Investigating the resistome, taxonomic composition, and mobilome of bacterial communities in hospital wastewaters of Metro Manila using a shotgun metagenomics approach

Jiaan Carlo E. Santos[1,5], Daphne Janelyn L. Go[3,5,6], Robert D. Unciano[4,5], Paul K. Yu[8], Angelyn R. Lao[2,5], Ma. Luisa D. Enriquez[1,5,7], Llewelyn M. Espiritu[1,5], and Anish M.S. Shrestha[3,5,6]

[1]Department of Biology, College of Science, De La Salle University, Manila
[2]Department of Mathematics and Statistics, College of Science, De La Salle University, Manila
[3]Department of Software Technology, College of Computer Studies, De La Salle University, Manila
[4]Institute of Arts and Sciences, Far Eastern University, Manila
[5]Systems & Computational Biology Unit, Center for Natural Sciences and Environmental Research, De La Salle University, Manila
[6]Bioinformatics Lab, Advanced Research Institute for Informatics, Computing, and Networking, De La Salle University, Manila
[7]Research & Biotechnology Division, St. Luke's Medical Center
[8]Bioinformatics and Genomics Program, Pennsylvania State University College of Medicine, Hershey, PA 17033, USA

Abstract

We profiled antibiotic resistance genes, antibiotic-resistant bacteria, and mobile genetic elements in untreated hospital wastewater from three tertiary hospitals in Metro Manila using shotgun metagenomic sequencing. The resistome profiles revealed high abundances of resistance genes against sulfonamides (*sul1*, *sul2*), aminoglycosides (*aadS*), and macrolides/streptogramins (*msrE*, *mphE*). To the best of our knowledge, this study provides the first report of antiseptic resistance genes (*qacE*Δ*1*, *qacL*) in hospital wastewater in the Philippines. Resistance genes of critical concern were also detected, including those conferring resistance to β-lactams (bla_{OXA}, bla_{TEM}, bla_{GES}, bla_{NDM}, bla_{KPC}), colistins (*mcr-5*), and tetracyclines (*tet(C)*, *tet(A)*, *tet(L)*, *tet(M)*). Bacterial composition was dominated by the phylum Pseudomonadota, with high abundances of the genera *Stenotrophomonas*, *Rhodococcus*, and *Pseudomonas*, while ESKAPEE pathogens were detected at lower levels. A wide diversity of mobile genetic elements associated with multiple drug classes, including antiseptic and disinfectant resistance genes was observed. Overall, this study demonstrates the utility of shotgun metagenomics for comprehensive profiling of the resistome, bacterial composition, and mobilome in hospital wastewaters and provides supplemental data to support AMR surveillance and mitigation in the Philippine healthcare system.

Keywords: metagenomics, antibiotic resistance, bioinformatics, hospital wastewater, resistome

1 Introduction

Antimicrobial resistance (AMR) is one of the most pressing global health threats. In 2019, an estimated 1.27 million deaths were directly attributed to bacterial AMR and 4.9 million deaths associated with it [2]. It is predicted that the economic burden of AMR-related healthcare costs will reach USD 1 trillion annually by 2050 [34]. The Philippines, a lower-middle-income country (LMIC), is among those disproportionately affected with an estimated 15,700 deaths attributed directly to resistant infections and 56,700 deaths associated with AMR in 2019 [2]. Effective surveillance methods are critical for tracing and understanding AMR risks, including the spread of antibiotic resistance genes (ARGs), discovery of novel resistance mechanisms, and identification of bacterial pathogens carrying these genes. The Philippine Department of Health established the Antimicrobial Resistance Surveillance Program (ARSP) in 1988 to monitor clinically significant antibiotic-resistant bacteria (ARB) from clinical samples. This program has reported an alarming increase in AMR in recent years. In 2023, the program documented rising resistance trends, for example, to carbapenems in *E. coli* and *K. pneumoniae*; to ceftazidime, piperacillin, carbapenems, and possible multidrug-resistance in *P. aeruginosa*; and to ceftazidime, carbapenems, piperacillin, and cotrimoxazole in *A. baumannii* [3].

Laboratories participating in the ARSP utilize culture-dependent methods for antibiotic susceptibility testing on clinically significant species. However, there remains a need for more comprehensive approaches in addressing the full scope of AMR. Shotgun metagenomics provides a powerful approach to analyze the genetic material of microorganisms, including unculturable bacteria, eliminating the biases inherent in culture-dependent methods. This approach enables comprehensive identification and profiling of microorganisms, ARGs, and mobile genetic elements (MGEs) that mediate transfer and spread of resistance genes.

