



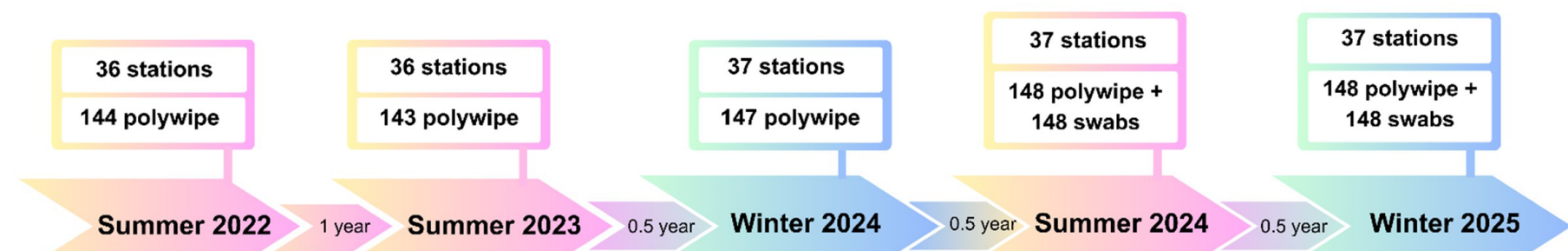
Metagenomics-Enabled Phylogeographic Mapping of Multidrug-Resistant Organisms (MDROs) and Antibiotic Resistance Genes (ARGs) in Hong Kong Urban Subway System

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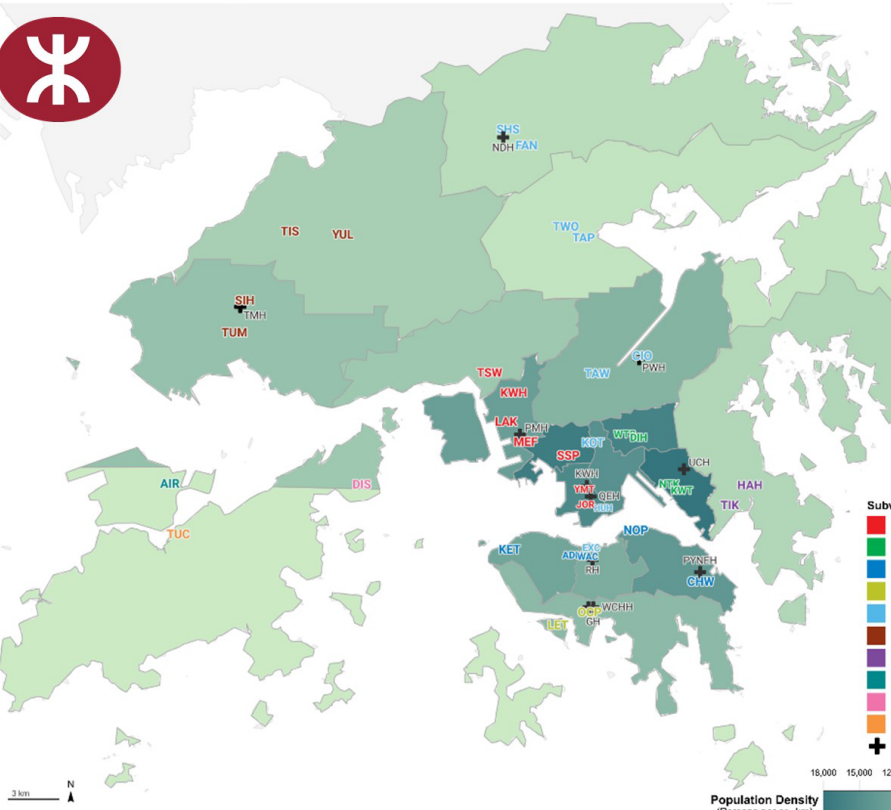
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INTRODUCTION

A. Study Timeline of the Hong Kong Subway System Microbiome and Resistome Surveillance



B. Subway station close to hospitals and interchange stations



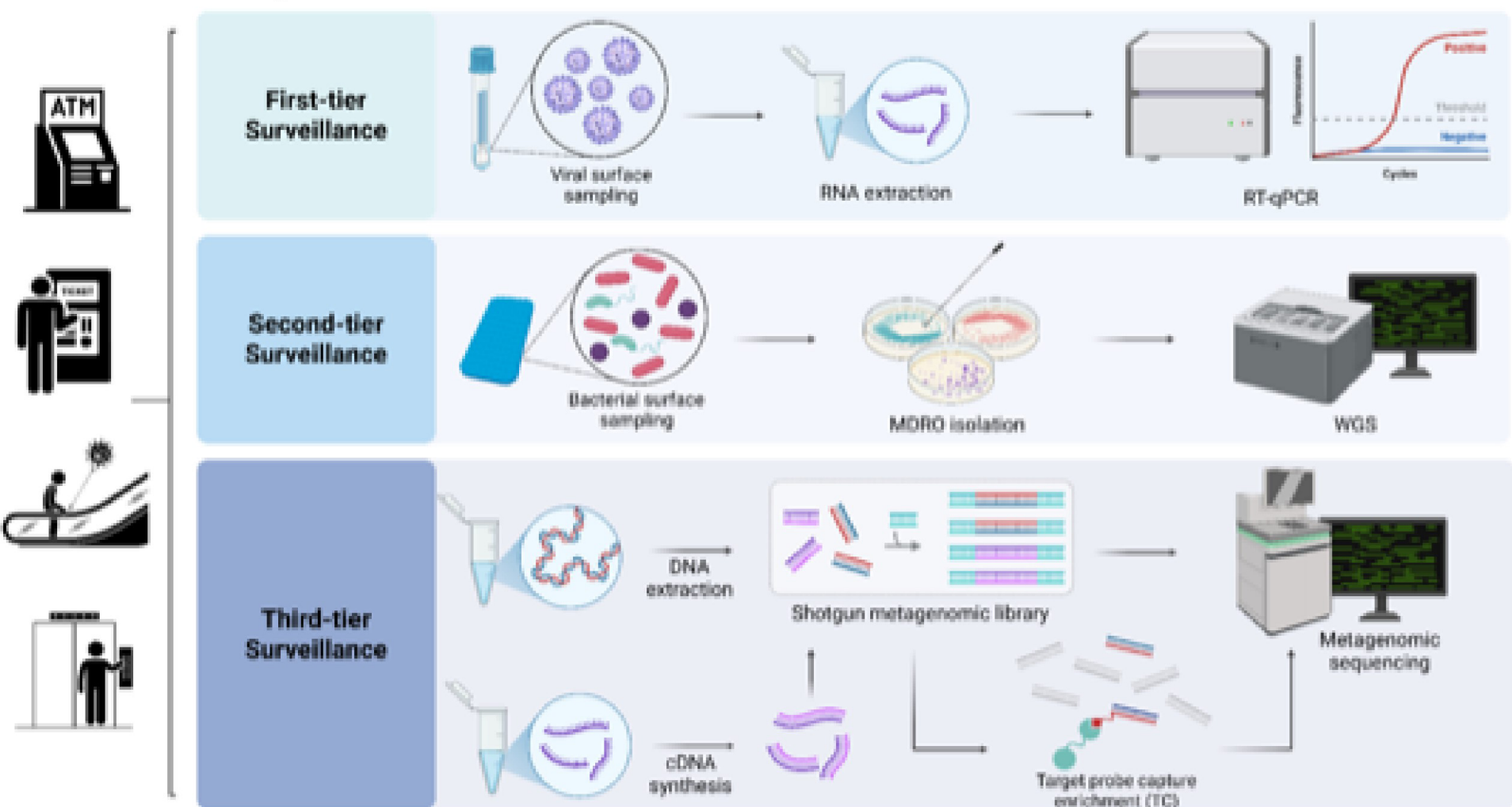
C. High-contact sites potential hotspot for MDROs and ARGs dissemination



