



Antimicrobial Resistance Profiles of *Acinetobacter Baumannii* and *Vibrio Cholerae* in Hospital Wastewater and Adjacent Household Water Sources

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Abstract

Antimicrobial resistance (AMR) in aquatic environments represents an important interface between healthcare-associated contamination and community water quality. This quantitative comparative cross-sectional study assessed the prevalence and antimicrobial resistance profiles of *Acinetobacter baumannii* and *Vibrio cholerae* in hospital wastewater and adjacent household water sources. A total of 240 environmental water samples were collected, of which 232 (96.7%) satisfied the quality criteria and were included in the final analysis, comprising 116 hospital wastewater and 116 household water samples. Laboratory analysis yielded 144 confirmed bacterial isolates, including 82 *A. baumannii* and 62 *V. cholerae*. *A. baumannii* was detected in 37.1% of hospital wastewater samples compared with 15.5% of household water samples, while *V. cholerae* prevalence was 26.7% and 14.7%, respectively. Among hospital wastewater *A. baumannii*, resistance was highest to cefepime (84.7%), ceftriaxone (81.4%), cefotaxime (79.7%), and ceftazidime (76.3%), while resistance to imipenem and meropenem reached 61.0% and 55.9%, respectively. Hospital wastewater *V. cholerae* showed 71.4% resistance to ampicillin and 64.3% to trimethoprim-sulfamethoxazole. Multidrug resistance (MDR) was identified in 72.9% of hospital wastewater *A. baumannii* compared with 43.5% of household-water isolates and in 57.1% of hospital wastewater *V. cholerae* compared with 30.0% of household isolates. Overall MDR prevalence was 66.3% among hospital wastewater isolates and 37.2% among household-water isolates. Mean multiple antibiotic resistance indices were also higher among hospital wastewater *A. baumannii* (0.57 ± 0.21) and *V. cholerae* (0.43 ± 0.20) than their household-water counterparts (0.38 ± 0.19 and 0.29 ± 0.16 , respectively). Multivariable analysis showed that hospital wastewater isolates had 2.94 times greater adjusted odds of MDR than household-water isolates (95% CI: 1.34–6.45, $p = 0.007$). The findings demonstrated a substantially greater antimicrobial resistance burden in hospital wastewater while confirming the occurrence of resistant target bacteria in adjacent household water environments.

Keywords

Antimicrobial resistance, *Acinetobacter baumannii*, *Vibrio cholerae*, Wastewater, Multidrug resistance.

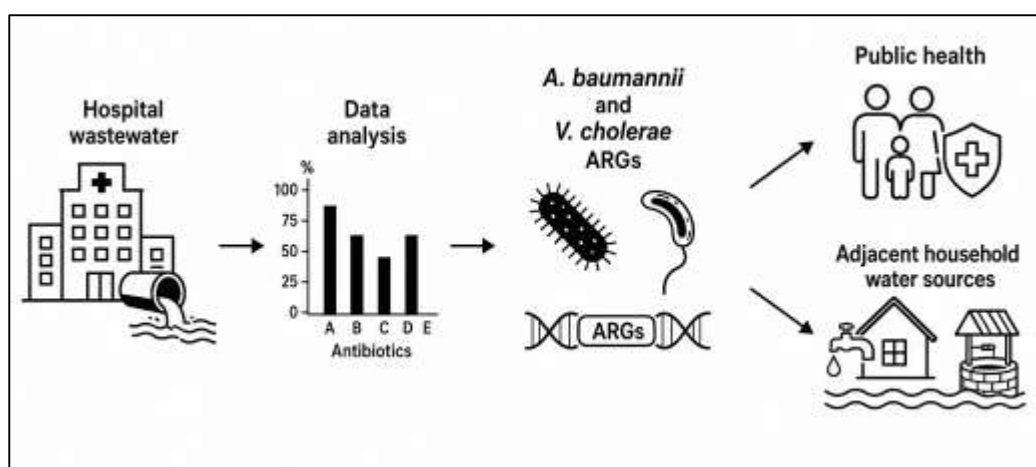
INTRODUCTION

Antimicrobial resistance (AMR) refers to the capacity of microorganisms to survive, multiply, or remain biologically active when exposed to antimicrobial agents that would ordinarily inhibit their growth or cause their death. In bacterial populations, antimicrobial resistance is commonly expressed as reduced susceptibility or complete resistance to one or more antibacterial agents and can arise through intrinsic biological characteristics, spontaneous genetic mutations, acquisition of resistance determinants, or transfer of mobile genetic elements between bacterial cells ([Nnadozie et al., 2017](#)). An antimicrobial resistance profile, often described through an antibiogram at the isolate or population level, represents the measurable pattern of susceptibility and resistance exhibited by a microorganism against a defined panel of antimicrobial agents. Such profiles permit quantitative characterization of resistance frequencies across antibiotic classes and enable bacterial isolates to be categorized according to the breadth of their resistance. Multidrug resistance generally describes non-susceptibility involving multiple antimicrobial categories, while more extensive resistance patterns indicate progressively narrower therapeutic options. Within environmental microbiology, AMR is not restricted to organisms recovered from infected patients because resistant bacteria, resistance genes, antimicrobial residues, disinfectants, and other selective substances can circulate through wastewater, surface water, groundwater, drainage systems, sediments, and domestic water networks ([Jian et al., 2021](#)). Hospital wastewater can be defined as liquid waste generated through healthcare activities, including wastewater originating from patient wards, toilets, laboratories, operating facilities, laundries, kitchens, cleaning activities, diagnostic units, and pharmaceutical handling areas. It represents a microbiologically complex matrix containing human-associated microorganisms, therapeutic antimicrobial residues, metabolites, organic matter, disinfectants, resistance genes, and other chemical constituents. Household water sources refer to water that households obtain or store for drinking, cooking, washing, bathing, cleaning, and other domestic purposes and may include piped supplies, wells, tube wells, boreholes, surface water, communal collection points, stored water, or locally distributed groundwater. The term adjacent household water sources in the present research context describes domestic water environments geographically associated with communities located near healthcare wastewater discharge pathways or receiving water systems ([Fouz et al., 2020](#)). Hospital wastewater and household water therefore represent two distinct but potentially connected environmental compartments through which antimicrobial-resistant organisms may be detected and quantitatively compared. International research on environmental AMR increasingly examines these compartments because water connects healthcare institutions, wastewater infrastructure, natural aquatic environments, and human populations within a shared microbial ecosystem. This conceptual relationship is particularly relevant to *Acinetobacter baumannii* and *Vibrio cholerae*, two Gram-negative organisms possessing different epidemiological characteristics but substantial abilities to persist in water-associated environments and acquire or maintain antimicrobial resistance.