Hospitals are recognized hotspots for AMR due to the frequent use of antibiotics in treating several pathogens. Hospital wastewater (HWW) in particular, contains a variety of waste materials including human waste, medical waste, hazardous chemicals, and antibiotic residues. This environment fosters a high concentration and diversity of ARGs, and bacterial pathogens, enabling mutations and horizontal gene transfer (HGT) between ARBs, making HWW an important medium for capturing a snapshot of AMR activity [92]. Indeed, several studies worldwide have applied shotgun metagenomics for AMR profiling in HWW (see e.g. [37], [55], [61], [47], [77]).

We employed shotgun metagenomics to profile the relative abundances of ARGs, bacterial composition, and MGEs in untreated HWW from three tertiary hospitals in Metro Manila, Philippines. We identified a broad range of ARGs, including those against aminoglycosides, macrolides, β-lactams, tetracyclines, sulfonamides, antiseptics and disinfectants. Notably, this is the first report of antiseptic and disinfectant resistance genes (*qacE*Δ*1* and *qacL*) in HWW in the Philippines. Our findings demonstrate that bacterial composition and ARG profiles in Philippine tertiary hospitals are similar to those observed globally. This research provides valuable data, describing the diversity and prevalence of ARGs and clinically relevant pathogens in healthcare-associated wastewater that inform future strategies for AMR surveillance and mitigation in the Philippines.

Sample ID	Hospital	Date of collection
A1, A2	A	May 2023
A3, A4	A	June 2023
A5, A6	A	July 2023
B1, B2	B	May 2023
B3, B4	B	June 2023
B5, B6	B	July 2023
C1, C2	C	May 2023
C3, C4	C	June 2023
C5, C6	C	July 2023

Table 1: Sample information

2 Methods

2.1 HWW sampling

HWW samples were collected biweekly over a three-month period (May to July 2023) between 8:00 and 11:00 AM from three hospitals (Hospital A, Hospital B, and Hospital C) located in Metro Manila. Untreated HWW samples were obtained from the collection points immediately prior to wastewater treatment, using sterile collection bottles with a total volume of 1000 mL. Samples A1-A6 were collected from Hospital A, from a collection pipe right before the primary collection tank of the treatment facility. Samples B1-B6 were collected from hospital B, from a manhole directly before the primary collection tank of hospital B's treatment facility. Samples C1-C6 were collected from hospital C from a collection chute right before the primary treatment tank of their treatment facility. Sample information is summarized in Table 1.

The surface of the sample bottles was sterilized with ethyl alcohol, placed in a zip bag, labeled, transferred on ice (2–4°C), and processed within 5 hours after collection. The collected HWW samples were subjected to vacuum filtration using 0.22 μm polyethersulfone (PES, Merck Millipore) membrane filters connected to a vacuum filtration assembly (AGAP Laboratories) powered by a portable vacuum pump. For each sample, 100 - 150 mL of HWW was filtered until clogging of the membrane occurred. The used filters were then transferred into 15 mL sterile conical centrifuge tubes (Falcon^{TM}) and stored at 2–4°C until DNA extraction.

2.2 DNA extraction

Bacterial DNA was extracted using the DNeasy® PowerWater® extraction kit (Qiagen, 2020) per the manufacturer's protocol. The quality and quantity of the extracted DNA were evaluated using a NanoDrop 2000c spectrophotometer, yielding >5 ng/μL concentration, purity ratios (A260/280) >1.8, and volumes >10 μL. DNA integrity was checked via electrophoresis (Vivantis) on a 1.2% (w/v) agarose gel run at 100 V for 30 minutes. All samples met the quality control criteria for high-quality DNA extracts. The DNA samples were resuspended in an elution buffer and stored at −80°C prior to sequencing. For sequencing, 20 μL aliquots of each sample were placed in 2 mL sterile microcentrifuge tubes sealed with parafilm, and submitted for sequencing.

2.3 DNA sequencing

A total of 18 DNA extracts were subjected to Nextera XT library preparation and shotgun metagenomic sequencing using the Illumina Novaseq6000 platform at the Macrogen Laboratories, South Korea. Shotgun metagenomic sequencing generated an average of 8.8 Gbp of raw sequence data per sample with a read length of 150 bp paired-end reads.

2.4 Quality control of sequence data

Quality assessment of the raw sequence data was performed using FastQC (v 0.12.1) [FastQC link] and MultiQC (v 1.14) [25]. Trimming of adapter sequences, primers, and low-quality reads was performed using Cutadapt (v 4.2) [48], with parameters provided by the sequencing facility based on the Nextera XT library preparation protocol. The following settings were used: `-a CTGTCTCTTATACACATCT -A CTGTCTCTTATACACATCT` for forward and reverse adapter sequences, respectively, `-O 10` to remove adapters with a minimum overlap of 10 bases, `-m 30` to discard reads shorter than 30 bases, and `-q 20` to trim bases with a quality score below 20.

2.5 ARG identification and quantification

Bowtie2 (v 2.5.1) [39] was used to align the sequencing reads against the Comprehensive Antibiotic Resistance Database (CARD) (v 3.2.6, accessed June 2023) [1]. The alignment was performed using the command `bowtie2 -x CARD-db -1 reads1 -2 reads2 -D 20 -R 3 -N 1 -L 20 -i S,1,0.50`, which corresponds to Bowtie2's very sensitive preset.