- Antimicrobial resistance (AMR) has become a pressing **global public health crisis**¹, yet surveillance efforts largely neglect urban built environments²
- Urban subway systems act as major hubs for **potential pathogen transmission** owing to their **high passenger density** and **frequent contact with high-touch surfaces**³
- This study presents a **2.5-year citywide surveillance** of the Hong Kong subway microbiome and resistome
- Bacterial** ($n = 730$) and **viral** ($n = 296$) swabs were collected from four high-contact sites in subway stations

METHODS

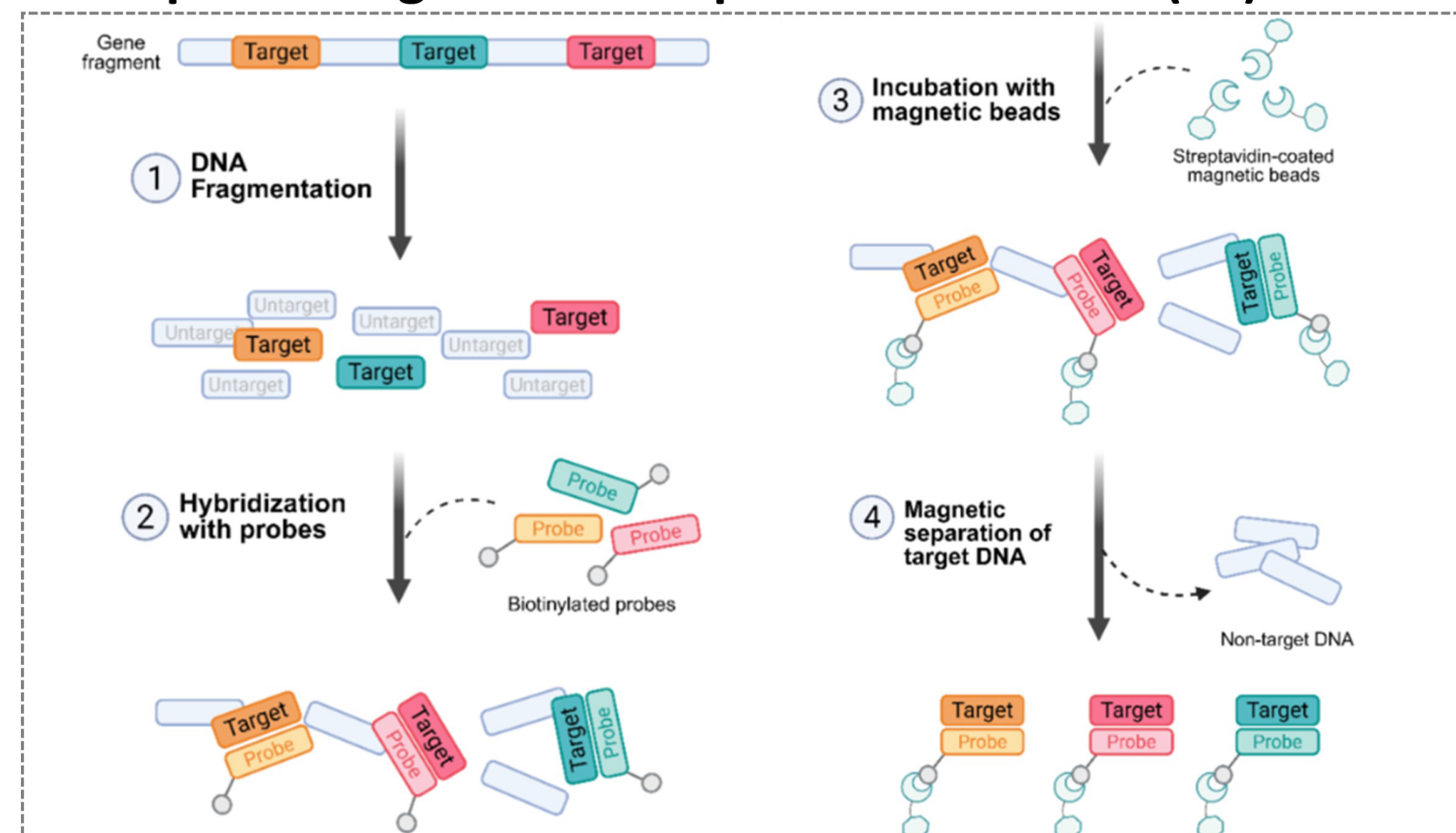
Three-tiered Surveillance Framework of MDRO and ARG in the Subway Environment



The pathogen surveillance of the subway environments adopted a **three-tiered framework**:

- 1st tier: Probe-based RT-qPCR assays** to screen for common respiratory viruses
- 2nd tier: Conventional culture-based methods** to isolate drug-resistant bacteria, followed by WGS of MDR isolates to identify their molecular subtypes and elucidate underlying resistance mechanisms
- 3rd tier: Culture-independent metagenomic approaches** including shotgun metagenomics and target probe capture enrichment (TC) to provide a comprehensive yet nuanced view of the subway microbiome and resistome

Principle of Target Probe Capture Enrichment (TC)



ACKNOWLEDGMENTS

I would like to thank members of Prof. Gilman Siu's Group for assistance in sample collection and processing.