Acinetobacter baumannii is a Gram-negative, non-fermentative, aerobic, non-motile coccobacillus belonging to a group of environmentally adaptable bacteria that can colonize humans and survive across clinical and non-clinical habitats ([Marathe et al., 2019](#)). It has acquired major international importance because of its association with healthcare-associated infections and its remarkable capacity to tolerate environmental stress while accumulating multiple mechanisms of antimicrobial resistance. Clinical infections involving this organism include ventilator-associated pneumonia, bloodstream infection, wound and burn infection, urinary tract infection, meningitis, and infections associated with invasive medical devices, with critically ill and immunocompromised patients representing important affected populations. Its antimicrobial resistance phenotype can involve β -lactams, cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, tetracyclines, and several other antimicrobial groups. Carbapenem-resistant *A. baumannii* occupies the critical-priority category of internationally recognized antibiotic-resistant bacterial pathogens because carbapenem resistance can substantially restrict treatment options ([Das et al., 2020](#)). Resistance is mediated through a combination of intrinsic and acquired processes that include β -lactamase production, carbapenem-hydrolyzing enzymes, alteration of membrane permeability, changes in antimicrobial targets, multidrug efflux systems, aminoglycoside-modifying enzymes, acquisition of plasmid-associated determinants, insertion sequences, integrons, transposons, and other mobile genetic structures. Genes associated with OXA-

type carbapenemases and metallo- β -lactamases have received substantial attention in environmental and clinical investigations because their occurrence is associated with clinically important carbapenem resistance.

Figure 1: Antimicrobial Resistance in Water



The organism also demonstrates natural competence and genomic plasticity, characteristics that facilitate acquisition and rearrangement of foreign genetic material under suitable environmental conditions. Biofilm formation provides another dimension of its persistence because biofilms permit bacterial populations to adhere to wet surfaces, pipes, drains, medical equipment, wastewater infrastructure, and other environmental substrates while creating microenvironments that can influence antimicrobial exposure and genetic exchange. Experimental and environmental studies have documented prolonged persistence of *A. baumannii* under conditions involving nutrient limitation, desiccation, fluctuating temperatures, and chemical stress (Zohra et al., 2021). Hospital wastewater therefore represents an important ecological location for examining the organism outside infected patients. International studies of hospital sewage, treatment systems, municipal receiving waters, rivers, and environmental reservoirs have identified *Acinetobacter* species and resistant *A. baumannii*, including isolates exhibiting multidrug-resistant and carbapenem-resistant phenotypes. Research in South Asia has similarly demonstrated environmentally recovered isolates with resistance to extended-spectrum cephalosporins, carbapenems, and other commonly used antimicrobial agents. Environmental water investigations in Bangladesh have detected *A. baumannii* in a substantial proportion of sampled water locations and have identified resistance to cefepime, ceftazidime, imipenem, and additional antibiotics (Carthy et al., 2021). These observations establish *A. baumannii* as both an important healthcare organism and a measurable component of aquatic AMR ecology, providing a microbiological basis for quantitatively comparing resistance profiles between hospital wastewater and household water sources.

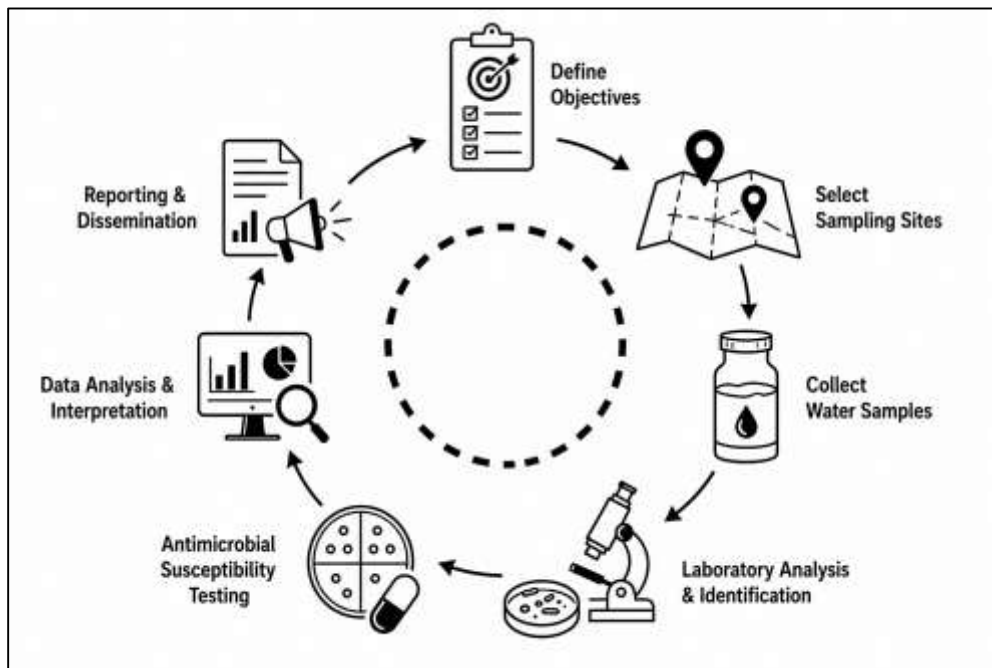
Vibrio cholerae is a Gram-negative, curved or comma-shaped, facultatively anaerobic, motile bacterium naturally associated with aquatic ecosystems and internationally recognized as the etiological agent of cholera when toxigenic strains capable of producing cholera toxin infect susceptible human populations. Its ecology differs fundamentally from that of a predominantly healthcare-associated opportunistic organism because *V. cholerae* can maintain environmental populations in freshwater, brackish water, estuarine systems, sediments, plankton-associated communities, aquatic organisms, wastewater, and other water-related habitats (Dewi et al., 2021). Toxigenic strains belonging mainly to epidemic-associated serogroups possess genetic determinants responsible for intestinal colonization and toxin production, while environmental populations contain extensive genetic diversity and include both toxigenic and non-toxigenic organisms. The aquatic ecology of *V. cholerae* is epidemiologically important because contaminated water remains a major route through which cholera transmission can occur. Bacterial survival can be supported by biofilm formation, association with plankton and chitinous surfaces, changes in physiological state, nutrient availability, salinity,