For an ARG gene G, its number of copies per genome in a sample [89] was estimated as follows:

$$n_G = \frac{150 \times r_G / l_G}{B \times \text{ average bacterial genome size}}, \tag{1}$$

where r_G is the number of reads mapped to G, l_G is the nucleotide length of G, B is the number of bases in the sample, and 150 refers to the length of the sequence reads. The relative abundance of G in that sample, denoted by a_G, was then computed using:

$$a_G = \frac{n_G}{\sum_H n_H}, \tag{2}$$

where the summation is across all ARG H in the sample.

All resistance genes in the CARD database were clustered into their corresponding drug classes. These drug classes categorize ARGs based on their mechanisms of action and the bacterial pathways they target. The clustering was done using the Antibiotic Resistance Ontology (ARO) numbers associated with each resistance gene. For a sample, the total count of ARGs belonging to a drug class D, denoted by n_D, was calculated using Equation 3.

$$n_D = \sum_{G \in D} n_G \tag{3}$$

The relative abundance of D in that sample, denoted by a_D, was then computed using:

$$a_D = \frac{n_D}{\sum_T n_T} \tag{4}$$

where the summation is across all Drug Classes T in the sample.

2.6 Bacterial Taxonomy Identification and Quantification

Taxonomic identification of bacterial communities was performed with Kraken 2 [84] using Standard Kraken 2 16GB Database. The relative abundances of various microbial genera were estimated using Bracken [44].

2.7 MGE Identification and Quantification

MGEs were identified by aligning the reads to three reference databases: ISFinder database for insertion sequences (IS) [72], PlasmidFinder database for plasmid replicons [15], and INTEGRALL database for integrases [52]. The alignment was performed using Bowtie2 (v2.5.1) with its very sensitive preset: `bowtie2 -x database_index -1 reads1 -2 reads2 -D 20 -R 3 -N 1 -L 20 -i S,1,0.50`, where `database_index` corresponds to the Bowtie2 index generated from the ISFinder, PlasmidFinder, or INTEGRALL databases.

The relative abundance of an IS I in a sample, denoted by a_I was computed using:

$$a_I = \frac{r_I/l_I}{\sum_H r_H/l_H}, \tag{5}$$

where r_I is the number of reads mapped to I, l_I is the nucleotide length of I, and the summation is across all insertion sequences H in the sample.

The relative abundances of plasmid replicons in PlasmidFinder and integrases in INTEGRALL were computed similarly.

3 Results and Discussion

3.1 *sul1*, *sul2*, *aadS*, *msrE*, *mphE*, *qacEΔ1*, and *qacL* were the predominant ARGs across all HWW samples.

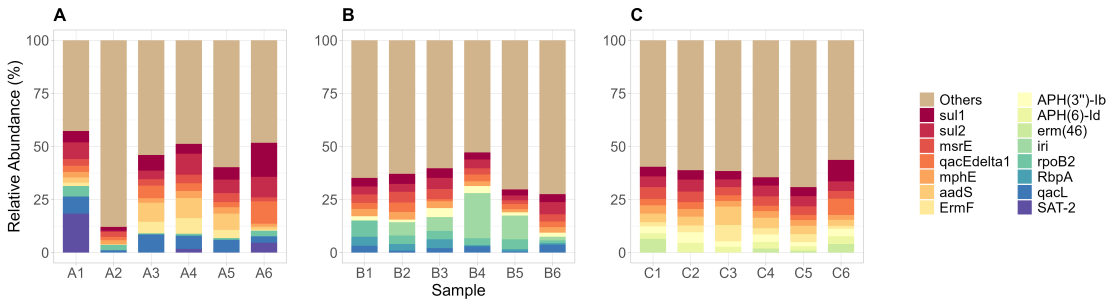

Figure 1: Relative abundances of the top ARGs from Hospital A (A), Hospital B (B), and Hospital C (C). ARGs not part of the top were grouped as "Others."

We observed a wide diversity of ARGs across all samples. Figure 1 shows the relative abundances of the top ARGs found at each hospital. The hospitals exhibited similar sets of high-abundance ARGs, which include *sul1*, *sul2*, *aadS*, *msrE*, *mphE*, *qacEΔ1*, and *qacL*.

Of these, *sul1* and *sul2* confer sulfonamide resistance by encoding alternative dihydropteroate synthase (DHPS) enzymes that reduce antibiotic efficacy [80]. These plasmid-mediated genes are

frequently identified in clinical isolates belonging to the genera *Acinetobacter*, *Escherichia*, *Klebsiella*, *Stenotrophomonas*, and *Staphylococcus* [1]. The prevalence of *sul* genes has been documented globally, including in LMICs such as South Africa and India, where they are predominant in HWW [77, 22].

