Quantifying Antibiotic Concentrations and Resistance Genes in Carriage Water of Ornamental Fish to Examine the Effect of Local Retail Antibiotic Misuse

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1 INTRODUCTION

The Hong Kong Fish Keeping Context & Retail Problem:

- **Ornamental fishkeeping** is the **third most popular** pet-keeping activity in Hong Kong, driven by cultural traditions and urban space constraints.
- **High-density** retail practices in areas like Mong Kok's Goldfish Market often feature **unhygienic** setups, **stressing fish** and **elevating disease risk**.
- Retailers frequently **misuse antibiotics**, as revealed by a previous study (Au-Yeung et al., 2024), to **reduce losses**, **fostering selection** of bacterial antibiotic resistance genes (**ARGs**) **in carriage water**.

The Regulatory and Research Gap:

- Unlike edible fish aquaculture, the ornamental fish trade is largely **unregulated** with respect to antibiotic use.
- Few studies have concurrently examined residues and ARGs in this context, leaving **household aquarists** who face daily carriage water exposure as an **overlooked at-risk population**.

Aim:

To investigate **antibiotic residues** and the **prevalence** of clinically important **ARGs** in fish retail **carriage water**, evaluating their correlation, co-selective dynamics and public health risk.

Objectives:

- Determine statistical **correlations** between **antibiotic residue** concentrations and **ARG** abundance profiles.
- Evaluate antibiotic-driven **selection pressure** on ARGs in fish shop carriage water.
- Assess the associated **risk** to humans.

2 METHODS

Sampling & pretreatment
- Purchase total 27 samples form Mong Kok and Tseun Wan
- Filter carriage water through 0.22μm membrane
- Filter paper: DNA extraction
- Filtrate: Acidify and spike with antibiotics

Antibiotic Concentration Analysis
SPE-LC-MS/MS analysis 22 target antibiotics from five classes

ARGs Quantifying
qPCR quantification of 15 ARGs and *Intl1* with 7 point std. curve and technical triplicate