temperature, and interactions with other microorganisms. Antimicrobial therapy is particularly relevant in severe cholera because effective agents can reduce bacterial shedding and the duration and severity of illness in appropriately selected clinical cases. Resistance can therefore affect the therapeutic characteristics of circulating strains (Carthy et al., 2021). International investigations have documented resistance or reduced susceptibility involving tetracyclines, trimethoprim-sulfamethoxazole, ampicillin, nalidixic acid, fluoroquinolones, macrolides, chloramphenicol, streptomycin, and other agents, with substantial geographical and temporal variation in resistance patterns. The resistance architecture of *V. cholerae* can include mutations affecting antimicrobial targets and permeability together with acquired determinants carried by plasmids, integrons, transposons, and integrative conjugative elements. SXT/R391-family integrative conjugative elements are particularly important in the antimicrobial resistance biology of *V. cholerae* because they can carry multiple resistance determinants and move through bacterial populations by horizontal genetic transfer. Aquatic environments can additionally support genetic exchange through biofilm-associated bacterial communities and naturally competent states, linking environmental persistence with genomic adaptation (Karumathil et al., 2014). Wastewater investigations conducted internationally have detected pathogenic and non-pathogenic *V. cholerae*, while environmental surveillance studies have examined sewage, wastewater-impacted rivers, drains, surface water, and drinking-water-related sources as indicators of community circulation. More than one hundred wastewater and environmental surveillance studies examining *V. cholerae* have been reported across multiple geographical regions, with a particularly substantial evidence base originating from South and Southeast Asia. Bangladesh has a long-established relationship with the aquatic epidemiology of cholera, where ponds, rivers, household water environments, drainage networks, and densely populated urban water systems form interconnected ecological settings for *Vibrio* circulation (Peng et al., 2014). Quantitative assessment of the antimicrobial susceptibility of environmental *V. cholerae* therefore provides information extending beyond simple bacterial detection by defining the proportions of isolates resistant to individual antimicrobial agents and combinations of antimicrobial classes.

Hospital wastewater has become an internationally significant environmental compartment in antimicrobial resistance research because healthcare institutions concentrate antimicrobial consumption, resistant microorganisms, infected and colonized patients, antimicrobial metabolites, disinfectants, diagnostic chemicals, and biologically active waste within relatively confined wastewater streams. Antimicrobials administered to patients may be partially metabolized and subsequently excreted through urine and feces, allowing parent compounds or active metabolites to enter hospital sewage. Resistant organisms originating from the intestinal tract, respiratory secretions, wounds, clinical samples, contaminated surfaces, and sanitation facilities may enter the same wastewater network (Bagińska et al., 2021). The resulting mixture produces a microbial environment in which antimicrobial-resistant bacteria coexist with susceptible bacteria, resistance genes, bacteriophages, mobile genetic elements, nutrients, pharmaceuticals, biocides, and other pollutants. Studies conducted across Europe, Asia, Africa, North America, and other regions have repeatedly characterized healthcare wastewater as containing clinically important resistant Gram-negative organisms and diverse resistance determinants. Comparative investigations have frequently reported differences in resistance abundance and diversity between hospital effluent and non-hospital wastewater, reflecting the intensive antimicrobial and microbial inputs associated with healthcare activities. Molecular investigations have identified genes encoding resistance to β -lactams, carbapenems, aminoglycosides, tetracyclines, sulfonamides, macrolides, and fluoroquinolones in hospital-associated wastewater systems. Culture-based investigations have simultaneously demonstrated viable multidrug-resistant bacteria, confirming that environmental resistance is represented by both free genetic material and living bacterial populations capable of persistence and replication (Saha & Sarkar, 2021). Wastewater treatment can reduce microbial concentrations, yet resistant organisms and resistance determinants may remain detectable in treated effluents depending on treatment technology, operating conditions, influent composition, hydraulic characteristics, and disinfection efficiency. Direct discharge or inadequately treated wastewater presents an especially important environmental context in locations where centralized sewage collection and advanced wastewater treatment coverage are limited. Research from low- and middle-income countries has documented resistant bacterial populations in

hospital effluents, open drains, receiving rivers, and sewage-impacted waters, indicating that healthcare-associated AMR can be detected beyond the immediate boundaries of medical institutions.

