Novel Research in Microbiology Journal (2024), 8(4): 2510-2525

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/

Review Article

DOI: 10.21608/NRMJ.2024.303546.1647

Influence of hospital wastewater on the development of antimicrobial resistance

Hend M. Radwan; Nagwan G. El Menofy*; Sahar M.R. Radwan

Microbiology and Immunology Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt

*Corresponding author E-mail: nagwan.elmenofy@azhar.edu.eg

Received: 24 June, 2024; Accepted: 30 July, 2024; Published online: 1 August, 2024

Abstract

Resistance to antimicrobial agents is a developing issue that requires calls for an integrated strategy from all nations. A significant contributor to antimicrobial resistance (AMR) is wastewater that is a vital resource for bacteria which provides a medium for gene transfer. Stressors like high concentration of hydrogen ions, antibiotics, and minerals can start and spread AMR in wastewater. Antimicrobial agents are present in the environment in varying amounts depending on the antimicrobial class and their frequent use. Evolution of antibiotic-resistant bacteria (ARB) poses a significant public health risk; especially in healthcare facilities and hospital's wastewater. Advanced wastewater treatment technologies should be implemented for effective treatment of hospital wastewater (HWW). Standardized phenotypic approaches are used to detect AMR in bacteria. Molecular approaches are now preferred to be used in laboratories instead of phenotypic approaches as they are faster and more accurate for detection of AMR's underlying genetic mechanisms. The most common molecular approaches for assessing AMR in water include quantitative polymerase chain reaction (PCR) and whole genome sequencing (WGS). Metagenomic WGS sequencing provides more extensive genomic data and taxonomic classification than quantitative PCR, as it makes sequencing for the entire genome of microorganisms. The objectives of this review were to demonstrate the primary mechanisms through which bacteria develop resistance to antimicrobials in water, and investigate the impact of hospital effluent water on the spread AMR and ARB. Additionally, various approaches for assessing AMR levels and guidelines for preventing and managing the transmission of AMR to the environment were discussed.

Keywords: Antibiotics, Bacteria, Sewage, Whole genome sequencing, Resistome

1. Introduction

One of the biggest issues and a significant public health concern is the development of AMR. AMR makes treating infections challenging and could be a source of pandemic risks and increases mortality rates worldwide (Soni *et al*., 2024). It is widely recognized that incorrect usage, misuse, and uncontrolled release of antimicrobials worsen AMR and cause the resistant microorganisms and their genes to reproduce. Following their exposure to reduced doses of antibiotics, bacteria develop resistance to the used antibiotics; however, although some sensitive bacteria become eliminated, the resistant cells withstand and proliferate (Amato *et al*., 2021; Bengtsson-Palme *et al*., 2021). A growing number of researches have asserted that AMR is a leading cause of death globally. According to the data published in 2023 by World Health Organization (WHO), AMR was responsible for approximately 1.05 million fatalities, including 250,000 in Africa, thus posing an exceptional health concern. As a result, AMR is a multifaceted issue that impacts social, human, and animal health, in addition to economics and the environment (Tang *et al*., 2023).

Water is a crucial linkage among nature, human, and animals. It mixes microorganisms from various ecosystems with humans and animals, serving as a tool for transfer of genetic materials among the microbial species. This is particularly applicable for wastewater that has high nutrient levels and bacterial burden, in addition to the presence of antibacterial agents and their metabolites (Sambaza and Naicker, 2023; Clarke *et al*., 2024). Hospitals are special spaces that use antibiotics extensively for several patients (Majlander *et al*., 2021). Antibiotic resistant bacteria concentrations may rise when patients are prescribed high average doses of antibiotics. The human body does not absorb about 30-90 % of antibiotics which in turn become discharged into wastewater treatment systems (Vo *et al*., 2019). In addition to antibiotic-resistant bacteria, hospital wastewater also contains medical wastes, antimicrobial agents used for cleanliness purposes, and antibiotics. This combination creates a reservoir that harbors antibiotic-resistant genes and further contributes to the presence of ARBs in wastewater (Sambaza and Naicker, 2023).This situation creates an ideal environment for bacteria to contact with drugs and disseminate antibiotic resistance (Haenni *et al*., 2022). Antibiotics interaction and their relative concentrations are important in AMR development in wastewater (Sutradhar *et al*., 2023).

2. Genetic mechanisms of antimicrobial resistance

Two major categories of AMR mechanisms exist including acquired resistance and natural resistance (Reygaert, 2018). Natural resistance is unaffected by prior antibiotic exposure and extends to genera and species of bacteria and their corresponding strains (Abushaheen *et al*., 2020). *Acinetobacter*, Enterococci, *Escherichia coli*, and *Pseudomonas aeruginosa* are some bacterial genera that naturally resist some classes of antibiotics. On the other hand, acquired resistance describes a potential resistance to all antimicrobial agents via major chromosomal DNA mutation pathways (Reygaert, 2018).

Bacteria can acquire antimicrobial resistant genes (ARGs) in two ways; mainly horizontal gene transfer (HGT) and vertical gene transfer (VGT). In HGT, the antimicrobial resistance-mediated genes can be located on transposons, integrons, bacteriophages, and plasmids. The second approach is VGT, which is the transfer of bacterial chromosomes containing altered genetic materials to succeeding generations of daughter cells (Rizzo *et al*., 2013). A specific biochemical acquired resistance mechanisms are encoded by the genetic determinants of resistant bacteria. These include drug inactivation through enzymatic means, structural modifications to the antibiotic target site, modifications that hinder the antimicrobial agent from reaching the active site in sufficient concentration, and/ or efflux pump removal

of an active antibiotic from the bacterial cell (Halawa *et al*., 2024).

