes (ARGs)

- A proxy for anthropogenic impact



Circular gene cassette

IntI mediated recombination

Cassette inserted into array

*intI*   Pc *attI*   *attC*   *attC*   *attC*

Integron-encoded promoter

Integron recombination site