3 RESULTS AND DISCUSSION

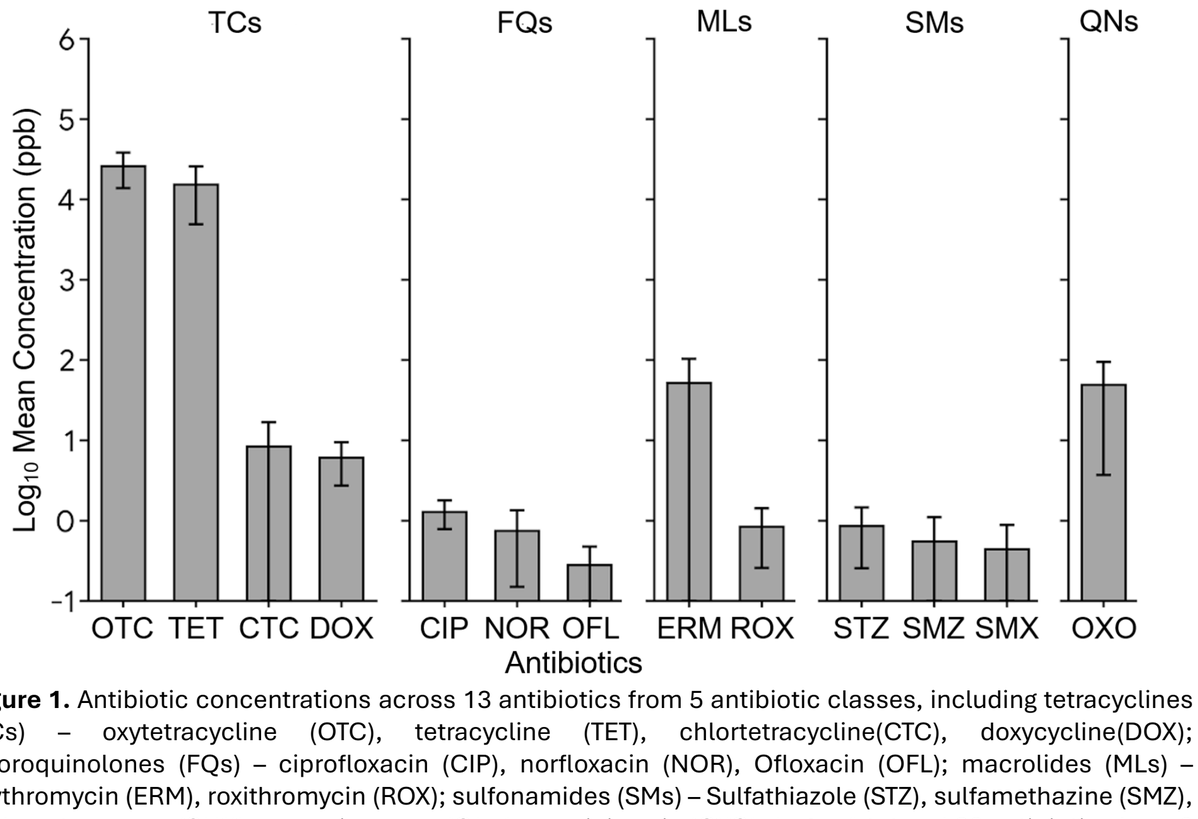


Figure 1. Antibiotic concentrations across 13 antibiotics from 5 antibiotic classes, including tetracyclines (TCs) – oxytetracycline (OTC), tetracycline (TET), chlortetracycline(CTC), doxycycline(DOX); fluoroquinolones (FQs) – ciprofloxacin (CIP), norfloxacin (NOR), Ofloxacin (OFL); macrolides (MLs) – erythromycin (ERM), roxithromycin (ROX); sulfonamides (SMs) – Sulfathiazole (STZ), sulfamethazine (SMZ), sulfamethoxazole (SMX); and quinolones (QNs) – oxolinic acid (OXO). **Note:** A total of 22 antibiotics from 6 classes, including β-lactams (BLs), were tested; those with concentrations below the detection limit are not shown in the figure.

Chemical Residue Profile:

- **In Figure 1**, **TCs dominated** carriage water contamination, with a mean **concentration reaching ppm levels** (OTC and TET), followed by ERM and OXO. β-lactams were below the detection limit.
- High concentrations reflect **heavy, unregulated prophylactic use** of cheap, stable aquaculture **drugs** by retailers.

