Does the Coexistence of Common Antibiotic and Metal Fuel Resistance Genes and Reshape Bacterial Community in Polluted Rivers?

A. Kashyap[1]*, V. Sonkar[1], S. Thatikonda[1]

[1]One Health Research Lab, Environmental Engineering Division, Department of Civil Engineering, IIT Hyderabad, Kandi, Sangareddy, Telangana 502285, India

*corresponding author:
e-mail: shashidhar@ce.iith.ac.in

Abstract. Surface water often receives effluent discharges from WWTPs, and agriculture runoffs reportedly contribute to sulfamethoxazole (SMX) and its resistance in river environment. SMX is commonly found antibiotics in the environment due to its overuse, causing persistent exposure to aquatic bacteria. SMX and Copper (Cu) can attribute to AMR emergence in the sensitive population, potentially inducing the community shift among the existing riverine bacteria. This study investigated the individual and combined effects of SMX and Cu on antibiotic resistance gene pattern and native populations in mesocosm reactors. Experiments were conducted with distinct conditions to monitor the water quality parameters, *uidA*, *sul2*, *intI1*, and bacterial diversity. The findings indicated that *uidA* declined significantly within 24 h in all reactors. Contrastingly, *sul2* and *intI1* did not decline drastically within 24 h, exhibiting a different trend, explaining their persistence up to 168 h. The 16S sequencing data across the reactors revealed the community shifts at genus level within 24 h, from predominance of *Arcobacter* (38%), *Bacteroides* (1.53%), and *Cloacibacterium* (1.12%) to *Arcobacter* (6%), *Bacteroides* (0.15%), and *Cloacibacterium* (0.22%). This study emphasizes that water quality parameters are significant determinants for the prevalence of ARGs in a highly contaminated riverine.

Keywords: Sulfamethoxazole; Copper; Antibiotic resistance genes; Aquatic Mesocosm; Microbial Diversity

1. Introduction. The coexistence of antibiotics such as sulfamethoxazole (SMX) and heavy metals like copper (Cu) in aquatic environments can significantly disrupt indigenous microbial communities and impair ecological functions such as nutrient cycling and organic matter decomposition [1]. The distribution of pathogenic and non-pathogenic bacteria may be differently impacted by such environmental stressors, which can faciliate the growth of resistant bacteria in the microbial community. These shifts are complicated by horizontal gene transfer (HGT), which allows for spreading AMR genes across different microbial taxa, which is not necessarily reflected in taxonomic changes [2]. Further, their dynamics are often associated with environmental variables including pH, temperature, dissolved oxygen (DO), chemical oxygen demand (COD), total organic carbon (TOC), and nutrient availability for the changes in bacterial community structure and regulation of AMR. Higher bacterial diversity inversely correlates with the establishment of antibiotic-resistant bacteria (ARB) and genes (ARGs), limiting HGT and reducing its dissemination potential [3]. Though long-term studies have significantly contributed to our understanding of AMR patterns in aquatic environments, the short-term microbial responses to SMX and Cu remain comparatively underexplored. Most existing research focuses on long-term exposure scenarios, overlooking the immediate shifts that may occur following acute contamination. In order to address these knowledge gaps, this study employs a mesocosm-based approach to explore how short-term exposures to SMX and/or Cu affect AMR gene abundance and bacterial diversity and the influence of water quality parameters on AMR dynamics in water columns. These short-term interactions are critical because they can initiate rapid changes in microbial community structure and transiently amplify ARG abundance even before stable resistance traits are established. Understanding these early-stage temporal dynamics is crucial for developing effective AMR intervention strategies in contaminated aquatic ecosystems. Early responses may serve as indicators of ecological tipping points, reveal vulnerabilities in microbial community resilience, and inform mitigation measures as part of the national action plan.

2. Methodology.

Reagents and Solvents. All chemicals (SMX and Cu) were of high purity (> 98%) analytical grade. Milli-Q water (18.2 MΩ) was used to prepare the stock solutions and stored in -20 °C.

Sample collection. Polluted Water from the downstream of the urban River Musi, Hyderabad, India (Latitude 17°22'56.43"N, Longitude 78°33'27.51"E) was collected 15 cm beneath the water surface in a pre-rinsed tanker during the dry season and transported to mesocosm setup. River sediment was collected from the top 10 cm and wet-sieved to 5 kg and transported to the laboratory in an ice box container.

Mesocosm Design and Monitoring. Cylindrical fiber reinforced plastic tanks (capacity: 0.3 m^3) with a 2 mm polypropylene lining were set up with 12,000 lumens artificial irradiation to mimic the natural sunlight. Six different conditions (**Fig. 1a**) were maintained in reactors with aerators ensuring homogeneity and explained in **Table 1**. Water samples were collected in 2 L HDPE

bottles periodically and their water quality parameters were monitored at 0, 4, 16, 24, 72, and 168 h as per the APHA guidelines, 2017 [4].