3. Emergence of bacterial resistance to antibiotics through aquatic settings

In aquatic environments, variation of the anti-microbial resistant genes is influenced by HGT, VGT, chromosomal mutations, and other genetic factors (Yu *et al*., 2022). Different classes of antibiotics and their corresponding ARGs found in wastewater are presented in Table (1) (Wang *et al*., 2024a; Zhang *et al*., 2024). One study demonstrated the factors and conditions that accelerate HGT and reported that the duration of bacterial incubation time emerged as the most critical parameter in this process, in addition to the presence of certain antibiotics such as kanamycin and nitrofurantoin, metallic ions; specifically $CuSO_4$ enhancing HGT at significantly lower concentrations (Dadeh Amirfard *et al*., 2024). Also net cages have extensive impacts on the accumulation of pollutants and subsequent dissemination of ARGs in aquatic setting (Wang *et al*., 2024b). Furthermore, wastewater contains metals, antibiotic residues, bacteria, disinfectant, and nutrients, which can operate as selection forces for microbial antibiotic resistance (Karkman *et al*., 2018). This is illustrated in Fig. (1). Wastewater treatment plants (WWTPs) are major hotspots for antibiotic resistance, where ARGs are propagated more easily in wastewater (Kunhikannan *et al*., 2021). Initially, WWTPs are intended to filter out wastewater from physical pollutants such as dirt, bacteria, and large amounts of organic matters, before being released into rivers, lakes, dams, and the ocean (Hammond *et al*., 2021). The fate of antibiotics, ARBs, and ARGs in WWTPs is influenced by many treatments including mechanical, biological, physical, and chemical processes, resulting in resistance spreading into the aquatic environment (Pazda *et al*., 2019). Despite technological advancements in WWTP treatment techniques, it is still hard to completely remove antimicrobial chemicals and other developing pollutants, as the emerging contaminants (EC) such as ARGs, ARB, and antibiotics, are rarely intended to be removed by traditional treatment methods and systems (Narciso-da-Rocha *et al*., 2018). Certain ARB can withstand chlorine disinfection and end up *via* distribution in the wastewater after chlorine treatment (Beattie *et al*., 2020). Additionally, plasmids, transposons, and integrons can be used to propagate and distribute ARGs in wastewater, which may lead to the development of novel ARBs (Karkman *et al*., 2018). Several common bacterial pathogens such as *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Enterobacter* spp., *Enterococcus faecium*, and *P. aeruginosa* are frequently found in sewage and have been identified as key drivers of AMR (Marano *et al*., 2021). Generally, wastewater's composition has varying levels of antimicrobial agents, ARG, and disease burden, based upon the sources of supply (Narciso-da-Rocha *et al*., 2018). Presence of antibiotics in wastewater can cause bacteria to become resistant to the drugs, even at concentrations below the minimum inhibitory concentration (MIC) (Clarke *et al*., 2024). Antibiotics and ARG that enter soils in a variety of ways such as wastewater irrigation and various emerging contaminants can pollute the soil. ARG and antibiotics might also be introduced to the terrestrial environment by several other means, including the use of fertilizer and manure, which would put selection pressure on the bacterial communities in the soil (Pan and Chu, 2018). Wastewaters are complex matrices with high quantities of organic and inorganic chemicals, inhibitors such as humic substances, and disinfectants, making a challenge to identify individual bacterial species and functional genes. A false negative result or an underestimate of the number of original targets in the environmental samples may arise from decreased detection sensitivity methods (Volkmann *et al*., 2007). A previous study conducted by Azuma *et al*., (2024) assessed the effectiveness of ozone (O_3)-based advanced wastewater treatment systems in treating the antimicrobials, ARB, and ARG present in wastewater from medical facilities and revealed that it successfully deactivated multiple antimicrobials (>99.9 %) and eliminated ARB within 10-30 min. of treatment. Furthermore, this treatment effectively removed ARGs in hospital wastewater.

Table 1: Different antibiotics and the respective antimicrobial resistance genes found in wastewater, adopted by Mutuku *et al*., (2022); Wang *et al*., (2024a); Zhang *et al*., (2024)

Antibiotic class	Mechanisms of action	Mechanisms of resistance	Types of detected antimicrobial resistance genes
ß-lactams (penicillin derivatives, cephalosporins carbapenems, and monobactams).	Inhibit the penicillin binding proteins (PBPs), which catalyze the transpeptidation process during peptidoglycan synthesis and thus prevent the cross-linking that forms the cell wall structure.	Alteration of target sites (mutations in PBPs). -Direct deactivation by β-lactamases.	bla_{OXA}, $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{CMY} bla_{IMP}, bla_{VIM}, bla_{KPC}, and bla_{NDM},
Aminoglycosides	They bind to the aminoacyl-tRNA recognition site (A-site), the decoding centre on the 16S rRNA of the ribosome inhibiting protein synthesis.	-Acquired inactivation enzymes (*i.e*., Aminoglycoside modifying enzymes (AME). 16S rRNA methyltransferases (RMTases).	*-AAC1, AAC2, AAC4* and *APH(6)* gene. *-ArmA, and RmtB* genes.
Quinolones and fuoroquinolones	They act on DNA gyrase and topoisomerase IV enzymes, bind to the cleaved-ligated active site, thereby intercalating into the DNA and blocking the ligation process.	-Mutations usually occur in the gyrase gene or in topoisomerase IV gene in some highly resistant isolates (Chromosomal) -Proteins encoded by *qnr* genes, which prevent quinolones from entering cleavage complexes by binding to DNA.	*-gyrA and parC* genes. *qnrA, qnrB, qnrS, qnrC,* and *qnrD.*
Sulfonamides and trimethoprim	They act by interfering with the two successive steps in folate biosynthesis.	-Metabolic alterations, overproduction of target enzyme or physiological metabolite *(pAB*). -Mutational changes in the target enzyme. -Changes in cell permeability -Synthesis of novel, drug-resistant bypass enzymes	*Sul1, sul2, sul3, dfrA,* and *dfrB*
Tetracyclines	They bind to the 30S ribosomal subunit and interfere with the association of aminoacyl-tRNA, inhibiting bacterial protein biosynthesis.	-Ribosomal protection mediated by large proteins coded by genes. -Decrease in intracellular drug concentration achieved through active efflux.	*tetA, tetA(C), tetB, tetC, tetE, tetF, tetH, tetK, tetL, tetM, tetN, tetO, tetQ,* and *tetS*
Macrolides	Bind to the 50S subunit of the bacterial ribosome and inhibit protein synthesis during the early stages	-Methylation to 23S rRNA by methylase enzyme. -Antibiotic inactivation by modifying enzymes-macrolide phosphotransferases. -Active efflux of the drug from the cell	*mphA, mphB, ereA2, ermA, ermB, ermF, ermO,* and *mefA*
Glycopepetides	Inhibit synthesis of cell well peptidoglycan and inhibit bacterial cell membrane permeability.	-Acquisition of operons that code for specific enzymes involved in the synthesis of low-affinity peptidoglycan precursors ending in D-Ala-D-Lactate or D-Ala-D-Serine	*vanA, vanB, vanC1, vanC2, vanC3,* and *vanD*
Chloramphenicols	Bind to the 50S ribosomal subunit and prevent peptide chain elongation that prevents the synthesis of proteins.	-Enzyme inactivation by Chloramphenicol acetyltransferase (CAT)	*catI,* and *catII*
Multidrug resistance (MDR)		-Bacteria that accumulate many genes encoding resistance to a single drug efflux pumps, and can remove a wide range of antibiotics from the cell.	*amrB, mdtG, mdtH, mexD,* and *qacEΔ1*