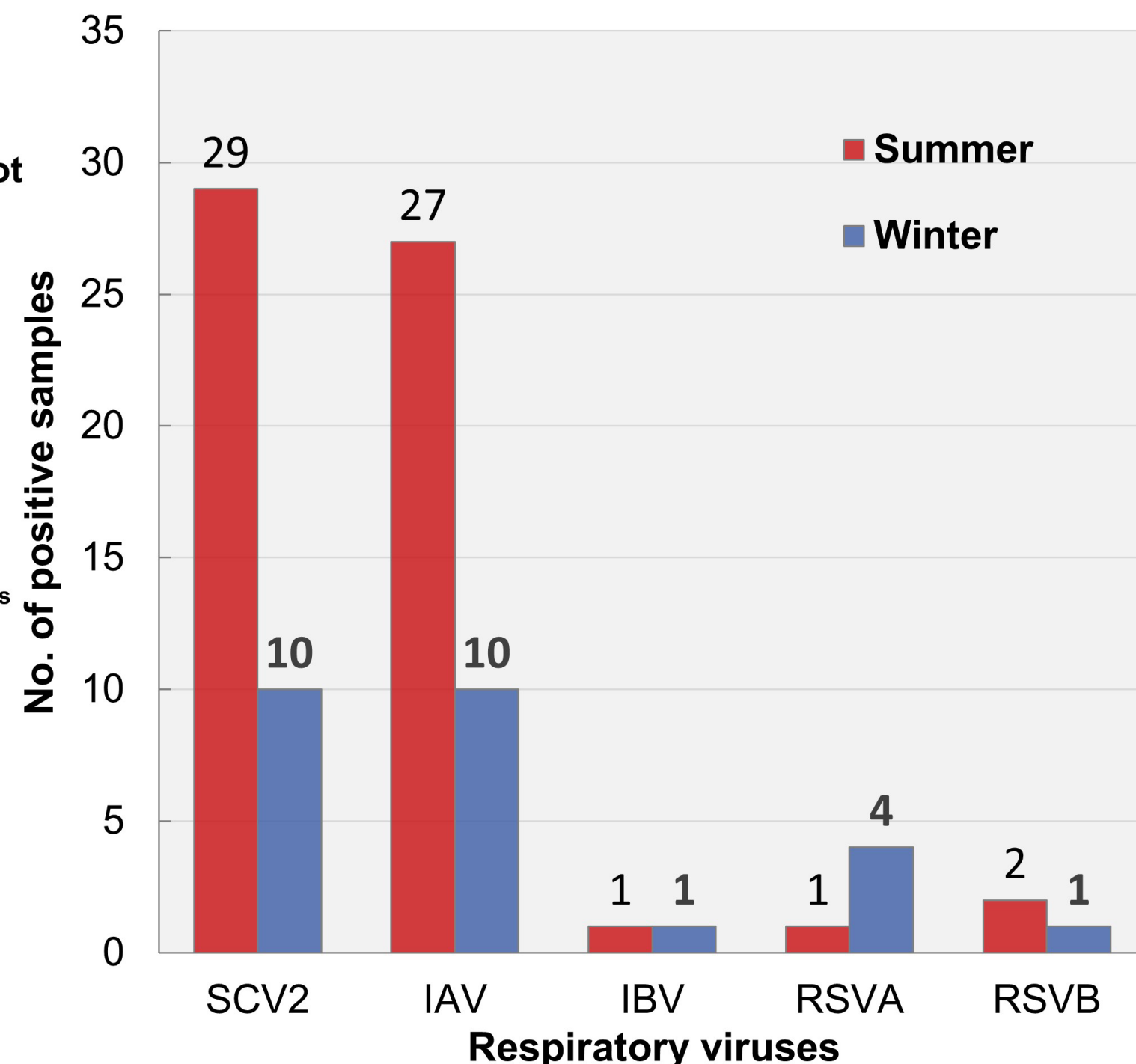
REFERENCES

- Naghavi, M., Vollset, S. E., Ikuta, K. S., Swetschinski, L. R., Gray, A. P., Wool, E. E., ... & Dekker, D. M. (2024). Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. *The Lancet*, 404(10459), 1199–1226.
- Essack, S. Y. (2018). Environment: the neglected component of the One Health triad. *The Lancet Planetary Health*, 2(6), e238–e239.
- Leung, M. H., & Lee, P. K. (2016). The roles of the outdoors and occupants in contributing to a potential pan-microbiome of the built environment: a review. *Microbiome*, 4(1), 21.

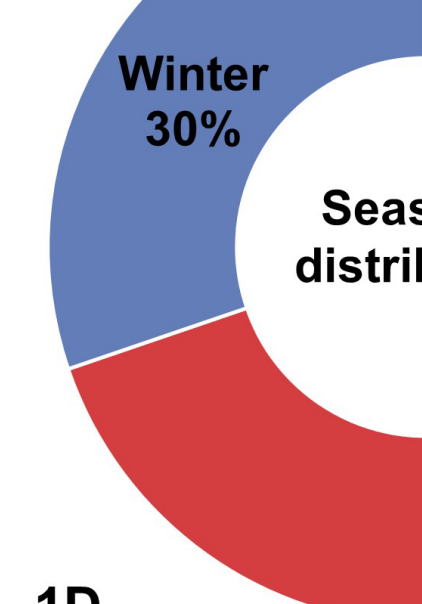
RESULTS

1) First-tier Surveillance: Detection of Five Common Respiratory Viruses in the HK Subway System

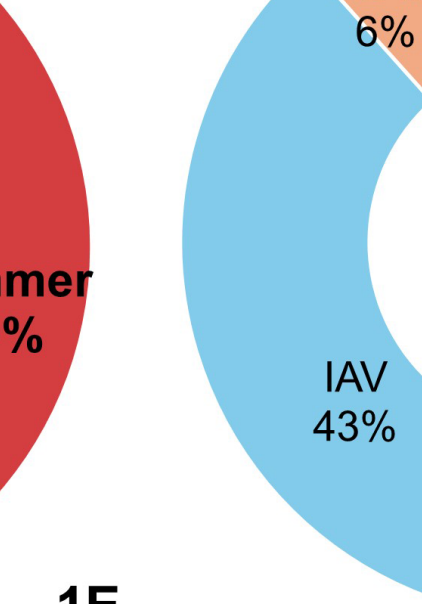
1A. No. of samples detected with respiratory viruses in summer and winter times.