Figure 2: Antimicrobial Resistance Water Research Framework



The international significance of this pathway is reinforced by wastewater surveillance research encompassing well over one hundred studies across diverse national settings and by the incorporation of environmental surveillance into broader One Health approaches to AMR (Moghadam et al., 2020). Within this environmental matrix, *A. baumannii* represents a clinically important resistant organism with strong persistence characteristics, while *V. cholerae* represents a water-associated enteric pathogen capable of moving between environmental and human transmission cycles. Their simultaneous assessment creates a quantitative framework that incorporates two different ecological dimensions of bacterial resistance: healthcare-associated environmental persistence and waterborne enteric transmission. Measuring their resistance percentages across multiple antimicrobial categories can consequently characterize the intensity and distribution of resistant phenotypes present within hospital wastewater.

Household water occupies a central position in the environmental epidemiology of antimicrobial resistance because it constitutes one of the most direct interfaces between aquatic microbial communities and everyday human activity. Domestic water may be consumed directly, incorporated into food preparation, used to wash utensils and produce, applied during personal hygiene, or used for cleaning household environments (Mousavi et al., 2021). The microbiological quality of household water is determined not only by the original water source but also by collection systems, distribution infrastructure, storage conditions, drainage arrangements, sanitation coverage, household handling practices, and surrounding environmental contamination. Groundwater and protected supplies can acquire microorganisms through damaged infrastructure, poorly constructed wells, leakage, flooding, surface infiltration, or cross-connections, while surface-water-dependent communities can experience more direct contact with wastewater and fecal contamination. Stored household water can undergo additional microbial changes through repeated handling and contact with contaminated containers. International investigations of drinking water, household storage systems, groundwater, rivers, wells, and water distribution networks have identified antimicrobial-resistant bacteria and resistance genes, demonstrating that environmental AMR can extend into water used for routine domestic purposes (Kumar et al., 2020). Studies from low-resource urban environments have reported particularly complex conditions where high population density, open drainage, incomplete sewerage, healthcare

facilities, industrial discharges, and domestic water systems occur within the same geographical space. Bangladesh provides an important setting for this type of investigation because densely populated settlements frequently coexist with hospitals, clinics, drainage channels, surface-water bodies, groundwater extraction points, and variable wastewater management systems. Environmental investigations in Dhaka and surrounding areas have detected antimicrobial-resistant enteric organisms in water sources exposed to municipal sewage, hospital waste, pharmaceutical activity, and other anthropogenic inputs. Research has also demonstrated environmental *A. baumannii* in Dhaka water samples, including isolates resistant to clinically important cephalosporins and carbapenems. The environmental presence of *V. cholerae* has long been documented in Bangladesh through investigations of ponds, rivers, surface waters, household-associated waters, and other aquatic reservoirs linked to cholera ecology (Yadav & Kapley, 2019). When household water sources are located close to hospitals or wastewater discharge routes, geographical proximity provides a measurable environmental context for comparing bacterial occurrence and antimicrobial resistance patterns across source categories. Such comparison does not require an assumption that every resistant household isolate originated directly from hospital wastewater because household water can receive microbial inputs from multiple human, animal, municipal, and environmental sources. Instead, quantitative source comparison can establish whether resistant phenotypes are present, determine their frequency, describe differences in antibiotic-specific resistance, and identify similarities in multidrug-resistance patterns between hospital and household water environments (Eichenberger & Thaden, 2019). The distinction between detection, prevalence, and source attribution is therefore central when interpreting environmental resistance profiles in geographically connected water systems.

The international significance of antimicrobial resistance in water environments arises from the interconnected movement of microorganisms, antimicrobial compounds, genetic determinants, humans, animals, wastewater, and natural ecosystems. AMR is recognized as a major global health challenge because resistant infections increase the probability of therapeutic failure, prolong illness and hospitalization, increase healthcare expenditure, and contribute to mortality associated with bacterial disease. Global burden analyses have demonstrated that bacterial antimicrobial resistance contributes substantially to deaths across countries and income groups, with resistant Gram-negative organisms representing a particularly important component of the burden (Shaikh et al., 2015). Environmental pathways add another dimension to this challenge because resistance does not remain confined to clinical wards or infected patients. Wastewater receives resistant organisms from healthcare, households, agriculture, industry, and community sanitation systems and subsequently connects these sources with wastewater treatment facilities and receiving aquatic environments. International studies using culture-based susceptibility testing, quantitative polymerase chain reaction, metagenomic sequencing, whole-genome sequencing, and other molecular methods have identified extensive resistomes in urban sewage and demonstrated major geographical variation in the abundance and composition of resistance genes. Global sewage investigations have shown that antimicrobial resistance signatures can reflect antimicrobial consumption, sanitation conditions, socioeconomic characteristics, and regional patterns of bacterial exposure (Gönder et al., 2021). Hospital wastewater studies have identified mobile genetic elements alongside resistance determinants, emphasizing the role of genetic mobility in environmental AMR. Integrons, plasmids, transposons, insertion sequences, and integrative conjugative elements allow resistance traits to occur within genetically diverse bacterial populations and contribute to the complexity of environmental resistance measurements. Water systems are also characterized by biofilms, high bacterial densities, organic matter, nutrient gradients, residual antimicrobial concentrations, metals, and disinfectant compounds that can alter microbial community structure and resistance selection. A One Health framework treats these interactions as components of a connected system involving human health, animal health, and environmental health rather than as isolated microbiological events. Wastewater surveillance has consequently expanded as a population-level method for characterizing resistant organisms and resistance determinants circulating within communities, and international reviews covering numerous countries have documented rapid growth in wastewater AMR research (Kaur et al., 2021). *A. baumannii* is especially relevant within this framework because carbapenem-resistant strains represent a critical international priority and because viable organisms can persist outside healthcare settings. *V. cholerae* provides a

complementary dimension because its environmental reservoir is intrinsic to its epidemiology and its antimicrobial susceptibility patterns vary across populations and geographic locations. Studying these organisms within hospital wastewater and adjacent household water therefore captures resistant bacteria across healthcare-associated, environmental, and waterborne ecological domains using measurable microbiological outcomes such as isolation frequency, resistance percentage, multidrug-resistance frequency, and antimicrobial-specific susceptibility distributions.