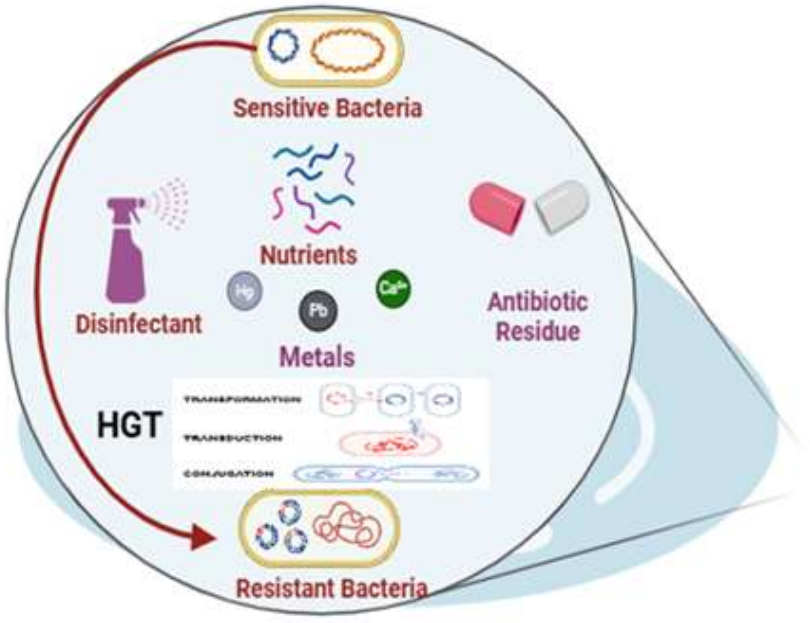

Fig. 1: Factors associated with emergence of AMR in hospital wastewater. The figure demonstrates that hospital wastewater contains metals, antibiotic residues, bacteria, disinfectant, and nutrients, which can operate as selection forces for HGT and conversion of sensitive bacterial strains to resistant ones

4. Hospitals and their impacts on the development of AMR

Hospitals are essential places for the transmission of ARGs, due to the predicted greater concentration of ARB and residual antibiotics in their wastewater sources. Hospital effluents greatly contribute to the antibiotic load of the wastewater effluent (Petrovich *et al*., 2020). Generally, about 20 %- 25 % of human medicine use is attributable to healthcare facilities, where antibiotics are part of this medicine that have the potential to upset the ecosystem's balance (Khan *et al*., 2021). Expansion of ARB and ARG in ecosystem represents a hazard to the microbial ecology and without appropriate treatment may provide a health risk for both humans and aquatic environments (Yao *et al*., 2021). Medical wastes, stool, and urine from patients are common components of hospital sewage. Some of these patients could have contributed to a significant amount of ARBs and ARGs to the sewage due to extensive antibiotic treatments (Unno *et al*., 2010). Many nations utilize wastewater for agricultural land irrigation due to their limited water supplies (Lahrich *et al*., 2021). When employing biologically treated and disinfected HWW effluents for direct utilization, there is a risk of micro-pollutant contamination (Khan *et al*., 2020). In a previous study, Mehanni *et al*., (2023) revealed the low impact of reusing of HWW effluent in agriculture irrigation compared to its greater risk in transferring multiple antibiotic resistant bacteria and resistance genes to the soil bacteria through natural transformation.

5. Guidelines for hospital wastewater regulation

The WHO's rules continue to be the most important rules for pre-treatment stage of wastewater, even if other international organizations have established their recommendations for wastewater treatment. WHO provides a framework for distinguishing the hazardous characteristics of these wastewater and suggests a

protocol for secure handling of HWW (Yan *et al*., 2020). The minimal prerequisites for discharging wastewater into municipal treatment drains include primary, secondary, and tertiary treatment units (Khan *et al*., 2021). Effective sewage treatment system should eliminate at least 95 % of microorganisms from wastewater and its connection to a central treatment plant is a prerequisite. After treatment, the sludge residue should be anaerobically digested to leave as few microscopic helminth eggs per liter as possible. Strict requirements must be upheld to ensure that the treated wastewater includes only minimal quantities of toxic pharmaceuticals, antimicrobial agents, radioactive materials, and medicines (Drane *et al*., 2024).

The unchecked raw wastewater is regularly dumped into wastewater drains. At some point, this wastewater travels into urban WWTPs and is blended with effluents before being treated (Samal *et al*., 2022). Government agencies, pharmaceutical industries, and academic institutions should collaborate to ensure effective HWW management, and these entities should utilize scientific data and systematic procedures (Khan *et al*., 2021). They should also take into account the unknown and novel sources of contaminants that have an effect on the physical and psychological health (Gavrilescu *et al*., 2015). The weak enforcement of rules and regulations and the low level of awareness in the public and private sectors, present the biggest challenges to this wastewater treatment strategy (Khan *et al*., 2021). So the environmental impact of ARGs in aquatic environments can be effectively mitigated through the implementation of various environmental protection measures, including monitoring, surveillance and reduction of antibiotic usage, effective waste management, education and awareness campaigns, and regulatory actions; especially in regions where net cages are employed (Wang *et al*., 2024b).

6. Methods for detection of antibiotic resistance in aquatic settings

6.1. Culture-based techniques

Culture-dependent analysis is used for traditional water quality monitoring and evaluations to screen for particular indicator microbial species, including bacterial pathogens and fecal coliforms as *E. coli* (McLain *et al*., 2016). These cultural methods are still in use today all around the world due to their availability and sensitivity (Lee *et al*., 2017). Such methods are based on screening numerous bacterial cultures found in an environment on non-selective media (e.g. blood agar and Mueller-Hinton agar) or more selective media (e.g. MacConkey agar, Mannitol Salt agar, Pseudomonas Cetrimide agar, *Salmonella Shigella* agar, and Thiosulfate Citrate Bile Salts Sucrose agar). This in addition to more specific media such as CHROM agar, ESBL medium used for selection of extended spectrum β-lactamase (ESBL)-producing microorganisms, and chromogenic modified membrane tolerant *E. coli* (mTEC) agar for detection and enumeration of *E. coli* in water (Katagiri *et al*., 2021; Mukherjee *et al*., 2021). The disk diffusion method is a qualitative approach for classifying samples as susceptible, intermediate, or resistant. Determination of minimum inhibitory concentration (MIC) is the most widely used method for determining the antimicrobial susceptibility, which quantifies the minimal dose of an antibiotic that inhibits the apparent growth of bacteria on agar and/ or in broth media (Galhano *et al*., 2021). Generally, the culture based methods facilitate identification of the functional ARGs and virulence factors and have also been combined with gene sequencing techniques to detect ARGs carried by particular bacteria (Drane *et al*., 2024).

The disadvantages of culture based techniques include long time lasting of cultivation and difficulty in detecting some microbial species if they are present in low concentration in the medium (Gheyas and Burt, 2013). Another drawback is that non-pathogenic environmental bacteria cannot be grown in standard culture media despite sustaining metabolic activity, attributable to existence of stresses, including temperature, pH, deficient substrates, and/or oxygen

concentrations, which don't meet the bacterial optimal requirements for growth (Galhano *et al*., 2021).