The streptomycin resistance gene *aadS* encodes an aminoglycoside acetyltransferase that inactivates aminoglycoside antibiotics [75, 1]. This gene is typically phenotypically silent in wild-type *Bacteroides*, with expression induced only by a chromosomal mutation [73]. Associated with the phylum Bacteroidota, *aadS* is commonly found on IS and transposons, indicating its potential role in HGT [79, 63]. Its presence has been documented in wastewater systems, including untreated HWWs from South Korea, Mexico, and several member states of the European Union [67, 28, 64].

The plasmid-associated *msrE* and *mphE* encode an ABC-F subfamily protein and a macrolide phosphotransferase respectively, which protect against and inactivate streptogramin and macrolide antibiotics. Studies have reported that both genes confer higher levels of resistance against second-generation macrolides compared to first-generation macrolides, underscoring the potential influence of increased clinical use of second-generation macrolides in recent years [24, 16]. Both ARGs are frequently associated with *Acinetobacter*, *Pseudomonas*, *Escherichia*, and *Klebsiella* among others [8, 1]. The prevalence of both genes has been documented in HWWs across various countries, including the Czech Republic and Scotland (UK) [37, 61]

The *qacEΔ1* and *qacL* genes confer resistance to quaternary ammonium compounds (QAC) widely used as surfactants, antimicrobials in disinfectants, and cleaning products[5]. Both genes have been mainly associated with *Acinetobacter*, *Klebsiella*, *Pseudomonas*, and *Stenotrophomonas* among others [1, 38]. Both ARGs are typically plasmid-encoded, enhancing their potential for HGT and environmental dissemination [43]. Their presence has been documented in HWWs from several countries, including China, Israel, and South Africa [82, 88, 62, 22].

3.2 Aminoglycoside, macrolide, β-lactam, tetracycline, and sulfonamide-associated ARGs had the highest combined relative abundances.

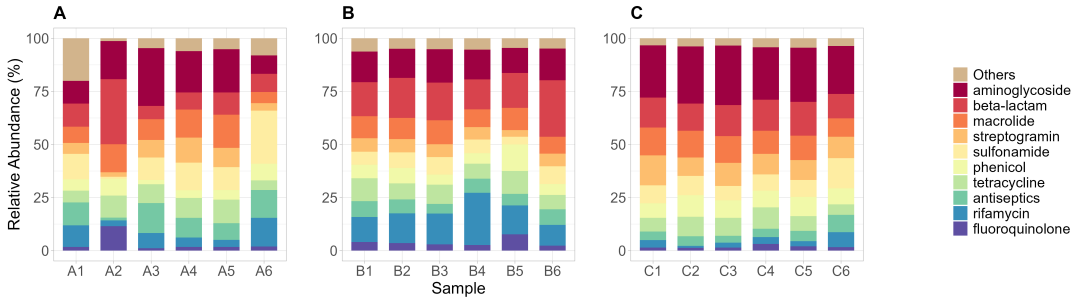

Figure 2: Top 10 drug classes based on combined relative abundances of associated ARGs identified in Hospital A (A), Hospital B (B), and Hospital C (C). Drug classes not part of the top 10 were grouped as "Others."

We consolidated the relative abundances of the ARGs based on the drug classes they are reported to be associated with in the CARD database, as shown in Equation 4. Figure 2 shows the top 10 drug classes with the consolidated abundances. The most abundant consolidated drug classes include aminoglycosides, macrolides, β-lactams, tetracyclines, and sulfonamides.

Among these, aminoglycosides are commonly used to treat severe Gram-negative bacterial infections, acting by binding to the 30S ribosomal subunit and inhibiting protein synthesis. Resistance is often mediated by ARGs encoding aminoglycoside-modifying enzymes (AMEs), including *aph(3")-Ib, aph(6)-Id, aac(6')-Ib7, aac(6')-IIa,* and *aac(6')-II* which were observed in our samples. These ARGs are plasmid-mediated increasing the likelihood of HGT across bacterial species. [83, 81, 42, 29]. The WHO classifies aminoglycosides as Critically Important Antimicrobials, highlighting the urgent need to preserve their clinical efficacy and prevent the potential spread of resistance [85].

Macrolides are commonly used to treat pneumonia and manage cystic fibrosis infections caused by multidrug-resistant, hospital-acquired *Pseudomonas aeruginosa* [31]. Multiple ARGs conferring resistance to macrolides were detected in our samples, including efflux pumps genes (*smeE*, *mefC*, *axyY*, *mexK*, *smeD*), ribosomal target modification genes (*erm(46)*), and antibiotic inactivation genes (*mphG*, *ereD*, *eraA2*, *mphF*). The widespread use of macrolides in both clinical and agricultural settings has contributed to the increasing prevalence of resistance among pathogenic organisms [57, 40, 33]. Like aminoglycosides, macrolides are classified by the WHO as Critically Important Antimicrobials. [85].