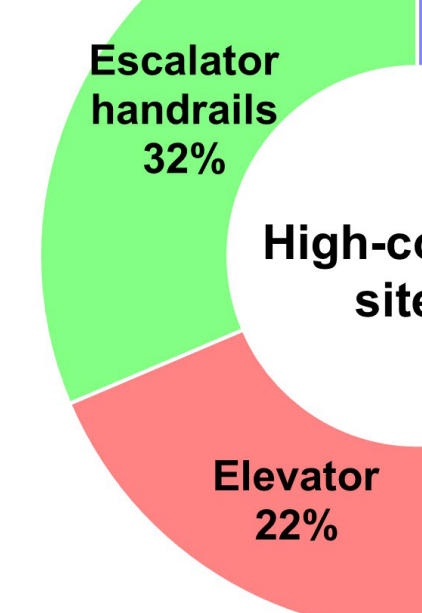
1B. Seasonal distribution



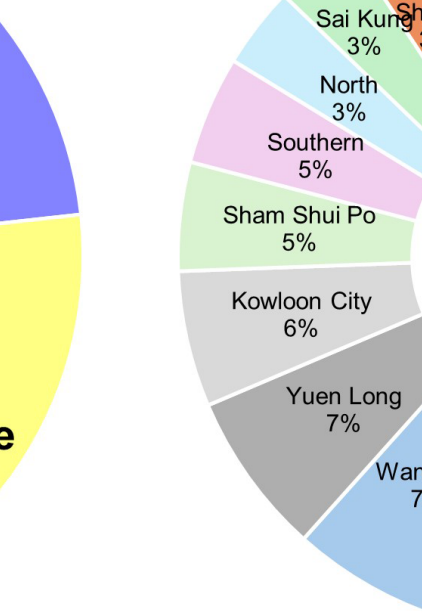
1C. Respiratory virus



1D. High-contact sites



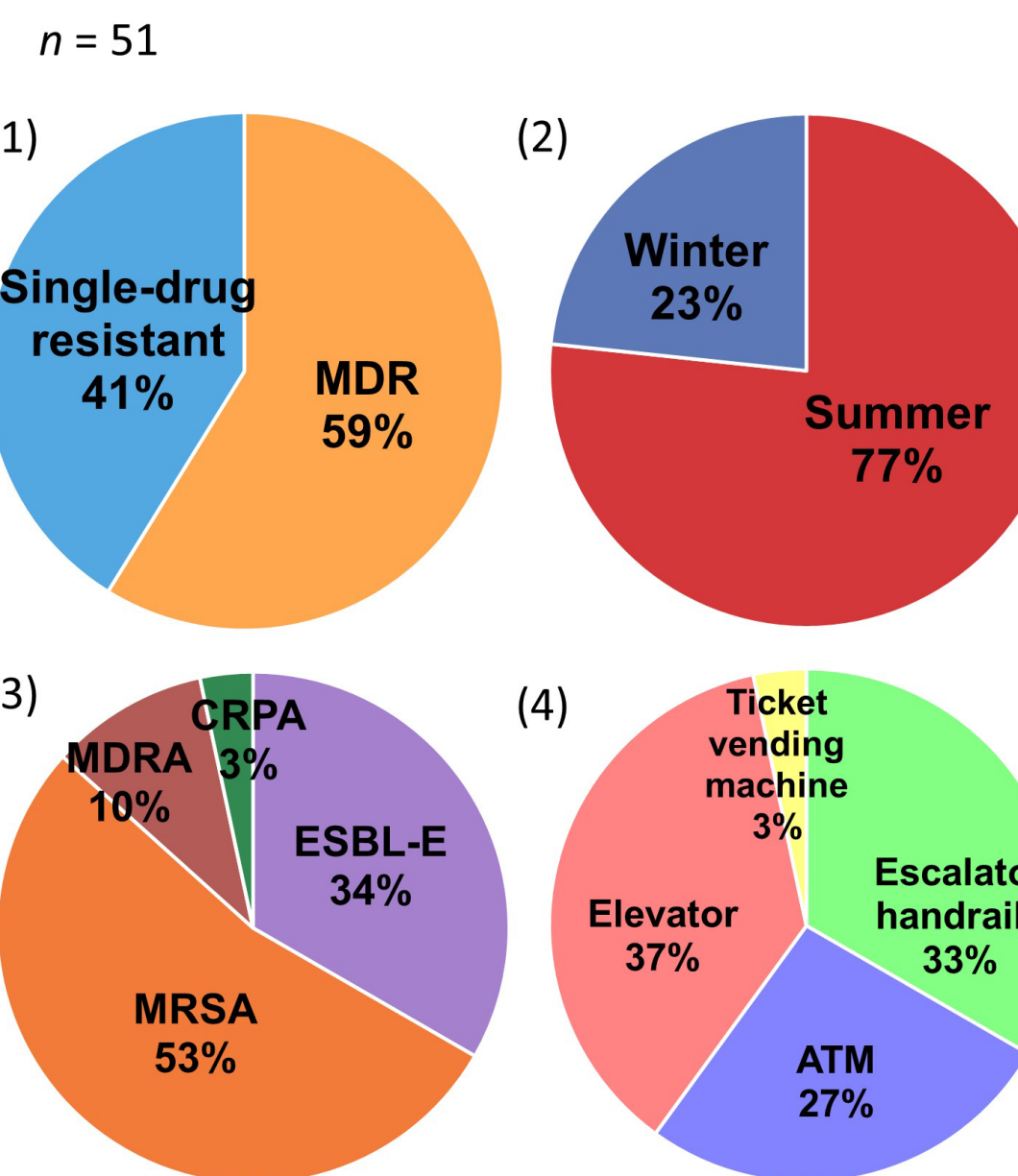
1E. Districts of HK



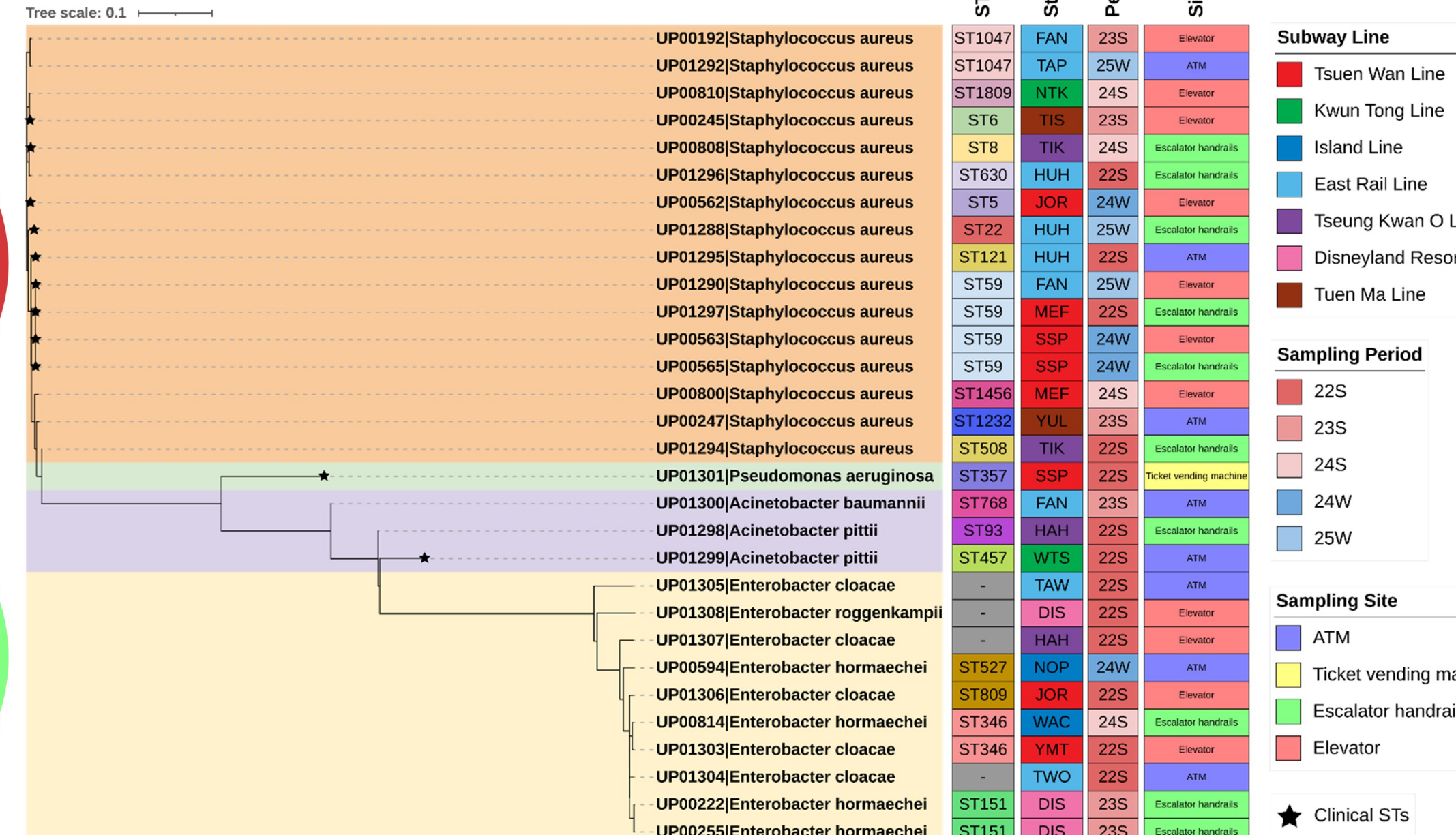
- 26%** surface samples were positive for **≥ 1 respiratory viruses** (Fig. 1A)
- 70%** detections occurred during **summer** times (Fig. 1B)
- SARS-CoV-2** and **Influenza A virus** were the two most prevalent respiratory viruses (Fig. 1C)
- Most viruses were detected from **escalator handrails**, making them a high-risk site for potential transmission (Fig. 1D)
- Tuen Mun district** had the highest number of detections (Fig. 1E)