6.2. Molecular based techniques

The challenges of culturing environmental microorganisms existing in surface water are managed using molecular techniques. AMR research in surface water examines bacterial DNA for ARGs and mobile genetic elements (MGEs) associated with AMR (Harbottle *et al*., 2006). However, simply knowing the existence of a specific gene doesn't help with analyzing the microbial threats to human health, because gene's presence doesn't always mean that it is expressed in that pathogen group or that a human microbial pathogen carries it. The most prevalent techniques used for evaluating AMR in surface waters are conventional PCR, high throughput quantitative PCR (HT-qPCR), metagenomics, and WGS (Franklin *et al*., 2021).

6.2.1. Polymerase chain reaction (PCR)

One of the most important scientific achievements of the 20th century is conventional PCR that acts as an advanced molecular biology technique by giving a quick and simple approach for isolating a gene of interest such as ARG (Powledge, 2004). The ease of constructing standard PCR primers has led to the widespread usage of PCRs for many detection purposes. Schwartz *et al*., (2003) conducted a study that examined the use of PCR to detect the presence of genes encoding for resistance to vancomycin (*vanA*), methicillin (*mecA*), and ampicillin (*ampC*) in wastewater and freshwater biofilms. They reported that all the detected genes were amplified predominantly from HWW biofilms. *VanA* and *ampC* genes were also detected in all other sewage samples (Schwartz *et al*., 2003). On the other hand, HT-qPCR is a more specific technique that operates at the nano-liter level. HT-qPCR requires only a small amount of DNA per sample when analyzing samples from aquatic environments, because DNA quantities per sample volume are typically lower compared to the other environmental components such as soil and sediment (Sander and Kalff, 1993). Utilization of the qPCR method to detect ARGs in wastewater offers the advantage of high-resolution quantification of specific ARG sequences within a given set; however, selectivity of the primers used in qPCR assays poses a limitation in identifying ARGs resistome (Elbait *et al*., 2024). The three most often used HT-qPCR technologies in previous studies investigating ARGs in surface waters are Takara Smartchip, Applied Biosystems, OpenArray, and BioMark Dynamic Array kits, which have high productivity and require different amounts of test reactions (Waseem *et al*., 2019).

6.2.2. Whole genome sequencing

In the clinical and non-clinical fields, AMR can be investigated utilizing a number of sequencing techniques (Forbes *et al*., 2017). Next-generation DNA sequencing (NGS) is the driving force behind advances in metagenomics, and is a crucial tool for researchers in various fields, including basic biology and clinical diagnostics (Satam *et al*., 2023). It enables complete genome sequencing, transcriptomics, epigenomics, metagenomics, and other omics investigations (Levy and Myers, 2016). Over the last 40 years, sequencing technologies have evolved in three generations. Sanger sequencing has established the first generation of DNA sequencing that required chemical breakdown and/ or enzymatic cleavage of the molecules to produce DNA pieces that could be examined independently (Heather and Chain, 2016). The second generation of sequencing systems involves Illumina and Ion Torrent, enabling simultaneous sequencing of thousands to millions of DNA fragments that allowed for high-throughput sequencing. They have considerably improved DNA sequencing throughput and quickness, allowing for diverse applications in genomics studies and medical diagnosis. The third-generation sequencing technologies (*i.e*., PacBio and Nanopor) provide long-read sequencing capabilities, enabling sequencing of much larger DNA fragments compared to the earlier methods. The third-generation sequencing technologies are the latest breakthroughs in DNA

sequencing that provide new ways to overcome limitations from prior generations (Satam *et al*., 2023) and are excellent for exploring AMR in isolated microbes and metagenomic samples as assembly difficulties become reduced (De Maio *et al*., 2019).

Whole genome sequencing (WGS) is widely used to characterize pathogens and better understand the emergence and spread of antibiotic resistance (Calero-Cáceres *et al*., 2023). It identifies bacterial species, resistance and virulence genes, genome annotation in a single laboratory procedure, allows for quick addition of new target sequences to the analysis database, and reanalysis of the already sequenced samples (Kekre *et al*., 2021). In addition, WGS eliminates the regular difficulties and disadvantages associated with growing ambient bacteria to identify AMR and could enhance AMR surveillance in surface waters (Franklin *et al*., 2021). Despite its advantages for pathogen identification; however, WGS implementation expenses and lack of competence may limit its usage in the clinical laboratories (Calero-Cáceres *et al*., 2023).

Pure culture isolation along with WGS has been and remains a significant tool to identify the phenotypic and genotypic correlations of ARBs, and allows detection of MGEs with which they are related (Xia *et al*., 2017). A previous study utilized a broth microdilution approach in conjunction with WGS to validate the presence of AMR, specifically for ESBL producers and waterborne *E. coli* in surface waters and wastewater sources (Haberecht *et al*., 2019). Metagenomics is a general approach to understand genetic diversity in the complicated environments and determines the relative number of genes or specific sequence categories, which enables studying the prevalence and proportions of different groups in the complex microbial populations (Satam *et al*., 2023). Metagenomics allows for simultaneous screening of known and new ARGs, thus eliminating the need to select targets as in PCR, where a specific gene becomes amplified that is targeted by specific primers. As new ARGs are often discovered and recorded, stored sequencing data can be evaluated to identify the developing genes or diseases (Davis *et al*., 2023). Integration of metagenomics with potent bioinformatic tools and platforms has made it easier to identify microbial communities and obtain genetic data (de Abreu *et al*., 2021). The disadvantages of metagenomics include the large volume of data generated and the need for specialist bioinformatics knowledge. Moreover, there are variations of reads among the different runs for the same sample that needs balancing of the number of repeats (Bengtsson-Palme *et al*., 2021), in addition to failure of detecting uncommon genetic elements, which is strongly dependent on the DNA sequencing depth (Yang *et al*., 2014). DNA sequencing depth can impact the quantity and diversity of ARGs found in a single sample. The various parts of the workflow, ranging from samples collection to NGS data creation and analysis, require careful attention, to be sure that monitoring objectives are achieved and data generated are consistent (Davis *et al*., 2023). The data generated by NGS instrument must be processed, analyzed, and interpreted, which require a variety of computational methods and algorithms, and tools for processing, alignment, gene transcription estimation, variation analysis, and other forms of analysis (Satam *et al*., 2023).

7. Pipelines and tools used for detection of AMR genes

Several tools for resistome analysis in WGS data are becoming more available for free. These tools are offered as website resources, standalone programs with a graphical user interface, and command-line utilities for Unix-based machines (Anjum *et al*., 2018). Table (2) summarizes the most widely used and freely accessible programs.

8. Solutions for the AMR problems arising from hospital wastewater

Suggestions have been made to address the threat posed by AMR, including raising public awareness, providing access to clean water and sanitary facilities, better surveillance, higher-quality tests, and more prudent use of antibiotics (Sharma *et al*., 2022).