β-lactam antibiotics are among the most widely used agents to combat a broad spectrum of Gram-positive and Gram-negative bacterial infections. They act by inhibiting cell wall synthesis through binding to essential penicillin-binding proteins[14]. We detected bla_{OXA}, bla_{GES}, bla_{TEM}, bla_{KPC}, and bla_{NDM} resistance genes in our samples. β-lactams, particularly 3rd- and 4th-generation cephalosporins, are classified as Highest Priority Critically Important Antimicrobials, as their proper use is essential to preserve effectiveness against the increasing threat of multidrug-resistant *Enterobacterales* worldwide [85].

Tetracyclines are broad-spectrum antibiotics that are commonly used to treat and manage various infections. They act by inhibiting the 30S ribosomal subunit, thereby halting bacterial growth and replication [17]. We identified several tetracycline resistance genes, including flavin-dependent monooxygenase (*tet*(X)), major facilitator superfamily efflux pump genes (*tet*(G), *tet*(C), *tet*(39), *tet*(A), *tet*(L)), and ribosomal protection proteins genes (*tet*(M), *tet*(O), *tet*(Q), *tet*(W)). The WHO classifies tetracyclines as Highly Important Antimicrobials, emphasizing the need for continuous surveillance to preserve their clinical efficacy [85].

Sulfonamides are used to treat a range of bacterial infections, including urinary tract infections, respiratory diseases, and opportunistic infections in immunocompromised patients[58]. They act as competitive inhibitors of DHPS in the bacterial folate synthesis pathway, thereby inhibiting their growth and development [80]. Specifically, the ARGs (*sul1*, *sul2*, and *sul4*) were detected in our samples.

3.3 ARGs of critical concern were observed in all HWW samples.

Previous studies have classified certain ARGs as being of "critical concern" based on their abundance, mobility, and host pathogenicity [90, 71]. Multiple ARGs of critical concern were identified across the three hospitals as shown in Figure 3. ARGs of "critical concern" are further classified into 4 categories (Highest Priority, High Priority, Highly Important, or Important) reflecting their clinical relevance and alignment with the WHO list of medically important antimicrobials for human medicine [85, 90, 71].

Multiple β-lactam resistance genes classified as Highest Priority ARGs were detected across all hospitals. The presence of these ARGs may be attributed to the frequent clinical use of β-lactam antibiotics, which corresponds to the increasing resistance rates among Gram-negative bacilli isolates from Philippine hospitals [3]. National surveillance studies have frequently reported β-lactam resistance genes such as bla_{AIM}, bla_{OXA}, and bla_{TEM}, whereas bla_{NDM} and bla_{KPC} have been detected

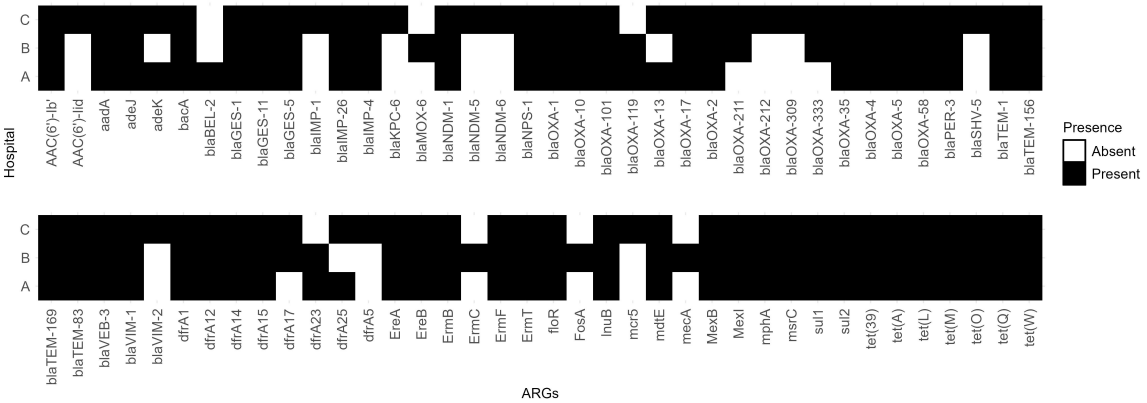

Figure 3: ARGs of critical concern. Black boxes indicate the presence of ARGs for each hospital A, B, and C.

in a few clinical isolates [3]. Of concern, bla_{NDM}, a carbapenemase that confers resistance to most β-lactam antibiotics, was first identified in India and has since spread globally [26]. Similarly, bla_{KPC}, first identified from a *K. pneumoniae* isolate in the United States, has been documented in multiple countries including France and Israel [10, 53, 54]. The detection of both bla_{NDM} and bla_{KPC} in our samples raises concern regarding the transmission of these clinically significant ARGs and warrants further investigation at the national level.