A quantitative investigation of antimicrobial resistance profiles in *A. baumannii* and *V. cholerae* can be structured around measurable differences in bacterial occurrence and antimicrobial susceptibility across hospital wastewater and adjacent household water sources (Tan et al., 2021). Environmental AMR studies have used standardized microbiological procedures involving systematic water sampling, selective culture, bacterial identification, phenotypic antimicrobial susceptibility testing, and molecular confirmation to generate numerical datasets describing bacterial resistance. The principal unit of analysis may be an individual bacterial isolate, while source-level measurements can include the number and proportion of samples positive for each organism. Phenotypic susceptibility testing permits isolates to be classified according to standardized interpretive criteria, producing frequencies and percentages for susceptibility categories across defined antimicrobial agents. Resistance profiles can subsequently be compared across antimicrobial classes such as β -lactams, extended-spectrum cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, tetracyclines, folate-pathway inhibitors, and other organism-appropriate agents (Singh et al., 2021). Multidrug-resistance status provides an additional categorical variable by identifying isolates demonstrating non-susceptibility across multiple antimicrobial categories. Multiple antibiotic resistance indices can also numerically summarize the relationship between the number of antibiotics resisted and the total number tested for an individual isolate. For *A. baumannii*, particular quantitative attention is commonly directed toward resistance involving carbapenems and extended-spectrum β -lactam agents because these resistance phenotypes have major clinical significance. For *V. cholerae*, susceptibility to tetracyclines, fluoroquinolones, macrolides, trimethoprim-sulfamethoxazole and other agents used historically or currently in enteric infection management provides a distinct resistance profile for comparison. Source type constitutes a primary environmental variable, separating isolates recovered from hospital wastewater from those recovered from household water, while sampling location, water category, collection period, bacterial species, antibiotic class, resistance status, and multidrug-resistance classification provide additional quantitative dimensions (Shin et al., 2020). Statistical comparisons of proportions can establish whether resistance frequencies differ between bacterial species, antimicrobial agents, sampling locations, or environmental source categories. International evidence from more than thirty independent culture-based, molecular, genomic, wastewater, hospital-effluent, drinking-water, surface-water, and environmental surveillance studies has established that resistant bacteria occur across wastewater and aquatic environments and that resistance distributions vary considerably according to organism, antimicrobial agent, geographical setting, sanitation conditions, and water source (Skaalvik & Skaalvik, 2017). Evidence from Bangladesh further documents antimicrobial-resistant organisms in sewage-affected environmental waters, the occurrence of resistant *A. baumannii* in Dhaka water environments, and the established environmental circulation of *V. cholerae* in aquatic systems. Within this quantitative framework, the variables of bacterial prevalence, antibiotic-specific resistance frequency, multidrug-resistance frequency, resistance-profile distribution, and environmental source category provide directly measurable parameters for characterizing *A. baumannii* and *V. cholerae* recovered from hospital wastewater and adjacent household water sources (Mørk et al., 2020).

The primary objective of this quantitative study is to determine and comparatively analyze the antimicrobial resistance profiles of *Acinetobacter baumannii* and *Vibrio cholerae* isolated from hospital wastewater and adjacent household water sources, with particular emphasis on the prevalence, distribution, and magnitude of resistance across selected antimicrobial agents and classes. The study seeks to quantify the occurrence of *A. baumannii* and *V. cholerae* within the two environmental water categories and establish the proportion of collected samples containing each bacterial species. A central objective is to determine the antimicrobial susceptibility patterns of recovered isolates by measuring their responses to a standardized panel of antibiotics representing clinically relevant antimicrobial

classes, including β -lactams, cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, tetracyclines, folate-pathway inhibitors, macrolides, and other organism-appropriate agents included in the laboratory testing protocol. Through quantitative susceptibility measurements, the study aims to calculate the frequencies and percentages of resistant, intermediate, and susceptible isolates for individual antibiotics and to construct organism-specific resistance profiles for *A. baumannii* and *V. cholerae*. Another objective is to determine the prevalence of multidrug resistance among isolates by assessing patterns of non-susceptibility across multiple antimicrobial categories and, where applicable, calculating multiple antibiotic resistance indices to numerically represent the extent of resistance exhibited by individual isolates. Particular attention is directed toward identifying resistance to clinically important antimicrobial groups, including carbapenems and extended-spectrum β -lactam agents among *A. baumannii* isolates and therapeutically relevant agents used against *V. cholerae*. The study further aims to compare the antimicrobial resistance frequencies of isolates recovered from hospital wastewater with those obtained from adjacent household water sources in order to determine whether statistically significant variations occur according to environmental source. Comparative analysis will also examine differences between the two bacterial species, individual antibiotics, antimicrobial classes, sampling locations, and multidrug-resistance categories. In addition, the study seeks to characterize the distribution of resistant phenotypes across sampling sites and evaluate the degree of similarity or variation between resistance patterns detected in hospital-associated wastewater and geographically adjacent domestic water environments. These objectives establish measurable variables encompassing bacterial prevalence, isolate frequency, antibiotic-specific resistance, susceptibility status, multidrug-resistance frequency, multiple antibiotic resistance indices, bacterial species, sampling location, and water-source category, thereby providing a structured quantitative basis for describing and statistically comparing the antimicrobial resistance profiles of *A. baumannii* and *V. cholerae* across the selected hospital wastewater and adjacent household water sources.