Table 2: Different open access bioinformatics' tools used for identification of the antimicrobial resistance genes, adopted by Anjum *et al*., (2018)

Method	Methods of gene detection	Files format and databases
Comprehensive Antibiotic Resistance Database (CARD)	The CARD is a web service and has two analysis options: BLAST and RGI. The BLAST option performs standard BLAST searches on smaller sequences uploaded by the user (but not whole genomes) against the CARD reference sequences. The RGI supports two detection model types, which are protein homolog models and protein variant models.	-FASTA format. -Four nucleotide databases with corresponding protein databases. - Resistance genes and mechanisms rRNA mutation genes. -Mutational genes. -Wild type genes.
ResFinder	ResFinder is a web server composed of a BLAST based alignment for detection of acquired AMR genes in assembled WGS data and a curated database in FASTA format containing the resistance genes. Possibly analyzes the assembled data or raw reads.	- FASTA format. -One database for each antimicrobial class with nucleotide sequences for acquired resistance genes. -Note file with phenotypic information on resistance genes.
Kmer Resistance	It is a mapping tool and is available both as a web server and as a command-line tool. Kmer Resistance performs mapping against the ResFinder gene database by examining the number of co-occurring *k-mers* between the raw sequence data and the database. A *k-mer* is a subsequence of the length k.	Uses the ResFinder database.
Short Read Sequence Typing for Bacterial Pathogens (SRST2)	SRST2 is a command-line tool based on the mapping tool Bowtie2. SRST2 maps raw sequence reads directly against an input database of preference and it enables further analysis of the identified genes, such as mutations compared to reference sequence.	Can only handle databases in FASTA format with a specific header format. Select between ARG-ANNOT database or ResFinder databases.
Antibiotic Resistance Gene Annotation (ARG-ANNOT)	ARG-ANNOT uses a local BLAST algorithm in conjunction with the BioEdit software sequences on a local computer without Internet access once the software is installed.	FASTA format. Three databases: Nucleotide sequences for acquired resistance gene database, Corresponding protein database, and Mutational gene database.

Many previous studies have provided solutions to this problem with wastewater such as eliminating the bacteria that carry these resistance genes and breaking down antibiotics in WWTPs. Ozonation, irradiation, and iron oxidation are other complex oxidation processes that have been employed by WWTPs over time to disinfect target water and inactivate all bacteria without distinction (Amin *et al*., 2013). The various wastewater treatment procedures have differing impacts on ARBs and related ARGs. The disinfection method that is still most frequently employed is chlorination due to its availability and efficiency (Sharma *et al*., 2019). The effectiveness of treating water with chlorine to inactivate ARG encoded by plasmid in external and internal forms has been assessed in a previous study conducted by Yoon *et al*., (2017), where significant amounts of ARG degradation have been recorded by the chlorination data, and these levels increased at rather high chlorine exposure rates. On the other hand, AMR induction

through HGT may takes place due to different reactions of ARB and ARG to the disinfection by-products (DBPs). A previous study has shown that DBPs can induce chromosomal alterations that lead to AMR at doses higher than MIC limits. There are several proposals for imposed complete prohibition on the use of chlorine-based disinfection agents due to the generation of DBPs and growth of bacteria resistant to chlorine (Li and Gu, 2019). Additionally, a membrane-based filtration has been utilized to remove ARB and related ARGs from wastewater. The effectiveness of these treatment processes is impacted by the higher fouling observed in several cases with an elevated concentration of ARB. Another effective and affordable way to get rid of ARGs is by coagulation (Yu *et al*., 2021). Alumina, polyacrylamide, and iron are examples of coagulants that have been used in wastewater treatment processes to remove different types of contaminants, which resulted in excellent effectiveness with affordable prices (Liang *et al*., 2021).

Conclusion

Healthcare facilities affect the sustainability and health of the environment by discharging ECs and bacteria into the water draining systems before treating them. Regarding the extremely toxic nature and amount of pollutants of HWW, appropriate disposal procedures should be established taking into account the catchment region and the kind of healthcare facility that discharges the material. Microbial community profiling and metagenomic sequence analysis are promising strategies for detecting AMR genes in aquatic settings. `

Acknowledgements

None.

Conflict of interests

All authors declare no conflicts of interest.

Funding

Non-fund was received for this study.

Ethical approval

Not applicable.

Author's Contributions

Investigation, Formal analysis, Writing Review and Editing: H.R.; Supervision, Conceptualization, Formal analysis, Revision and Editing: N.G.E.; Supervision, Conceptualization and Revision: R.S.

9. References

Abushaheen, M.A.; Fatani, A.J.; Alosaimi, M.; Mansy, W.; George, M.; Acharya, S. et al. (2020). Antimicrobial resistance, mechanisms and its clinical significance. Disease-a-Month. 66(6): 100971. https://doi.org/10.1016/j.disamonth.2020.100971.

Amato, M.; Dasí, D.; González, A.; Ferrús, M.A. and Castillo, M.Á. (2021). Occurrence of antibiotic resistant bacteria and resistance genes in agricultural irrigation waters from Valencia city (Spain). Agricultural Water Management. 256: 107097. https://doi.org/10.1016/j.agwat.2021.107097.

Amin, M.M.; Hashemi, H. and Bovini, A.M. (2013). A review on wastewater disinfection. International Journal of Environmental Health Engineering. 2(1). https://doi.org/10.4103/2277-9183.113209.

Anjum, M.F.; Zankari, E. and Hasman, H. (2018). Molecular methods for detection of antimicrobial resistance. Microbiology Spectrum. 5(6). https://doi.org/10.1128/microbiolspec.arba-0011-2017.

Azuma, T.; Usui, M. and Hayashi, T. (2024). Inactivation of antibiotic-resistant bacteria in hospital wastewater by ozone-based advanced water treatment processes. Science of the Total Environment. 906: 167432. https://doi.org/10.1016/j.scitotenv.2023.167432.

Beattie, R.E.; Skwor, T. and Hristova, K.R. (2020). Survivor microbial populations in post- chlorinated

wastewater are strongly associated with untreated hospital sewage and include ceftazidime and meropenem resistant populations. Science of the Total Environment. 740: 140186. https://doi.org/10.1016/j.scitotenv.2020.140186.

Bengtsson-Palme, J.; Jonsson, V. and Heß, S. (2021). What is the role of the environment in the emergence of novel antibiotic resistance genes? A modeling approach. Environmental Science & Technology. 55 (23): 15734-15743. https://doi.org/10.1021/acs.est.1c02977.

Calero-Cáceres, W.; Ortuño-Gutiérrez, N.; Sunyoto, T.; Gomes-Dias, C.A.; Bastidas-Caldes, C.; Ramírez, M.S. et al. (2023). Whole-genome sequencing for surveillance of antimicrobial resistance in Ecuador: Present and future implications. Revista Panamericana de Salud Pública. 47: e8. https://doi.org/10.26633/RPSP.2023.8.