The mobile colistin resistance gene (*mcr-5*) was also identified in our samples. Colistin serves as a last-resort treatment against multidrug-resistant Gram-negative infections and is classified as a Highest Priority Critically Important Antimicrobial, highlighting the need to preserve its clinical effectivity [76, 85]. Although clinical studies in the Philippines have primarily reported *mcr-1*, the detection of *mcr-5* in our study is noteworthy due to its higher resistance activity and greater potential for HGT, underscoring the need for continued surveillance of this resistance gene [91, 3].

Tetracycline ARGs, classified as Highly Important, were detected across all hospitals. We identified genes encoding major facilitator superfamily antibiotic efflux pumps (*tet(39)*, *tet(A)*, *tet(L)*) and tetracycline-resistant ribosomal protection proteins (*tet(M)*, *tet(O)*, *tet(Q)*, *tet(W)*). Previous studies in the Philippines have reported the prevalence of ARGs *tet(A)*, *tet(C)*, *tet(M)*, and *tet(L)* in *E. coli* and *P. aeruginosa* isolates, highlighting the similarities of our results with national surveillance efforts [3, 69].

3.4 Antiseptic and disinfectant resistance genes (*qacEΔ1* and *qacL*) were identified for the first time in Philippine HWW samples.

To the best of our knowledge, this is the first report of *qacE*Δ1 and *qacL* genes in HWW from the Philippines. Both genes confer resistance to QACs, which are commonly used as disinfectants and cleaning agents[5]. The increased use of QAC-containing products during the COVID-19 pandemic has resulted in greater release of these compounds into the environment [93, 9]. The extensive use of QAC-based disinfectants in hospitals during the pandemic may have contributed to the high abundance of *qacEΔ1* and *qacL* resistance genes in our samples.

The presence of these genes in hospital wastewater has also been documented in China, Israel, Australia, and South Africa [82, 88, 62, 22, 70]. The detection of antiseptic and disinfectant resistance

genes in our samples underscores the need for further investigation in the Philippines to evaluate the potential risks of antiseptic resistance in both clinical and environmental contexts.

3.5 Phyla Pseudomonadota, Actinomycetota, and Bacteroidota dominated the HWW microbial community.

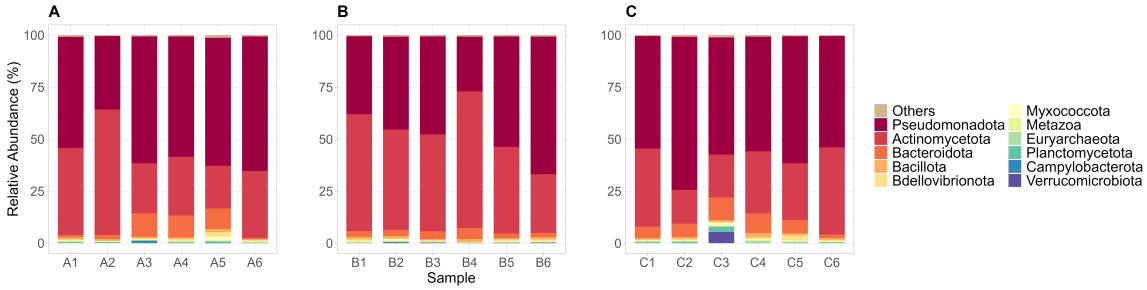

Figure 4: Relative abundance of the top phyla from Hospital A (A), Hospital B (B), and Hospital C (C). Phyla not part of the top were grouped as "Others."

We assessed the bacterial taxonomic composition in our samples. Pseudomonadota (synonym Proteobacteria), Actinomycetota (synonym Actinobacteria), and Bacteroidota (synonym Bacteroidetes) were the most abundant phyla in all HWW samples (Figure 4). These were also reported to be most abundant in HWW samples from other LMICs such as Burkina Faso, Benin, and India [47, 77], highlighting similarities in phylum-level bacterial composition to our HWW samples. Members of Actinomycetota and Bacteroidota are known to be the major constituents of the human gut microbiome and are present in wastewaters [4, 55]. Members of Pseudomonadota are known human pathogens harboring various resistance genes against aminoglycosides, sulfonamides, β-lactams, and tetracyclines [56, 68, 51]. Under the phylum Pseudomonadota, ESKAPEE pathogens were detected at relatively lower abundances in our samples. These pathogens are responsible for a wide range of human infections and include strains that exhibit resistance to most antibiotics, prompting their classification as "high-priority pathogens" by the WHO [86, 21, 23, 59, 50].