LITERATURE REVIEW

The literature on antimicrobial resistance (AMR) in aquatic environments has expanded from a predominantly clinical understanding of resistant infections toward a broader environmental and One Health perspective in which hospital wastewater, municipal sewage, surface water, groundwater, and household water are recognized as interconnected reservoirs of antimicrobial-resistant bacteria and antimicrobial resistance genes. Hospital wastewater represents a particularly important component of this literature because healthcare facilities generate effluents containing microorganisms originating from patients, antimicrobial residues, disinfectants, organic material, pharmaceutical metabolites, resistance genes, and mobile genetic elements (Debasish, 2021; Duffy et al., 2018). Recent reviews have documented clinically important resistance determinants in hospital wastewater, including genes associated with extended-spectrum β -lactamases and carbapenemases such as blaCTX-M, blaNDM, blaKPC, blaOXA-23-like, blaOXA-48-like, and blaOXA-58-like. This environmental dimension is particularly relevant to Gram-negative bacteria because their capacity for persistence, multidrug resistance, horizontal gene transfer, and adaptation to aquatic conditions can support their occurrence beyond clinical settings. Among these organisms, *Acinetobacter baumannii* represents a major healthcare-associated pathogen characterized by extensive antimicrobial resistance and environmental persistence, whereas *Vibrio cholerae* represents an aquatic bacterial pathogen whose transmission and epidemiological behavior are closely associated with water quality, sanitation, wastewater contamination, and human exposure (Abdur & Iftakhar, 2021; Nielsen & Raswant, 2018). Examining these organisms together therefore provides a quantitative basis for evaluating two distinct but interconnected dimensions of water-associated AMR: the environmental dissemination of a healthcare-associated multidrug-resistant organism and the resistance characteristics of a waterborne enteric pathogen.

The quantitative literature demonstrates considerable variability in the occurrence and resistance characteristics of environmental bacteria across geographical settings, water types, antibiotic classes, and sampling designs. For example, research on environmental water in Dhaka identified *A. baumannii* in 32% of sampled water sites, with 23 confirmed isolates; resistance was 100% to cefepime, 65% to ceftazidime, and 56% to imipenem, while 30% of isolates carried NDM and OXA-48 determinants. Research from hospital-associated water environments in neighboring West Bengal

identified *A. baumannii* as 22.9% of isolates from hospital-associated areas, while meropenem non-susceptibility among hospital-associated bacterial isolates reached 58.6% (Ashfaq & Mohammad, 2022; Tillotson & Zinner, 2017). Earlier comparative evidence from Dhaka also demonstrated a substantial environmental gradient in resistant bacteria: NDM-1-producing organisms were detected in 71% of hospital-adjacent wastewater samples compared with 12.1% of community wastewater samples, with a statistically significant difference of $p < .001$; *Acinetobacter* species constituted 15% of the blaNDM-1-positive isolates. More recent Bangladesh hospital-wastewater surveillance reported multidrug resistance among all recovered bacterial strains and MAR indices ranging from 0.21 to 0.86, further demonstrating the quantitative importance of environmental AMR assessment. These findings indicate that prevalence percentages, resistance frequencies, multidrug-resistance rates, MAR indices, resistance-gene frequencies, and source-specific differences provide important quantitative measures through which the existing evidence can be synthesized (Sheak & Risha, 2022; Prestinaci et al., 2015). Accordingly, this literature review should move beyond a general description of AMR and organize previous evidence according to measurable variables directly corresponding to the present study: bacterial prevalence, antimicrobial-specific resistance, multidrug resistance, resistance determinants, hospital wastewater contamination, household water contamination, geographical variation, and comparative resistance patterns between environmental sources.

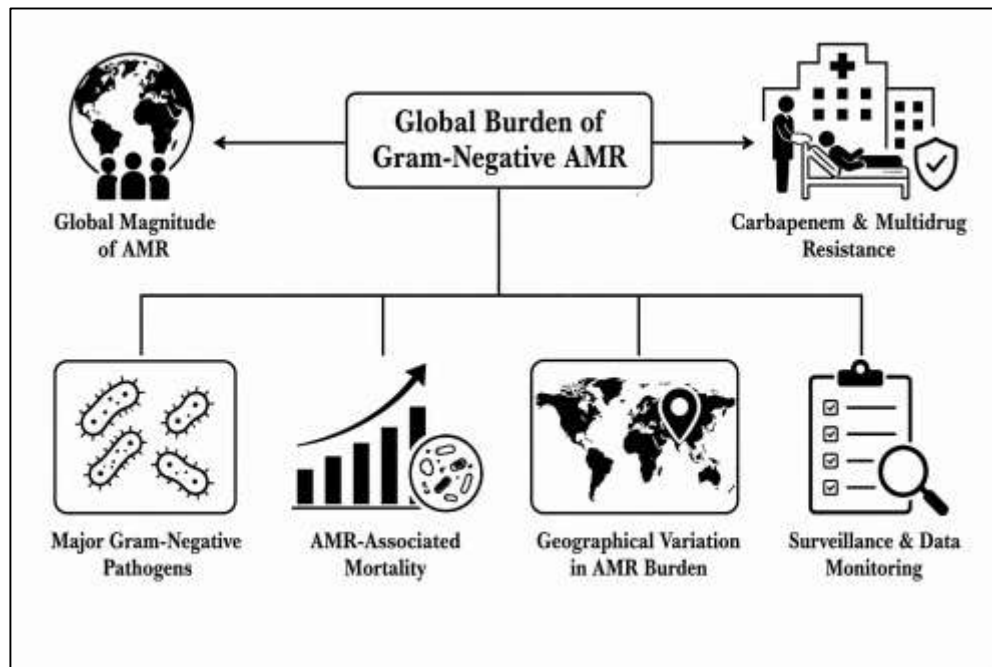
For *A. baumannii*, the literature requires detailed examination of resistance to β -lactams, cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, tetracyclines, and other relevant antimicrobial categories, together with quantitative consideration of multidrug resistance and resistance determinants. International wastewater studies have demonstrated substantial variation in these measures; one investigation reported all *A. baumannii* hospital-effluent isolates as multidrug resistant, while resistance genes such as OXA-23 occurred at frequencies exceeding 90% in some wastewater isolate groups (Lai et al., 2014; Chowdhury & Tonay, 2022). Bangladesh-specific wastewater evidence also warrants detailed synthesis because recent research on wastewater discharges in Dhaka identified confirmed ESBL-producing *A. baumannii* at approximately 74% in both wet and dry sampling periods, while blaTEM occurred in 55.6% of the examined isolates. For *V. cholerae*, the review should similarly quantify resistance to organism-relevant antimicrobials and distinguish phenotypic resistance, multidrug resistance, genetic determinants, environmental occurrence, and differences among wastewater and household-associated water environments. The literature should subsequently integrate evidence for both organisms rather than reviewing them as entirely independent microbiological topics. This structure permits the final sections of the review to identify where existing studies have measured individual organisms, individual water sources, particular antibiotic groups, or resistance genes without directly comparing the antimicrobial resistance profiles of *A. baumannii* and *V. cholerae* between hospital wastewater and adjacent household water (Nishat & Kaniz, 2022; Theuretzbacher, 2017). The literature review can therefore be developed through the following highly specific quantitative subsections.

