Single-cell genomic profiling of antimicrobial resistance in *Escherichia coli* from the Densu River, Ghana

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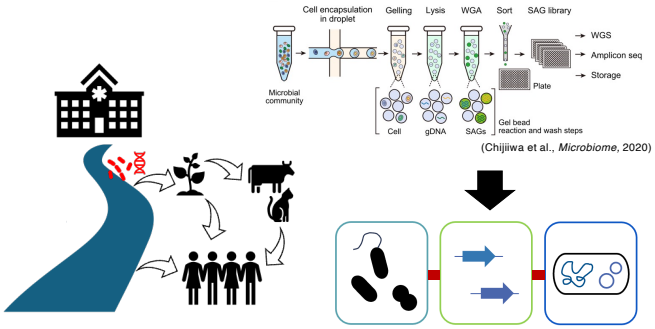
Runa Furuya[1], Isaac Prah[1], Yusuke Ota[1], Samiratu Mahazu[1], Anthony Ablordey [2], Ryoichi Saito[1]

1 Department of Molecular Microbiology and Immunology, Graduate School of Medicine and Dental Science, Institute of Science Tokyo, Japan

2 Department of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon P. O. Box LG 25, Ghana

Introduction

River water serves as a natural reservoir for antimicrobial resistance (AMR) factors. Although environmental AMR poses a global threat to public health as it spreads to local communities through the microbiome in aquatic environments, the actual situation remains clear, especially in developing countries. In this study, we attempted to obtain microbiome data, including AMR information, for multiple bacterial strains from river water samples in Ghana, using a single-cell genomics platform.



(Chijiiwa et al., *Microbiome*, 2020)

Materials and methods

Sample collection → Enrichment → DNA extraction

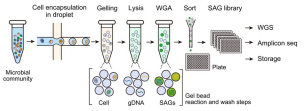
On 6/14/2021 from the Densu River in Ghana

In tryptic soy broth with or without 32 µg/mL ampicillin and 16 µg/mL sulbactam (A/S)

Using Magattract (Quiagen) or NucleoSpin Tissue kit (Takara Bio)

16S rRNA amplicon sequencing → Single-cell genomics

Amplified 16S rRNA V3-V4 region and assessed bacterial composition



Obtained single-cell amplified genomes (SAGs) and QC with CheckM

Sequencing-based analysis

Gene prediction: eggNOG-mapper v2.1.12
Pan-genome analysis: Anvi'o v8
Taxonomic annotation: GTDB-Tk v1.7.0
The ARGs and plasmids: Staramr v0.7.2
Virulence factors: VirulenceFinder 2.0
The average nucleotide identity (ANI) values: FastANI v1.34
A maximum-likelihood phylogenetic tree: kSNP4.1 and iTOL v7

Results

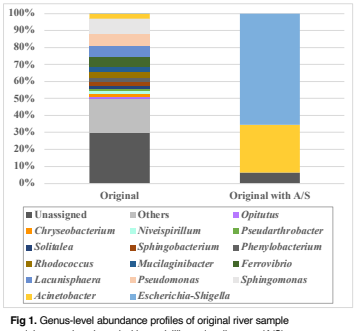


Fig 1. Genus-level abundance profiles of original river sample and the sample selected with ampicillin and sulbactam (A/S).

High prevalence of *E. coli* in the river sample under antimicrobial pressure

We conducted 16S rRNA gene amplicon sequencing to analyze the microbiome of the river sample, with or without antimicrobial selection, to assess the diversity of bacterial species harboring ESBL and carbapenemase genes. The genus *Sphingomonas* was predominant in the original sample. The sample selected with A/S showed 65.4% abundance of the genus *Escherichia-Shigella* and 28.8% of the genus *Acinetobacter* (Fig. 1).