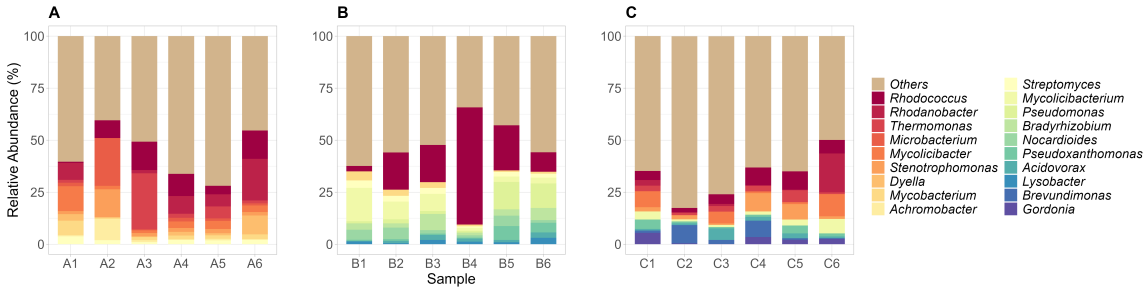

Figure 5: Relative abundance of the top genera from Hospital A (A), Hospital B (B), and Hospital C (C). Genera not part of the top were grouped as "Others."

Among the genera under these three phyla, *S.*, *Rhodococcus*, and *Pseudomonas* were consistently among the most abundant across all samples (Figure 5). *Stenotrophomonas* are opportunistic pathogens commonly infecting humans, with known multidrug-resistant species such as

Stenotrophomonas maltophilia [30, 20, 12, 11]. This genus has been documented in various hospital plumbing systems, including sinks, showers, and toilets, which likely contributes to their presence in HWWs [87]. Similarly, reports in India, Singapore and the UK have also identified the genus in HWW [77, 55, 18]. *Rhodococcus* is typically found in soil, water, and animal hosts with a relatively low prevalence in hospital environments. Interestingly, in our study this genus was detected at high abundance in HWW samples. Previous reports of human infections caused by several *Rhodococcus* species indicate their increasing clinical relevance and potential establishment within hospital settings [6, 13, 19]. Similarly, a study in China has detected the presence of *Rhodococcus* in HWWs [94], while other studies have identified them from patient samples [66, 78]. *Pseudomonas* are clinically significant opportunistic pathogens capable of causing a wide range of human infections in hospital settings. They are frequently reported to exhibit resistance to most antibiotics, especially against β-lactams. [23, 21].

Other highly abundant genera included *Achromobacter*, *Brecundimonas*, *Acidovorax*, *Microbacterium*, *Mycolicibacterium*, *Nocardoides*, *Rhodanobacter*, and *Thermomonas*. These genera have been previously described as components of HWW bacterial communities that shape the HWW environment and contribute to the removal of organic and inorganic compounds from wastewaters [77, 47, 18, 45, 32]. Interestingly, *Mycolicibacter* and *Pseudoxanthomonas* were present at high abundance in our samples. Both genera are recognized as environmental contaminants, and their members are typically non-pathogenic to humans. However, recent studies have reported certain species with the potential to cause human infections which may lead to severe diseases [36, 7, 46].

3.6 Diverse MGEs were detected across all HWW samples.

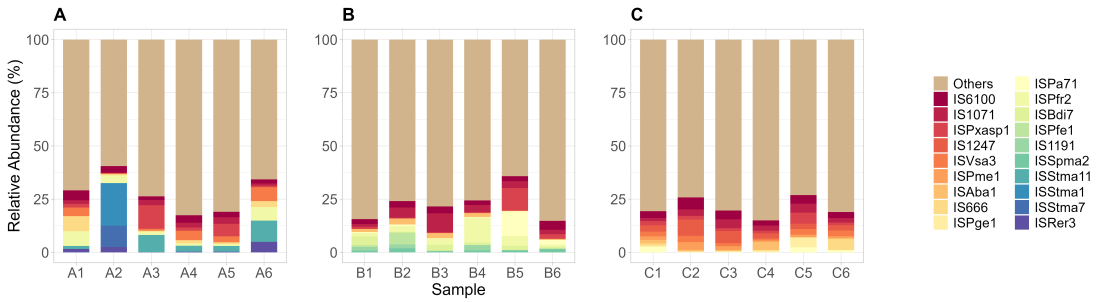

Figure 6: The relative abundance of the top IS from Hospital A (A), Hospital B (B), and Hospital C (C). IS not part of the top were grouped as "Others."