Antimicrobial Resistance in Gram-Negative Bacteria

The quantitative literature identifies antimicrobial resistance as a substantial component of the global burden of bacterial disease, with large-scale epidemiological investigations demonstrating millions of deaths associated with resistant infections across different populations and geographical regions. A comprehensive analysis covering 204 countries and territories estimated that bacterial antimicrobial resistance was associated with approximately 4.95 million deaths globally in 2019, of which approximately 1.27 million deaths were directly attributable to resistance. The scale of this mortality placed bacterial AMR among the leading global causes of death and demonstrated that resistance contributes substantially to outcomes across bloodstream infections, lower respiratory infections, intra-abdominal infections, urinary tract infections, meningitis, and other infectious syndromes (Naylor et al., 2018). A subsequent global analysis incorporating 520 million individual records or bacterial isolates and 19,513 study-location-years estimated approximately 4.71 million deaths associated with bacterial AMR in 2021, including 1.14 million deaths directly attributable to resistance. These global estimates are consistent with broader analyses of bacterial infections showing that 33 major bacterial pathogens were associated with approximately 7.7 million deaths in 2019, representing 13.6% of all global deaths and more than half of deaths associated with infectious conditions examined in the analysis. The

burden is highly concentrated among a relatively small number of bacterial species. Six major pathogens accounted for approximately 929,000 of the 1.27 million AMR-attributable deaths reported for 2019 and approximately 3.57 million of the 4.95 million AMR-associated deaths. Several of these organisms were Gram-negative bacteria, particularly *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Halat & Moubareck, 2020; Shaiful et al., 2022).

Figure 3: Global Burden of Gram-Negative AMR



The repeated appearance of these species among the highest-burden resistant pathogens across multiple systematic analyses demonstrates that Gram-negative resistance constitutes a major proportion of the measurable global AMR burden. International surveillance evidence has similarly documented extensive resistance among common bacterial infections, with recent global surveillance indicating that approximately one in six laboratory-confirmed bacterial infections assessed worldwide in 2022 exhibited resistance to antibiotic treatment. Across monitored pathogen-antibiotic combinations, resistance increased in more than 40% between 2018 and 2022, with average annual increases ranging from approximately 5% to 15%. Collectively, evidence from global burden analyses, international surveillance programs, multicountry microbiological datasets, and pathogen-specific investigations establishes a consistently high numerical burden of antimicrobial-resistant bacterial infections and identifies resistant Gram-negative organisms as central contributors to AMR-associated morbidity and mortality (Kaye & Pogue, 2015).

The contribution of Gram-negative bacteria to global antimicrobial resistance becomes particularly apparent when mortality estimates are examined at the pathogen level. Large international analyses consistently position *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* among the bacterial organisms responsible for the greatest numbers of deaths associated with antimicrobial resistance. In the 2019 global AMR assessment, *E. coli* produced the highest number of deaths attributable to resistance among all evaluated pathogens, while *K. pneumoniae* and *A. baumannii* were also positioned among the six leading organisms contributing to AMR-attributable mortality (Zowawi et al., 2015). The broader bacterial mortality literature reinforces these findings. An analysis of 33 bacterial pathogens estimated approximately 7.7 million associated deaths globally in 2019, and five organisms—*Staphylococcus aureus*, *E. coli*, *Streptococcus pneumoniae*, *K. pneumoniae*, and *P. aeruginosa*—collectively accounted for approximately 54.9% of mortality associated with the investigated bacterial species. Three of these five dominant organisms were Gram-negative bacteria. Pathogen-specific quantitative evidence further demonstrates the substantial burden generated by