Clarke, L.; O'Brien, J.; Murray, A.; Gaze, W. and Thomas, K. (2024). A review of wastewater-based epidemiology for antimicrobial resistance surveillance. Journal of Environmental Exposure Assessment. 3(7): 1-18. https://dx.doi.org/10.20517/jeea.2023.29.

Dadeh Amirfard, K.; Moriyama, M.; Suzuki, S. and Sano, D. (2024). Effect of environmental factors on conjugative transfer of antibiotic resistance genes in aquatic settings. Journal of Applied Microbiology. 135 (6). https://doi.org/10.1093/jambio/lxae129.

Davis, B.C.; Brown, C.; Gupta, S.; Calarco, J.; Liguori, K.; Milligan, E. et al. (2023). Recommendations for the use of metagenomics for routine monitoring of antibiotic resistance in wastewater and impacted aquatic environments. Critical Reviews in Environmental Science and Technology. 53(19): 1731-1756. https://doi.org/10.1080/10643389.2023.2181620.

Elbait, G.D.; Daou, M.; Abuoudah, M.; Elmekawy, A.; Hasan, S.W.; Everett, D.B. et al. (2024). Comparison of qPCR and metagenomic sequencing methods for quantifying antibiotic resistance genes in wastewater. Plos one. 19(4): e0298325. https://doi.org/10.1371/journal.pone.0298325.

de Abreu, V.A.C.; Perdigão, J. and Almeida, S. (2021). Metagenomic approaches to analyze antimicrobial resistance: An overview. Frontiers in Genetics. 11. https://doi.org/10.3389/fgene.2020.575592.

De Maio, N.; Shaw, L.P.; Hubbard, A.; George, S.; Sanderson, N.D.; Swann, J. et al. (2019). Comparison of long-read sequencing technologies in the hybrid assembly of complex bacterial genomes. Microbial Genomics. 5(9). https://doi.org/10.1099/mgen.0.000294.

Drane, K.; Sheehan, M.; Whelan, A.; Ariel, E. and Kinobe, R. (2024). The Role of Wastewater Treatment Plants in Dissemination of Antibiotic Resistance: Source, Measurement, Removal and Risk Assessment. Antibiotics. 13(7): 668. https://doi.org/10.3390/antibiotics13070668.

Forbes, J.D.; Knox, N.C.; Ronholm, J.; Pagotto, F. and Reimer, A. (2017). Metagenomics: The next culture-independent game changer. Frontiers in Microbiology. 8:1069. https://doi.org/10.3389/fmicb.2017.01069.

Franklin, A.M.; Brinkman, N.E.; Jahne, M.A. and Keely, S.P. (2021). Twenty-first century molecular methods for analyzing antimicrobial resistance in surface waters to support one health assessments. Journal of Microbiological Methods. 184: 106174. https://doi.org/10.1016/j.mimet.2021.106174.

Galhano, B.S.P.; Ferrari, R.G.; Panzenhagen, P.; de Jesus, A.C.S., and Conte-Junior, C.A. (2021). Antimicrobial resistance gene detection methods for bacteria in animal-based foods: A brief review of highlights and advantages. Microorganisms. 9(5): 923. https://doi.org/10.3390/microorganisms9050923.

Gavrilescu, M.; Demnerová, K.; Aamand, J.; Agathos, S. and Fava, F. (2015). Emerging pollutants in the environment: present and future challenges in

biomonitoring, ecological risks and bioremediation. New Biotechnology. 32(1): 147-156. https://doi.org/10.1016/j.nbt.2014.01.001.

Gheyas, A.A. and Burt, D.W. (2013). Microarray resources for genetic and genomic studies in chicken: A review. Genesis. 51(5): 337-356. https://doi.org/10.1002/dvg.22387.

Haberecht, H.B.; Nealon, N.J.; Gilliland, J.R.; Holder, A.V.; Runyan, C.; Oppel, R.C. et al. (2019). Antimicrobial-resistant *Escherichia coli* from environmental waters in Northern Colorado. Journal of Environmental and Public Health. 2019(1): 3862949. https://doi.org/10.1155/2019/3862949.

Haenni, M.; Dagot, C.; Chesneau, O.; Bibbal, D.; Labanowski, J.; Vialette, M et al. (2022). Environmental contamination in a high-income country (France) by antibiotics, antibiotic-resistant bacteria, and antibiotic resistance genes: Status and possible causes. Environment International. 159. https://doi.org/10.1016/j.envint.2021.107047.

Halawa, M.; Fadel, M.; Al-Rabia, W.; Behairy, A.; Nouh, N.A.; Abdo, M.; et al. (2024). Antibiotic action and resistance: updated review of mechanisms, spread, influencing factors, and alternative approaches for combating resistance. Frontiers in Pharmacology. 14: 1305294. https://doi.org/10.3389/fphar.2023.1305294.

Hammond, P.; Suttie, M.; Lewis, V.T.; Smith, A.P. and Singer, A.C. (2021). Detection of untreated sewage discharges to watercourses using machine learning. npj Clean Water. 4(1): 18. https://doi.org/10.1038/s41545-021-00108-3.

Harbottle, H.; Thakur, S.; Zhao, S. and White, D.G. (2006). Genetics of antimicrobial resistance. Animal Biotechnology. 17(2): 111-124. https://doi.org/10.1080/10495390600957092.

Heather, J.M. and Chain, B. (2016). The sequence of sequencers: The history of sequencing DNA. Genomics. 107(1): 1-8. https://doi.org/10.1016/j.ygeno.2015.11.003.

Karkman, A.; Do, T.T.; Walsh, F. and Virta, M.P. (2018). Antibiotic-resistance genes in wastewater. Trends in Microbiology. 26(3): 220-228. https://doi.org/10.1016/j.tim.2017.09.005.

Katagiri, M.; Kuroda, M.; Sekizuka, T.; Nakada, N.; Ito, Y.; Otsuka, M. et al. (2021). Comprehensive genomic survey of antimicrobial-resistance bacteria in the sewage tank replacement with hospital relocation. Infection and Drug Resistance. 14: 5563-5574. https://doi.org/10.2147/IDR.S336418.

Kekre, M.; Arevalo, S.A.; Valencia, M.F.; Lagrada, M.L.; Macaranas, P.K.V.; Nagaraj, G. et al. (2021). Integrating scalable genome sequencing into microbiology laboratories for routine antimicrobial resistance surveillance. Clinical Infectious Diseases. 73(4): S258-S266. https://doi.org/10.1093/cid/ciab796.

Khan, M.T.; Shah, I.A.; Ihsanullah, I.; Naushad, M.; Ali, S.; Shah, S.H. A. et al. (2021). Hospital wastewater as a source of environmental contamination: An overview of management practices, environmental risks, and treatment processes. Journal of Water Process Engineering. 41-1990. https://doi.org/10.1016/j.jwpe.2021.101990.