2) Second-tier Culture-based Methods Identified Clinically Relevant STs and Dominant ARG Class among MDRO Isolates from the HK Subway System

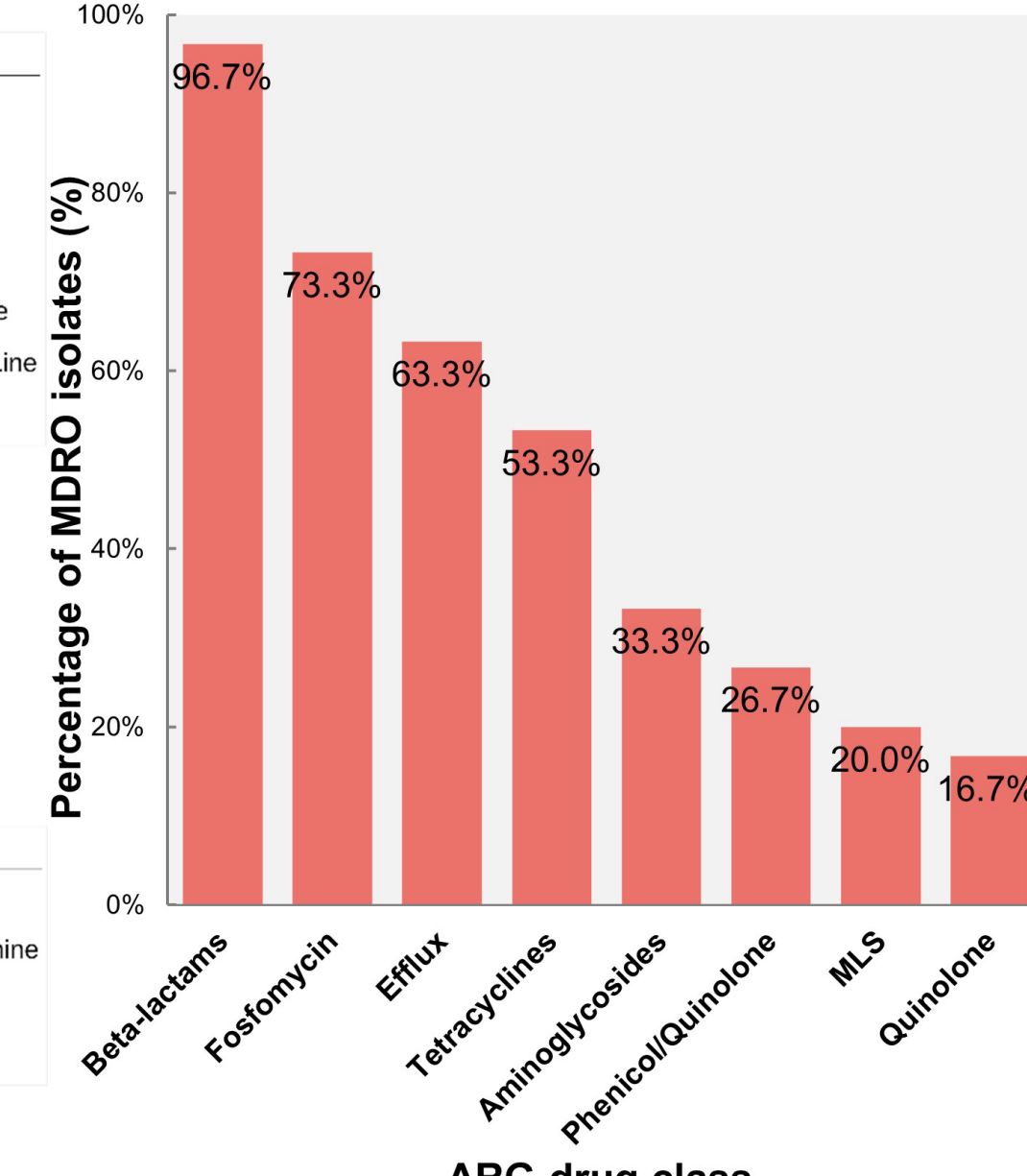
2A. Prevalence and distribution of MDROs in the HK subway system.



2B. Phylogenetic tree of all MDRO isolates recovered from high-contact surfaces of the HK subway system.