Results

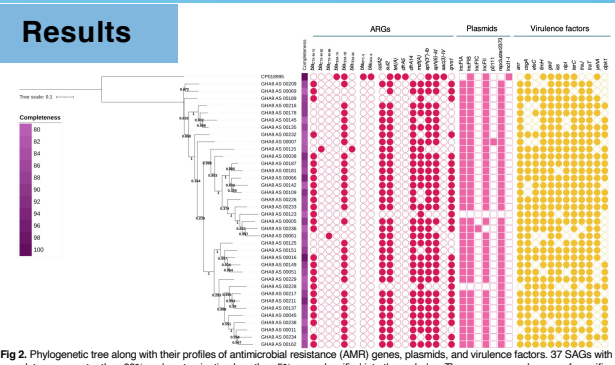


Fig 2. Phylogenetic tree along with their profiles of antimicrobial resistance (AMR) genes, plasmids, and virulence factors. 37 SAGs with completeness greater than 80% and contamination less than 5% were classified into three clades. The presence or absence of specific AMR genes, plasmids, and virulence factors is shown on the right.

Phylogenetic and AMR features of *E. coli* genomes

Using 37 SAGs classified as *E. coli* with completeness greater than 80% and contamination less than 5%, we performed a phylogenetic analysis using an single nucleotide polymorphism (SNP)-based maximum-likelihood method (Fig. 2). Based on the bootstrap values in the tree, the SAGs appeared to be divided into multiple distinct populations. The presence or absence of specific ARGs, plasmids, and virulence factors revealed different gene content profiles for each SAG, although they were all classified as the same species.

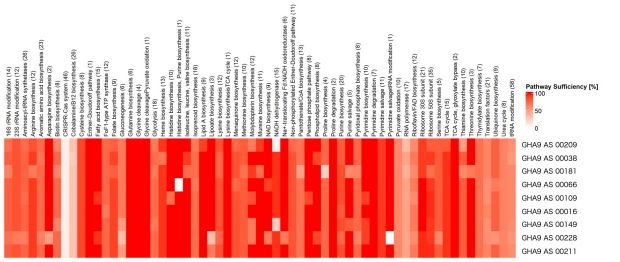


Fig 3. COG pathway sufficiency on 9 SAGs. The heatmap illustrates the percentage of sufficiency for each COG pathway, with darker red shades indicating higher levels of completeness. The numbers in parentheses indicate the number of genes assigned to each respective pathway.

Comparative functional analysis using COG classification

We also analyzed the COG pathways of *E. coli* to determine the functional composition and potential metabolic capabilities of the genomes (Fig. 3). Pathways, such as glycine cleavage and fatty acid biosynthesis, consistently exhibited high sufficiency across most samples, indicating that these core metabolic functions are well conserved. In contrast, pathways, such as NADH dehydrogenase (6.67–93.3%) and molybdopterin biosynthesis (66.7–100%) showed lower and more variable sufficiency levels, indicating potentially specific functional limitations or adaptations. These results highlight the functional heterogeneity among genomes and suggest that while essential metabolic pathways are largely intact, accessory functions related to biosynthesis and horizontal gene transfer may vary significantly between SAGs.

Discussion

This study highlights the critical utility of single-cell genomics in environmental AMR surveillance, particularly in complex and under-monitored ecosystems such as river water in Ghana. Unlike conventional metagenomic approaches, which are often not appropriate for associating AMR genes with specific microbial taxa or genomic contexts, single-cell genomics enables direct linkage of AMR traits to individual microbial genomes. This precision is especially valuable in aquatic environments where microbial communities are highly diverse and many species remain unculturable (Takhampunya et al., 2023). Our findings reveal that even within a single species, *E. coli*, substantial genomic and functional heterogeneity exists, including the presence of globally significant resistance genes, such as $bla_{CTX\text{-}M\text{-}15}$, ESBL variants associated with clinical treatment failure. This indicates that even within *E. coli* strains possessing the same CTX-type genes, genetic diversity exists, which is challenging to detect using conventional culture methods or metagenomic approaches, highlighting the utility of single-cell genomic analysis.

The study had some additional limitations. The number of samples was relatively small, which may limit the generalizability of our findings. In addition, we focused on environmental samples, which prevented us from assessing potential relationships with clinical or animal sources.

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