Khan, N.A.; Ahmed, S.; Farooqi, I.H.; Ali, I.; Vambol, V.; Changani, F. et al. (2020). Occurrence, sources and conventional treatment techniques for various antibiotics present in hospital wastewaters: A critical review. Trends in Analytical Chemistry. 129-115921. https://doi.org/10.1016/j.trac.2020.115921.

Kunhikannan, S.; Thomas, C.J.; Franks, A.E.; Mahadevaiah, S.; Kumar, S. and Petrovski, S. (2021). Environmental hotspots for antibiotic resistance genes. Microbiology Open. 10(3): e1197. https://doi.org/10.1002/mbo3.1197.

Lahrich, S.; Laghrib, F.; Farahi, A.; Bakasse, M.; Saqrane, S. and El Mhammedi, M.A. (2021). Review on the contamination of wastewater by covid-

19 virus: Impact and treatment. Science of the Total Environment. 751. https://doi.org/10.1016/j.scitotenv.2020.142325.

Lee, H.W.; Yoon, S.R.; Kim, S.J.; Lee, H.M.; Lee, J.Y.; Lee, J.H. et al. (2017). Identification of microbial communities, with a focus on foodborne pathogens, during kimchi manufacturing process using culture-independent and -dependent analyses. LWT - Food Science and Technology. 81: 153-159. https://doi.org/10.1016/j.lwt.2017.04.001.

Levy, S.E. and Myers, R.M. (2016). Advancements in next-generation sequencing. Annual Review of Genomics and Human Genetics. 17(17): 95-115. https://doi.org/10.1146/annurev-genom-083115-022413.

Li, D. and Gu, A.Z. (2019). Antimicrobial resistance: A new threat from disinfection byproducts and disinfection of drinking water. Current Opinion in Environmental Science & Health. 7: 83-91. https://doi.org/10.1016/j.coesh.2018.12.003.

Liang, C.; Wei, D.; Zhang, S.; Ren, Q.; Shi, J. and Liu, L. (2021). Removal of antibiotic resistance genes from swine wastewater by membrane filtration treatment. Ecotoxicology and Environmental Safety. 210: 111885. https://doi.org/10.1016/j.ecoenv.2020.111885.

Majlander, J.; Anttila, V.J.; Nurmi, W.; Seppälä, A.; Tiedje, J. and Muziasari, W. (2021). Routine wastewater-based monitoring of antibiotic resistance in two finish hospitals: Focus on carbapenem resistance genes and genes associated with bacteria causing hospital-acquired infections. Journal of Hospital Infection. 117: 157-164. https://doi.org/10.1016/j.jhin.2021.09.008.

Marano, R.B.M.; Gupta, C.L.; Cozer, T.; Jurkevitch, E. and Cytryn, E. (2021). Hidden resistome: Enrichment reveals the presence of clinically relevant antibiotic resistance determinants in treated wastewater-irrigated soils. Environmental Science & Technology. 55(10): 6814- 6827. https://doi.org/10.1021/acs.est.1c00612.

McLain, J.E.; Cytryn, E.; Durso, L.M. and Young, S. (2016). Culture-based methods for detection of antibiotic resistance in agroecosystems: Advantages, challenges, and gaps in knowledge. Journal of Environmental Quality. 45(2): 432-440. https://doi.org/10.2134/jeq2015.06.0317.

Mehanni, M.M.; Gadow, S.I.; Alshammari, F.A.; Modafer, Y.; Ghanem, K.Z.; El-Tahtawi, N.F. et al. (2023). Antibiotic-resistant bacteria in hospital wastewater treatment plant effluent and the possible consequences of its reuse in agricultural irrigation. Frontiers in Microbiology. 14: 1141383. https://doi.org/10.3389/fmicb.2023.1141383.

Mukherjee, M.; Laird, E.; Gentry, T.J.; Brooks, J.P. and Karthikeyan, R. (2021). Increased antimicrobial and multidrug resistance downstream of wastewater treatment plants in an urban watershed. Frontiers in Microbiology. 12: 657353. https://doi.org/10.3389/fmicb.2021.

Mutuku, C.; Gazdag, Z. and Melegh, S. (2022). Occurrence of antibiotics and bacterial resistance genes in wastewater: resistance mechanisms and antimicrobial resistance control approaches. World Journal of Microbiology and Biotechnology. 38(9): 152. https://doi.org/10.1007/s11274-022-03334-0.

Narciso-da-Rocha, C.; Rocha, J.; Vaz-Moreira, I.; Lira, F.; Tamames, J.; Henriques, I. et al. (2018). Bacterial lineages putatively associated with the dissemination of antibiotic resistance genes in a full-scale urban wastewater treatment plant. Environment International. 118: 179-188. https://doi.org/10.1016/j.envint.2018.05.040.

Pan, M. and Chu, L.M. (2018). Occurrence of antibiotics and antibiotic resistance genes in soils from wastewater irrigation areas in the pearl river delta region, southern china. Science of the Total Environment. 624: 145-152. https://doi.org/10.1016/j.scitotenv.2017.12.008.

Pazda, M.; Kumirska, J.; Stepnowski, P. and Mulkiewicz, E. (2019). Antibiotic resistance genes identified in wastewater treatment plant systems – a review. Science of the Total Environment. 20: 697:134023. https://doi.org/10.1016/j.scitotenv.2019.134023.

Petrovich, M.L.; Zilberman, A.; Kaplan, A.; Eliraz, G.R.; Wang, Y.; Langenfeld, K. et al. (2020). Microbial and viral communities and their antibiotic resistance genes throughout a hospital wastewater treatment system. Frontiers in Microbiology. 11. https://doi.org/10.3389/fmicb.2020.00153.

Powledge, T.M. (2004). The polymerase chain reaction. Advances in Physiology Education. 28(2): 44-50. https://doi.org/10.1152/advan.00002.2004.

Reygaert, W.C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiology. 4(3): 482-501. https://doi.org/10.3934/microbiol.2018.3.482.

Rizzo, L.; Manaia, C.; Merlin, C.; Schwartz, T.; Dagot, C.; Ploy, M. et al. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. Science of the Total Environment. 447: 345-360. https://doi.org/10.1016/j.scitotenv.2013.01.032.

Samal, K.; Mahapatra, S. and Hibzur Ali, M. (2022). Pharmaceutical wastewater as emerging contaminants (EC): Treatment technologies, impact on environment and human health. Energy Nexus. 6: 100076. https://doi.org/10.1016/j.nexus.2022.100076.

Sambaza, S.S. and Naicker, N. (2023). Contribution of wastewater to antimicrobial resistance: A review article. Journal of Global Antimicrobial Resistance. 34: 23-29. https://doi.org/10.1016/j.jgar.2023.05.010.

Sander, B.C. and Kalff, J. (1993). Factors controlling bacterial production in marine and freshwater sediments. Microbial Ecology. 26(2): 79-99. https://doi.org/10.1007/BF00177045.