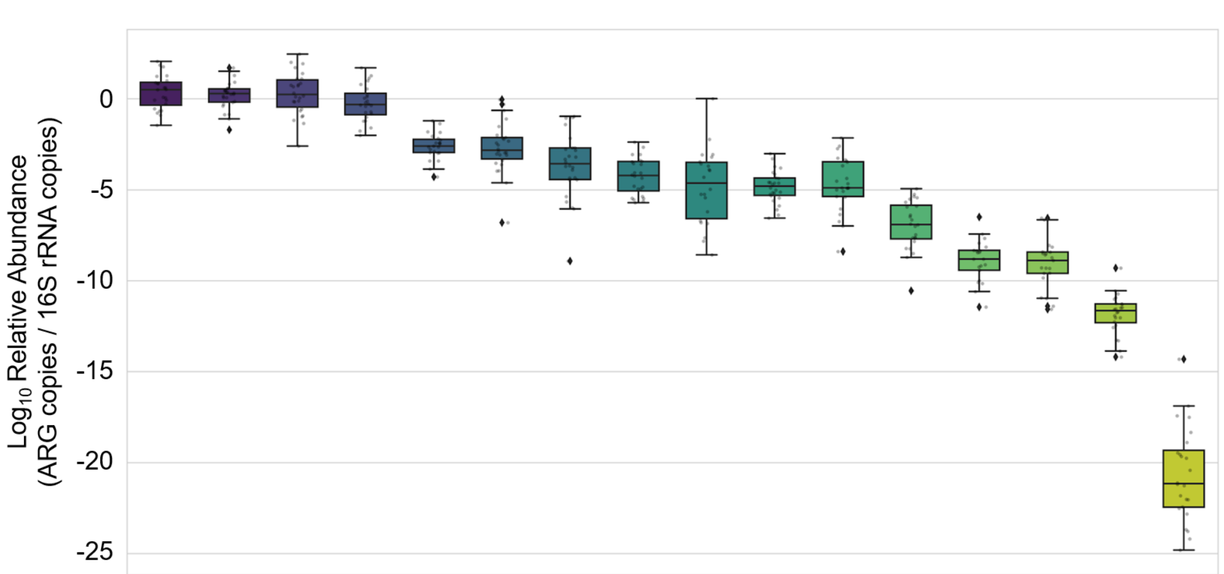


Figure 2. Log_{10} relative abundance of the number of copies of 15 ARG and *intl1* gene normalised per 16s rRNA copies.

Cont. RESULTS AND DISCUSSION

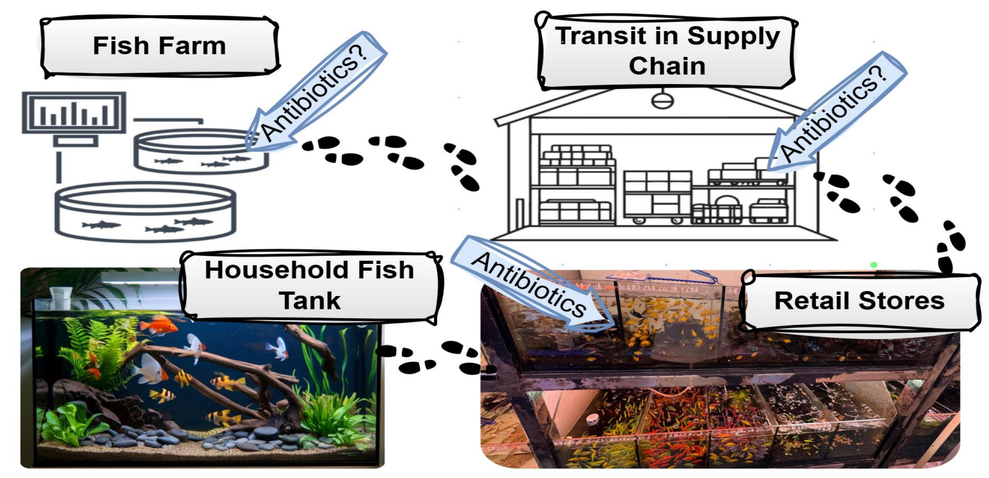


Figure 3. Diagram demonstrating the supply chain of the ornamental fish trade

Resistome Baseline & Transfer Potential:

- **In Figure 2**, SMs (***sul1* and *sul2***) and QNs (***qnrS1***) resistance genes exhibited **high relative abundance**, matching the Class 1 integron (*intl1*), while clinic critical BLs resistance genes (*bla*TEM, *bla*KPC and *bla*NDM) persisted despite being lower than the methods' detection limit.
- This disconnected points to a **pre-existing** effect from **upstream supply chain** (**Figure 3**) where antibiotic misuse selects for ARGs, which are subsequently maintained and spread via *intl1*-mediated horizontal gene transfer in retail and household environments.