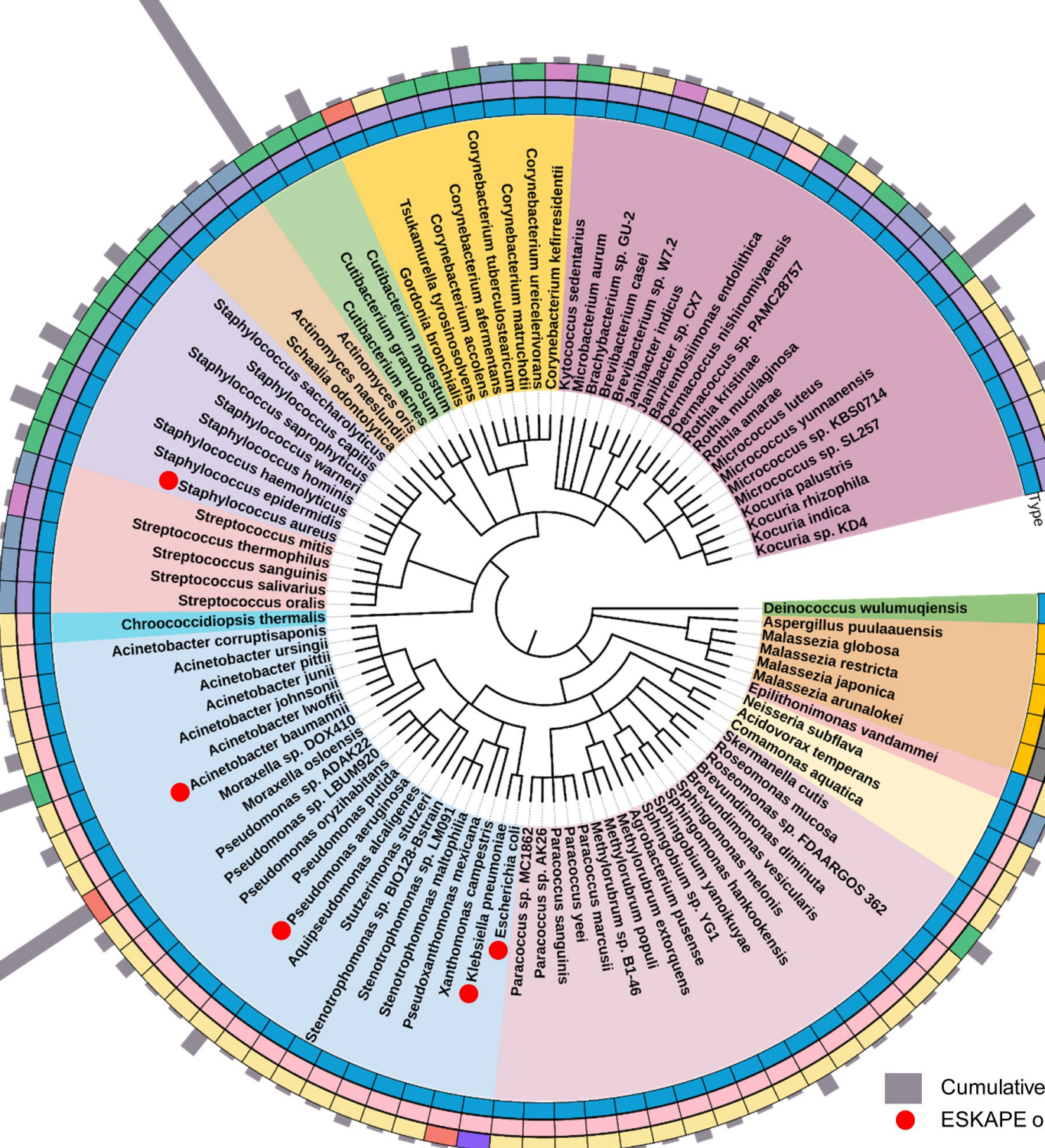
2C. Prevalence of ARG drug class among MDRO isolates.



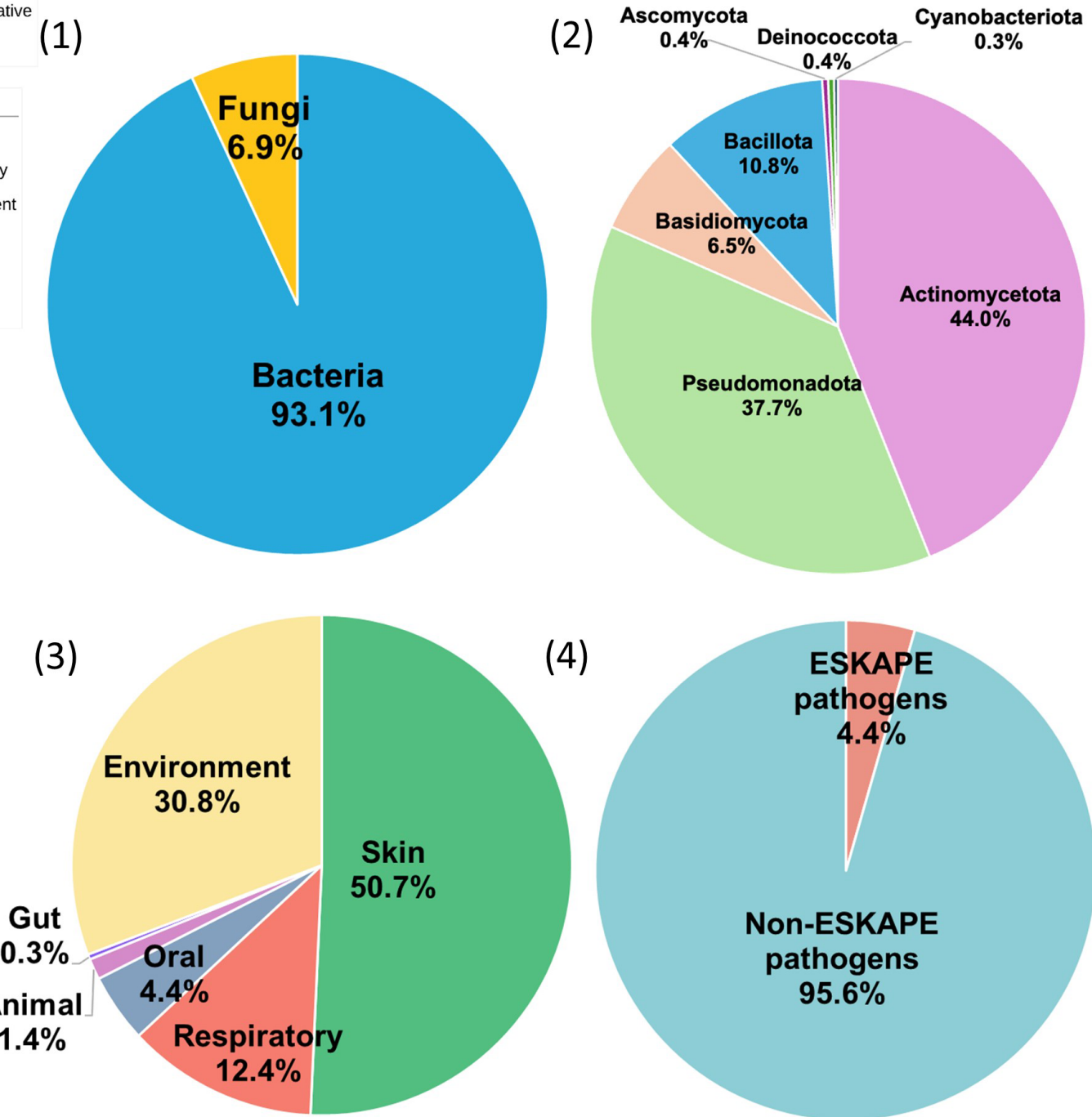
- Conventional culture-based methods recovered **51 viable drug-resistant isolates (7%)** (Fig. 2A.1)
- Most MDR isolates were recovered during **summer** times and **MRSA** was the most prevalent MDRO, comprising 53% ($n = 16$) of isolates (Fig. 2A.2-3)
- Elevators** and **escalator handrails** are potential reservoirs for MDRO (Fig. 2A.4)
- Clinical important STs were detected, including the first report of **MRSA ST8 SCCmecIV** in the HK subway system (Fig. 2B)
- Beta-lactam resistance genes** were the most common ARGs, harbored by **96.7%** of the MDRO isolates (Fig. 2C)

3) Culture-independent Metagenomic Approach Reveals Clinical Taxa and Dominant Antibiotic Resistance Genes