We identified a broad diversity of MGEs of IS and transposons, integrons, and plasmids. Among the most abundant IS and transposons (Figure 6), we observed the presence of IS1071, ISPasp1, IS1247, ISVsa3, ISAba1, and ISStma11. These IS are known to carry ARGs against β-lactams, tetracyclines, aminoglycosides, sulfonamides, macrolides, and disinfecting agents among others. They are known to be associated with the genera *Klebsiella*, *Pseudomonas*, *Stenotrophomonas*, *Rhodococcus*, and *Mycobacterium* among others. [65, 41, 35, 27]. Class 1 integrons (intI1) were the most abundant type of integrons detected in our samples. These integrons are frequently associated with Gram-negative ARBs in clinical settings. Class 1 integrons often contain ARGs conferring resistance to β-lactams, aminoglycosides, sulfonamides, and disinfectants [60]. We have also identified a diversity of plasmid types that carry ARGs including IncQ2 (NCBI accession FJ696404), IncP6 (NCBI

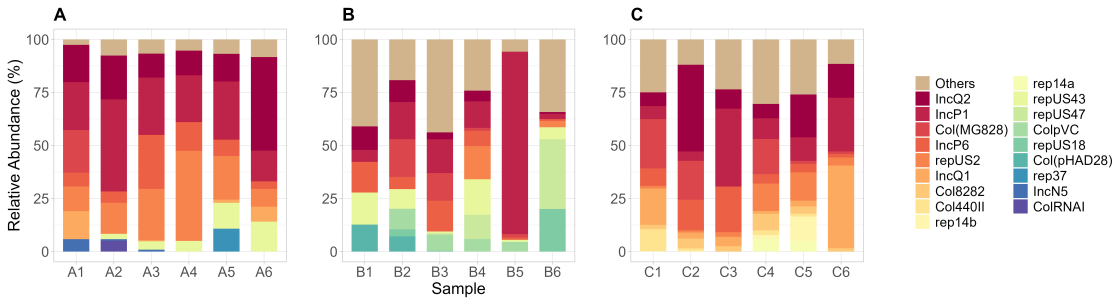

Figure 7: The relative abundance of the top plasmid replicons from Hospital A (A), Hospital B (B), and Hospital C (C). Plasmid replicons not part of the top were grouped as"Others."

accession JF785550), repUS43 (NCBI accession CP003584), Col440II (NCBI accession CP023921), Col(pHAD28) (NCBI accession KU674895) and ColRNAI (NCBI accession DQ298019) among others (Figure 7). These plasmids can carry multiple ARGs against β-lactams, tetracyclines, carbapenems, macrolides, and disinfectants among others [60, 74, 49].

3.7 Our data supplement the findings of the Philippine Antibiotic Resistance Surveillance Program

The Philippine Department of Health (DOH) established the Antimicrobial Resistance Surveillance Program (ARSP) in 1988, to monitor the prevalence and trends of antimicrobial resistance in selected bacterial pathogens. The ARSP reports phenotypic resistance rates of 11 species (*S. pneumoniae*, *H. influenzae*, *S. enterica*, *Shigella*, *V. cholerae*, *N. gonorrhoeae*, *S. aureus*, *Enterococcus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*) against commonly used antibiotics [3].

ARBs such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* among others showed resistance rates above 50% against drug classes of aminoglycosides, β-lactams, tetracyclines, and sulfonamides among others [3]. The detection of abundant ARGs in our samples conferring resistance to aminoglycosides, β-lactams, tetracyclines, and sulfonamides supplements ARSP reports by providing insights into genotypic resistance mechanisms.

The ARSP reports on clinically significant bacterial species that play a role in the spread of AMR in hospital settings. The presence of other ARBs such as *Rhodococcus*, *Mycolicibacter*, and *Pseudoxanthomonas* in our samples underscores the potential of shotgun metagenomics to identify underexplored ARBs for AMR surveillance. These organisms may also contribute to AMR dissemination and represent possible emerging pathogens warranting further investigation.

4 Conclusion

Our study investigated the diversity and abundance of ARGs, bacterial taxa, and MGEs in HWW in three hospitals in Metro Manila, Philippines. ARGs conferring resistance to critically important antibiotics including β-lactams, colistin, and tetracyclines were present in our samples. Interestingly, this is the first report of antiseptic and disinfectant resistance genes (*qacE*Δ*1*, *qacL*) in HWW in the Philippines. Notable bacterial genera such as *Stenotrophomonas*, *Rhodococcus*, *Mycolicibacter*, and *Pseudoxanthomonas* were detected across all sampling sites. MGEs associated with clinically important drug classes were also detected. These findings are consistent with global studies across

various economic settings, suggesting shared challenges in AMR management. Our HWW resistome profiles provide a more comprehensive view of ARGs and bacterial pathogens circulating in healthcare environments and can supplement data generated by national surveillance programs.

Overall, our results highlight the pressing need for strengthened antimicrobial stewardship and targeted control strategies within healthcare systems. The detection and characterization of ARGs of critical concern, emerging bacterial pathogens, and MGEs are critical to deepen our understanding of AMR interaction in hospital settings. These insights provide valuable evidence to inform national policy, enhance AMR monitoring, and support the development of more effective interventions to mitigate the growing threat of AMR in the country.

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7 Availability of data and materials

The datasets generated during the current study are available in the DDBJ Sequence Read Archive under the accession number PRJDB37674 [currently on Hold-Until-Published arrangement]. The computational pipeline used for data analysis is available at the following URL: `https://github.com/bioinfodlsu/attack_amr_pipeline`.

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