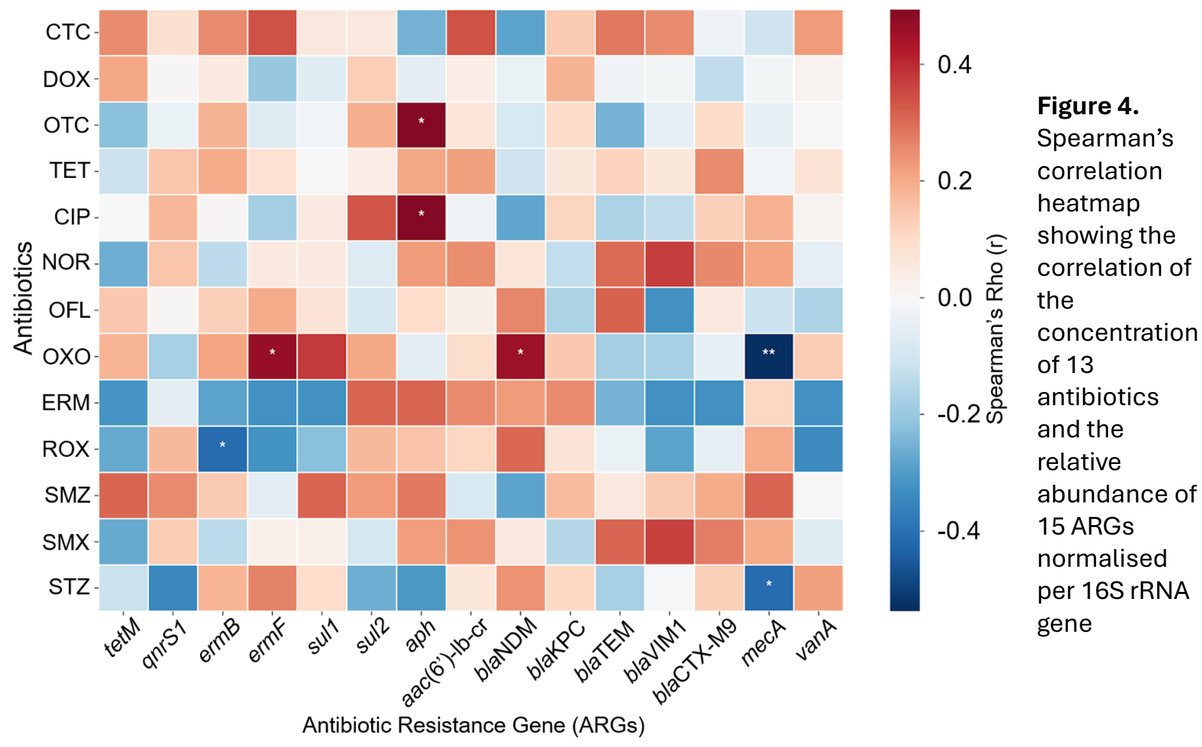


Figure 4. Spearman's correlation heatmap showing the correlation of the concentration of 13 antibiotics and the relative abundance of 15 ARGs normalised per 16S rRNA gene

Evidence of Co-Selection:

- **In Figure 4**, Correlation analysis revealed significant **unmatched positive correlations** ($r > 0.4$, $p < 0.05$) between OXO and *bla*NDM/*ermF*, as well as OTC/CIP with *aph*.
- This provides evidence of **co-selection**; application of **antibiotic** drugs may **increase selective pressure** that **cross-selects** for and maintains **clinical traits** with shared mobile genetic elements.