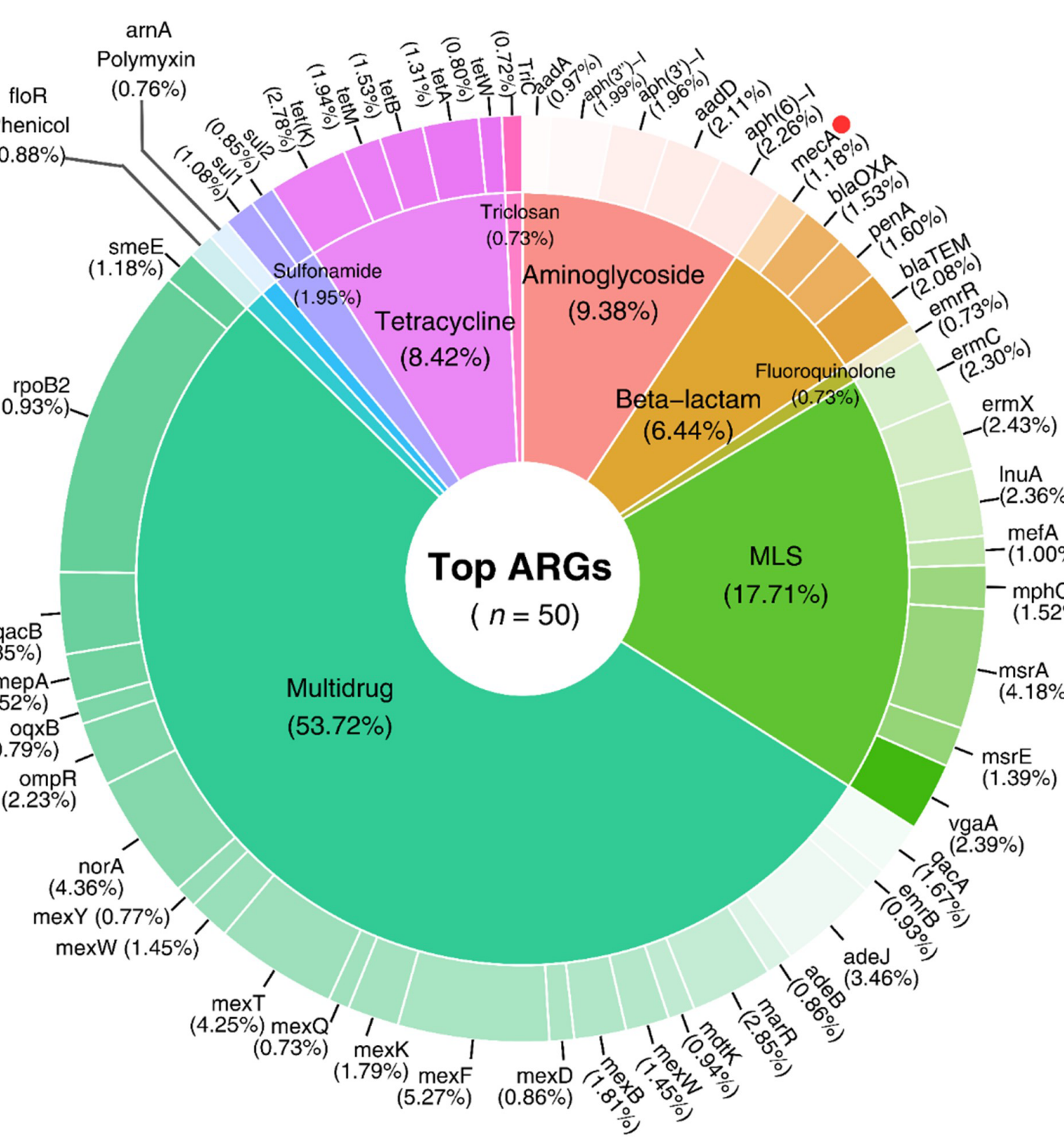
3A. Top 100 dominant species of the HK subway system



3B. Proportions of reads assigned to (1) bacterial and fungal taxa, (2) phyla, (3) sources, and (4) ESKAPE and non-ESKAPE pathogens