Table 1. Mesocosm Reactor Details and Conditons

S. No.	Reactor Annotation	Reactor Condition
1	Tap Water: TW	Tap water
2	Musi River Water: MW	Water from Downstream of Urban Hyderabad, Telangana, India
3	Musi Water with SMX: MW.SMX	SMX (Initial concentration: 6.4 mg/L)
4	Musi River Water with Cu: MW.Cu	Cu (Initial concentration: 4 mg/L)
5	Musi River Water with SMX and Cu: MW.SMX.Cu	Same concentration of SMX and Cu as in Reactor 3 and 4.
6	Musi River Water with Sediment, SMX and Cu: MW.Sed.SMX.Cu	SMX and Cu concentration as in reactor 5 with sediment inside the tank.

Bacterial Enumeration, ARG Quantification, 16S Amplicon Sequencing. *E. coli* was chosen as a marker for fecal contamination. A selective agar plate with and without SMX were utilized for colony counting after 16 h of incubation at 44 ± 2 °C. DNA from water and sediments was extracted and gene targets like *uidA*, *intI1*, *sul2* and *16Sr DNA* were measured using quantitative polymerase chain reaction (CFX Bio-Rad) [4]. 16S rRNA sequencing libraries were prepared on the Illumina MiSeq sequencing platform (Illumina, USA) by amplifying the V3–V4 region using universal primer sets (341F and 806R) from extracted DNA.

3. Result and Discussion. This study found that the anthropogenic pollution marker gene *intI1* and the ARG *sul2* remained persistent for up to 168 h, regardless of the initial conditions in the test reactors (**Fig. 1b**). In contrast, the *uidA* gene declined by 2-fold sharply within 72 h, suggesting that a reduction in COD, likely driven by aeration, may have contributed to decreased fecal contamination. Additionally, the *16Sr DNA* gene declined to 1.5-fold gradually at 168 h, explaining the further decrease in heterotrophic bacteria (**Fig. 1b**). Although the test reactors displayed a similar reduction trend for *sul2*, its decay rates under different initial conditions varied. The reactors containing sole SMX and in combination with Cu demonstrated a slow decay rate for *sul2* (-0.090 – -0.094) compared to the decay rate in MW. In contrast, the test reactor with Cu alone showed a 2-fold increase in decay rate (-0.172), suggesting that the addition of SMX (6.4 mg/L) impacted the persistence of the *sul2*.

16S rRNA sequencing data from the test reactors revealed a shift in the bacterial community from a predominance of pathogenic taxa to non-pathogenic groups (Decomposers and organic degraders). At 0 h, the community was dominated by *Arcobacter* (38%), *Bacteroides* (1.53%), and *Cloacibacterium* (1.12%). By 24 h, their relative abundances had decreased substantially to *Arcobacter* (6%), *Bacteroides* (0.15%), and *Cloacibacterium* (0.22%) (**Fig. 1c**), indicating a drastic shift in community structure during early exposure. Furthermore, redundancy analysis (**Fig. 1d**) was used to evaluate the relationships between water quality parameters (pH, DO, COD, TOC, nitrate, and ammonia) and bacterial genera. Among these, pH and DO were positively associated with decomposer and organic-degrading taxa such as *Novosphingobium* and *Fluviicola*. In contrast, elevated levels of TOC were more strongly linked with potential pathogens, including *Arcobacter* and *Paludibacter*.

4. Conclusion. This study shows that the persistence of AMR genes such as *intI1* and *sul2* under various initial conditions was driven the water quality parameters. However, the variability of their decay rates across treatments suggests complex contaminant interactions that merit further investigation. Futhermore, the short-term exposure to SMX and Cu can drastically change the composition of the microbial community, inhibiting heterotrophic and fecal bacteria while enhancing resistant and non-pathogenic taxa. This mesocosm-based approach limits real-world understanding of AMR dynamics, and the study's short-term focus overlooks long-term AMR persistence. Therefore, addressing one health intersections, encouraging coordinated interventions across the environmental, human, and animal sectors, and incorporating their probable outcomes into national action plans may improve the real time mitigation of AMR.

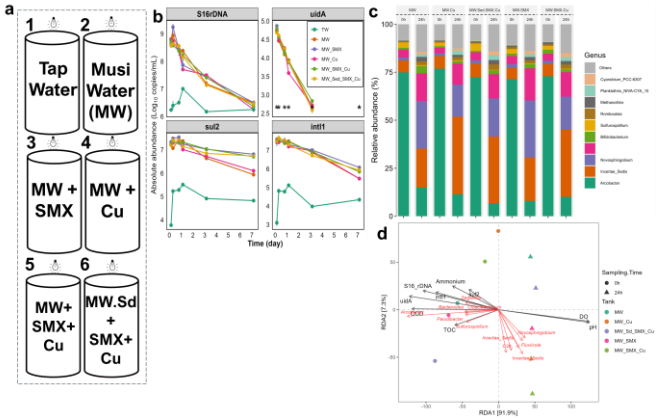

Fig. 1 a) Scheme of each reactor, b) Removal of targeted genes, c) Microbiome shift in reactors, d) Correlation between abiotic and biotic parameters.

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