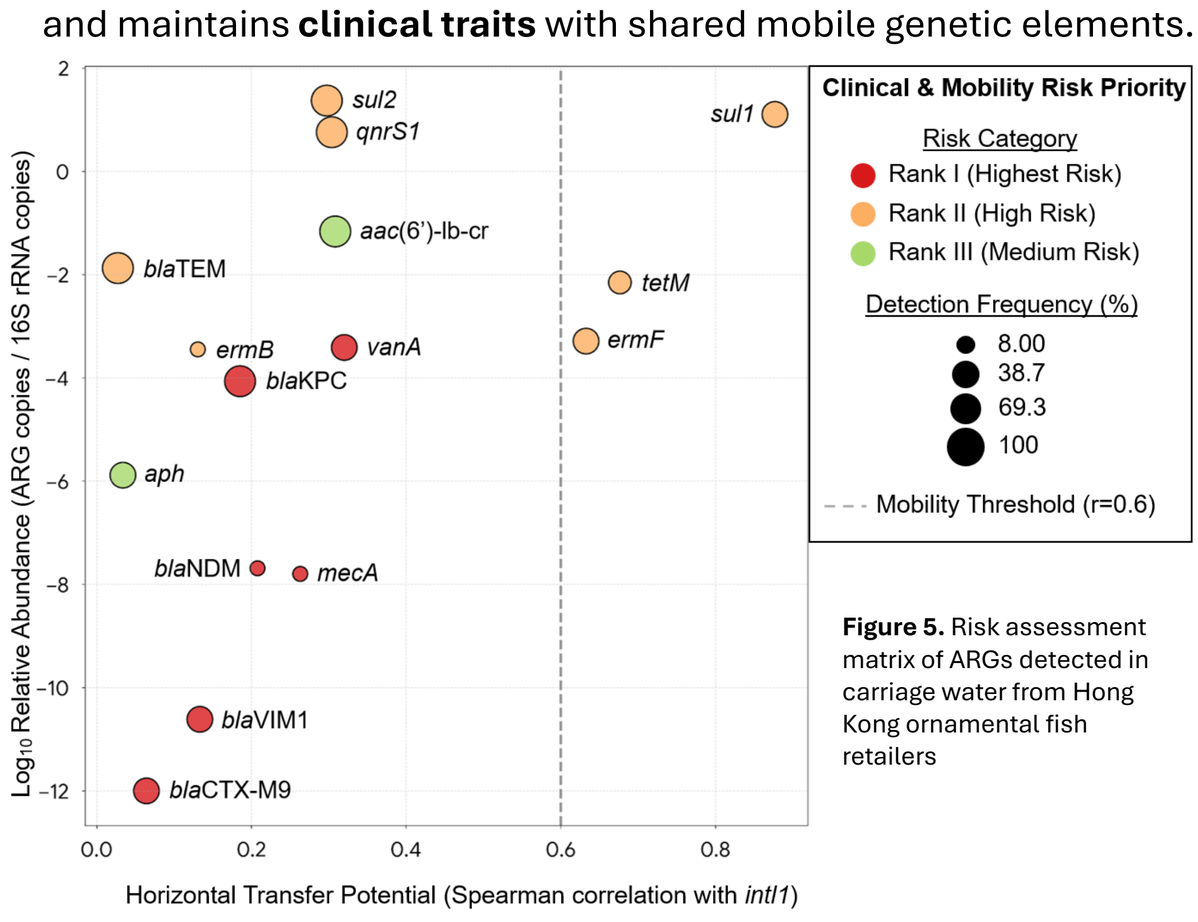


Figure 5. Risk assessment matrix of ARGs detected in carriage water from Hong Kong ornamental fish retailers

Public Health Risk Prioritisation:

- **In Figure 5**, Risk matrix modelling identified ***sul1*** might be the **highest immediate threat** due to extreme mobility potential ($r = 0.88$), while **last-line drug ARGs** *bla*KPC and *vanA*, despite low motility, the high abundance made them a **threat to public health**.
- Widespread and highly mobile ARGs create a permanent bacterial reservoir in the water, **directly exposing** household hobbyists to **dangerous clinical threats** during routine tank maintenance.

4 CONCLUSION

- Disconnects between local antibiotic residues and high ARG abundances, notably for BLs and SMs, demonstrate the **co-selection** potential, and the upstream supply chain may act as the primary source of ARGs, which are then maintained and **spread via *intl1***.

The presence of Rank I ARGs threatening last-line clinical antibiotics highlights an overlooked urban exposure pathway, calling for urgent regulatory oversight and enhanced import surveillance across the ornamental fish trade.

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