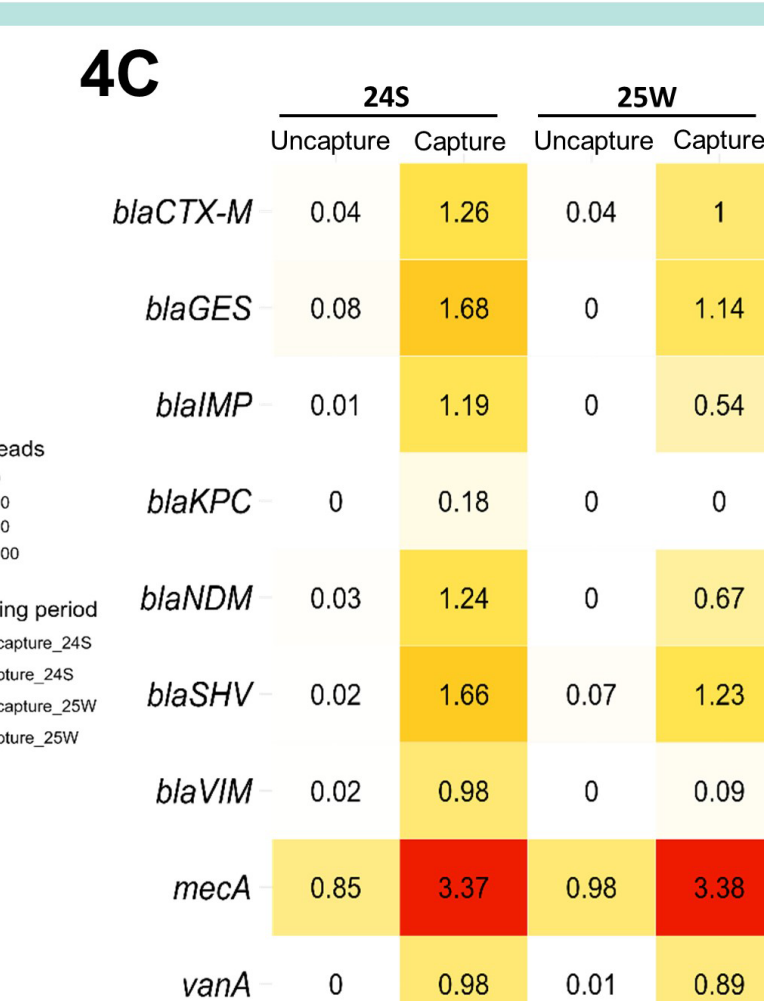
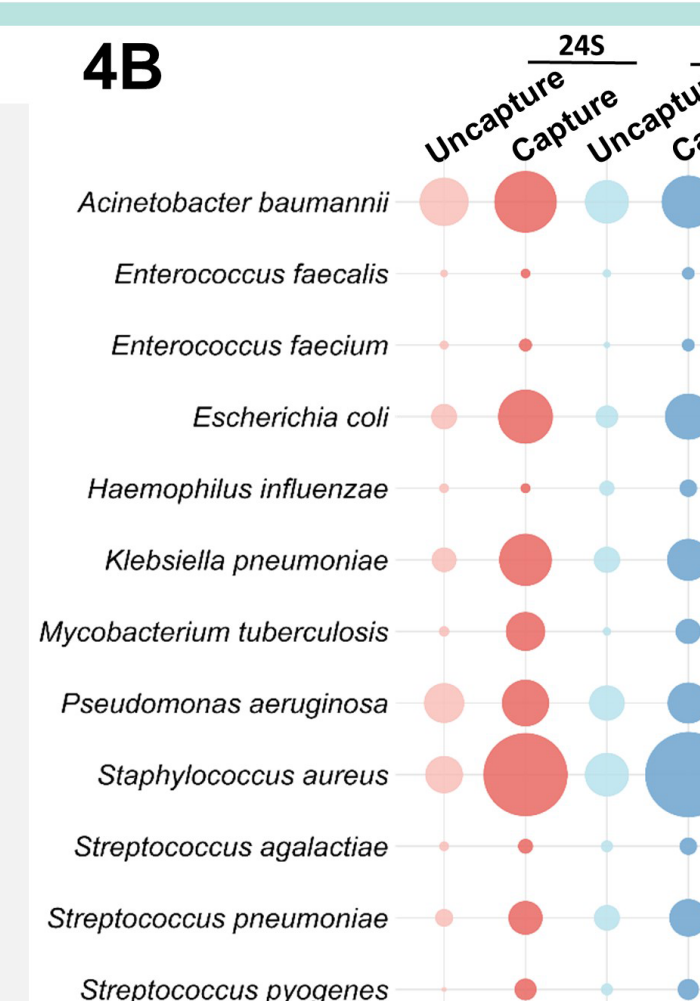
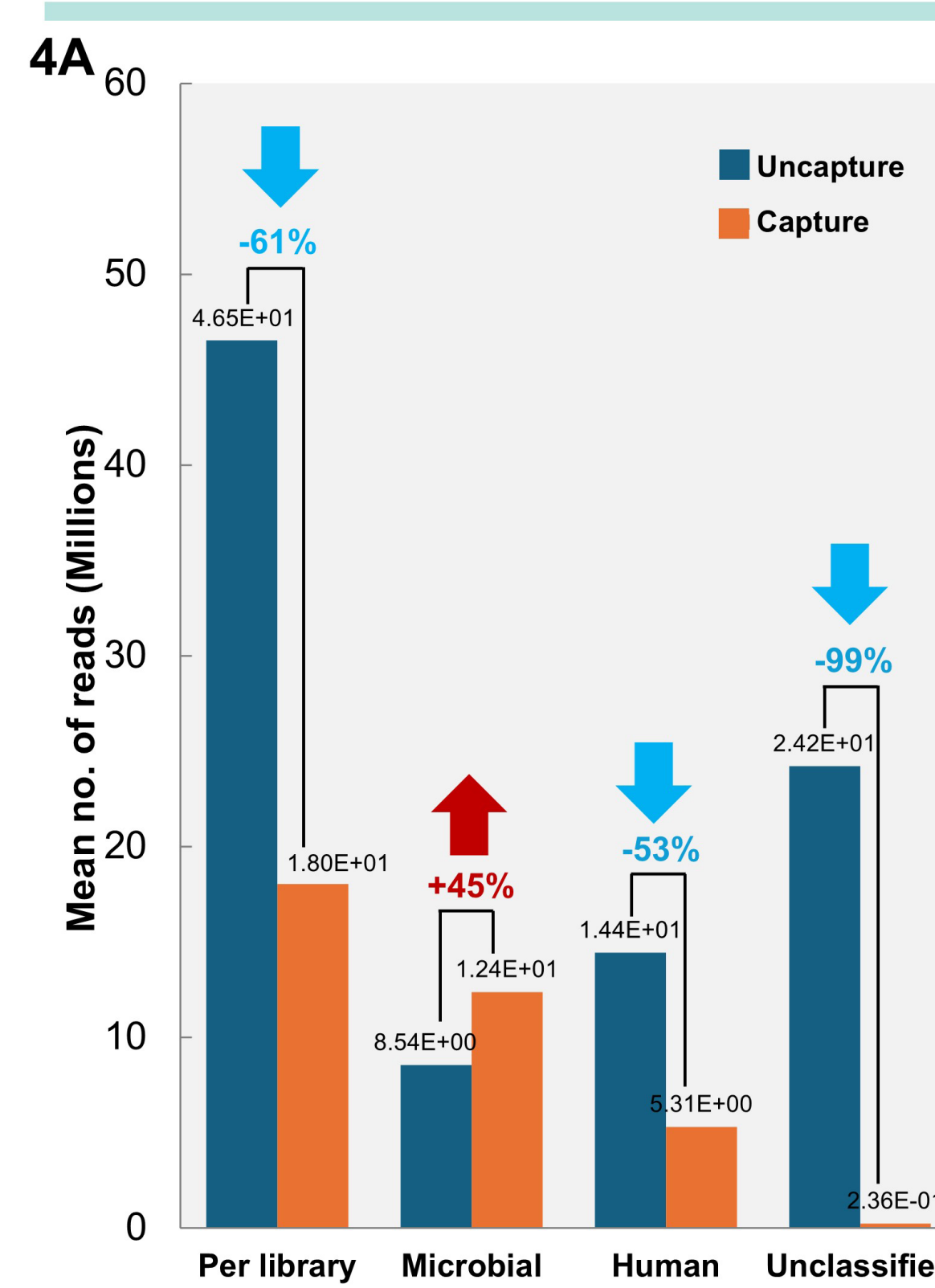


3C. Top 50 dominating ARGs of the HK subway system



- HK subway microbiome is dominated by **bacteria** (Fig. 3B.1)
- Skin** and **environment** are two major source making up the subway microbiome (Fig. 3B.3)
- Five ESKAPE-E organisms were found but at a low abundance (4.4%) (Fig 3B.4)
- ARGs conferring **multidrug resistance (53.72%)** were the most prevalent class (Fig. 3C)
- rpoB2**, conferring rifamycin resistance, is most frequently detected ARG
- mecA** (ranked 33rd), the hallmark of MRSA, was the only gene with high clinical relevance

4) Target Capture Probe Enrichment (TC) Enhances the Detection of Clinically Relevant Microbial Taxa and ARGs



- Proportion of **microbial reads** increased by **45%** after TC was performed (Fig. 4A)
- Proportion of **human and unclassified reads** reduced by **53%** and **99%**, respectively (Fig. 4A)
- No. of reads assigned to clinically important taxa and ARGs increased by **5.3** and **349.1-fold** (Fig. 4B,C)

CONCLUSIONS

- HK subway microbiome is dominated by **bacterial species** of **human skin** and **environmental** origin
- HK subway system is a **low-risk environment** for MDRO infections harboring a **modest AMR burden**
- Three-tiered surveillance** provides a comprehensive and nuanced understanding of the subway microbiome & resistome, which enables **actionable public health insights**
- Incorporation of TC approach into conventional metagenomic workflow enhances the detection of **rare but clinically important targets**