Satam, H.; Joshi, K.; Mangrolia, U.; Waghoo, S.; Zaidi, G.; Rawool, S. et al. (2023). Next-generation sequencing technology: Current trends and advancements. Biology. 12(7): 997. https://doi.org/10.3390/biology12070997.

Schwartz, T.; Kohnen, W.; Jansen, B. and Obst, U. (2003). Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiology Ecology. 43(3): 325-335. https://doi.org/10.1111/j.1574-4966941.2003.tb01073.x.

Sharma, A.; Singh, A.; Dar, M.A.; Kaur, R.J.; Charan, J.; Iskandar, K. et al. (2022). Menace of antimicrobial resistance in LMICs: Current surveillance practices and control measures to tackle hostility. Journal of Infection and Public Health. 15(2): 172-181. https://doi.org/10.1016/j.jiph.2021.12.008.

Sharma, V.K.; Yu, X.; McDonald, T.J.; Jinadatha, C.; Dionysiou, D.D. and Feng, M. (2019). Elimination of antibiotic resistance genes and control of horizontal transfer risk by UV- based treatment of drinking water: A mini review. Frontiers of Environmental Science & Engineering. 13(3): 37. https://doi.org/10.1007/s11783-019-1122-7.

Soni, J.; Sinha, S. and Pandey, R. (2024). Understanding bacterial pathogenicity: a closer look at the journey of harmful microbes. Frontiers in Microbiology. 15: 1370818. https://doi.org/10.3389/fmicb.2024.1370818.

Sutradhar, I.; Ching, C.; Desai, D.; Heins, Z.; Khalil, A. and Zaman, M. (2023). Effects of antibiotic interaction on antimicrobial resistance development in wastewater. Scientific Reports. 13(1): 7801. https://doi.org/10.1038/s41598-023-34935-w.

Tang, K.W.K.; Millar, B.C. and Moore, J.E. (2023). Antimicrobial resistance (AMR). British Journal of Biomedical Science. 80: 11387. https://doi.org/10.3389/bjbs.2023.

Unno, T.; Han, D.; Jang, J.; Lee, S.N.; Kim, J.H.; Ko, G. et al. (2010). High diversity and abundance of antibiotic- resistant *Escherichia coli* isolated from humans and farm animal hosts in Jeonnam province, South Korea. Science of the Total Environment. 408(17): 3499-3506. https://doi.org/10.1016/j.scitotenv.2010.04.046.

Vo, T.K.Q.; Bui, X.T.; Chen, S.S.; Nguyen, P.D.; Cao, N.D.T.; Vo, T.D.H. et al. (2019). Hospital wastewater treatment by sponge membrane bioreactor coupled with ozonation process. Chemosphere. 230: 377-383. https://doi.org/10.1016/j.chemosphere.2019.05.009.

Volkmann, H.; Schwartz, T.; Kirchen, S.; Stofer, C. and Obst, U. (2007). Evaluation of inhibition and cross-reaction effects on real-time PCR applied to the total DNA of wastewater samples for the quantification of bacterial antibiotic resistance genes and taxon-specific targets. Molecular and Cellular Probes. 21(2): 125-133. https://doi.org/10.1016/j.mcp.2006.08.009.

Wang, Z.; Cai, M.; Du, P. and Li, X. (2024a). Wastewater surveillance for antibiotics and resistance genes in a river catchment: spatiotemporal variations and the main drivers. Water Research. 251: 121090. https://doi.org/10.1016/j.watres.2023.121090.

Wang, M.; Masoudi, A.; Wang, C.; Wu, C.; Zhang, Z.; Zhao, X. et al. (2024b). Impacts of net cages on pollutant accumulation and its consequence on antibiotic resistance genes (ARGs) dissemination in freshwater ecosystems: Insights for sustainable urban water management. Environment International. 183: 108357. https://doi.org/10.1016/j.envint.2023.108357.

Waseem, H.; Jameel, S.; Ali, J.; Saleem Ur Rehman, H.; Tauseef, I.; Farooq, U. et al. (2019). Contributions and challenges of high throughput qPCR for determining antimicrobial resistance in the environment: A critical review. Molecules. 24(1): 163. https://doi.org/10.3390/molecules24010163.

Xia, Y.; Li, A.D.; Deng, Y.; Jiang, X.T.; Li, L.G. and Zhang, T. (2017). Minion nanopore sequencing enables correlation between resistome phenotype and genotype of coliform bacteria in municipal sewage. Frontiers in Microbiology. 8: 527. https://doi.org/10.3389/fmicb.2017.02105.

Yan, S.; Zhang, X.L.; Tyagi, R.D. and Drogui, P. (2020). Guidelines for hospital wastewater discharge. In: Tyagi, R.D.; Sellamuthu, B.; Tiwari, B.; Yan, S.; Drogui, P.; Zhang, X. et al. (Editors), Current developments in biotechnology and bioengineering, pp. 571-597. https://doi.org/10.1016/B978-0-12-819722-6.00016-X.

Yang, Y.; Li, B.; Zou, S.; Fang, H.H.P. and Zhang, T. (2014). Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach. Water Research. 62: 97-106. https://doi.org/10.1016/j.watres.2014.05.019.

Yao, S.; Ye, J.; Yang, Q.; Hu, Y.; Zhang, T.; Jiang, L. et al. (2021). Occurrence and removal of antibiotics, antibiotic resistance genes, and bacterial communities in hospital wastewater. Environmental Science and Pollution Research. 28(40): 57321-57333. https://doi.org/10.1007/s11356-021-14735-3.

Yoon, Y.; Chung, H.J.; Wen Di, D.Y.; Dodd, M.C.; Hur, H.G. and Lee, Y. (2017). Inactivation efficiency of plasmid-encoded antibiotic resistance genes during water treatment with chlorine, UV, and UV/H_2O_2. Water Research. 123: 783-793. https://doi.org/10.1016/j.watres.2017.06.056.

Yu, Z.; Wang, Y.; Henderson, I.R. and Guo, J. (2022). Artificial sweeteners stimulate horizontal transfer of extracellular antibiotic resistance genes through natural transformation. The ISME Journal. 16(2): 543-554. https://doi.org/10.1038/s41396-021-01095-6.

Yu, K.F.; Li, P.; Li, H.; Zhang, B.; Yang, J.; Huang, F.Y. et al. (2021). Potential of coagulation to remove particle-associated and free-living antibiotic resistome from wastewater. Journal of Hazardous

Materials. 406: 124295. https://doi.org/10.1016/j.jhazmat.2020.124295.

Zhang, Y.; Hu, Y.; Li, X.; Gao, L.; Wang, S.; Jia, S. et al. (2024). Prevalence of antibiotics, antibiotic resistance genes, and their associations in municipal wastewater treatment plants along the Yangtze River basin, China. Environmental Pollution. 348: 123800. https://doi.org/10.1016/j.envpol.2024.123800.