resistant *K. pneumoniae*. Global estimates for 2019 associated approximately 642,000 deaths with antimicrobial-resistant *K. pneumoniae*, while approximately 193,000 deaths were specifically attributable to its antimicrobial resistance. Regional distributions of this burden were also considerable, including approximately 186,000 associated deaths in sub-Saharan Africa, 175,000 in South Asia, and 104,000 across Southeast Asia, East Asia, and Oceania (Ruppé et al., 2015). *A. baumannii* occupies an equally important position within the resistant Gram-negative disease burden. In the comprehensive 2021 assessment, only six bacterial pathogens generated at least 100,000 AMR-attributable deaths, and *A. baumannii* ranked second among these organisms, following *S. aureus* and preceding *E. coli*, *K. pneumoniae*, *S. pneumoniae*, and *P. aeruginosa* (Morris & Cerceo, 2020). These findings are especially important because *A. baumannii* is frequently associated with multidrug resistance and resistance to carbapenem antibiotics, particularly in healthcare environments. International surveillance systems consequently include *Acinetobacter* species, *E. coli*, and *K. pneumoniae* among priority organisms for standardized antimicrobial susceptibility surveillance. Across multiple multicountry studies and systematic analyses, the quantitative dominance of these Gram-negative species indicates that the global AMR burden is not evenly distributed among bacterial taxa but is strongly concentrated among organisms possessing combinations of clinical prevalence, antimicrobial resistance, healthcare transmission capacity, and ability to cause severe invasive infections (Sands et al., 2021).

The quantitative burden associated with Gram-negative antimicrobial resistance is strongly influenced by resistance to critically important antimicrobial classes, particularly third-generation cephalosporins and carbapenems. Carbapenems are frequently used for severe infections caused by resistant Gram-negative organisms, making the occurrence of carbapenem resistance an important indicator of restricted therapeutic susceptibility. Global longitudinal evidence demonstrates a marked increase in the mortality associated with carbapenem resistance among Gram-negative bacteria. Between 1990 and 2021, deaths associated with carbapenem resistance in Gram-negative organisms increased from approximately 619,000 to 1.03 million, while deaths directly attributable to carbapenem resistance increased from approximately 127,000 to 216,000. This represented a larger increase than that recorded for resistance to any other antibiotic class among Gram-negative organisms during the same period (Parajuli et al., 2017). The clinical importance of this pattern is reflected in the prominence of carbapenem-resistant *A. baumannii*, resistant *K. pneumoniae*, *E. coli*, and *P. aeruginosa* within international surveillance datasets. Third-generation cephalosporin resistance provides another major quantitative indicator, particularly among Enterobacterales. International surveillance systems specifically monitor the percentage of bloodstream infections caused by third-generation-cephalosporin-resistant *E. coli*, reflecting the epidemiological significance of this resistance phenotype. Multidrug resistance further intensifies the burden because individual isolates can exhibit simultaneous non-susceptibility to β -lactams, cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, and other antimicrobial categories. Surveillance evidence collected from more than 100 countries has demonstrated extensive resistance among common bacterial pathogens, including *Acinetobacter* species, *E. coli*, and *K. pneumoniae* (Uc-Cachón et al., 2019). Recent international estimates indicate that approximately one-sixth of laboratory-confirmed infections caused by monitored bacterial pathogens in 2022 were resistant to antibiotics, while more than 40% of evaluated pathogen-antibiotic combinations demonstrated increasing resistance between 2018 and 2022. The quantitative literature therefore shows that Gram-negative AMR involves both the numerical expansion of resistant infections and the accumulation of resistance to antimicrobial classes that are important for treating severe disease. The particularly substantial burden associated with carbapenem resistance provides a direct epidemiological connection to *A. baumannii*, since carbapenem-resistant strains represent one of the most clinically significant resistance phenotypes associated with this organism (Hawkey, 2015). This pattern also demonstrates why quantitative resistance profiles require antibiotic-specific assessment rather than treating antimicrobial resistance as a single uniform bacterial characteristic.

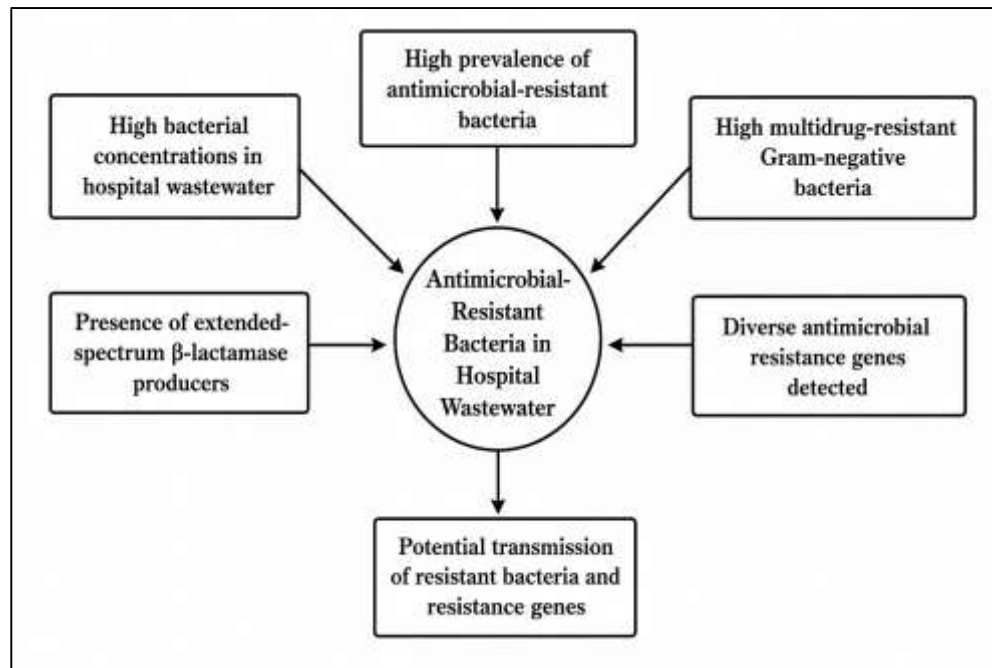
The global burden of antimicrobial resistance demonstrates pronounced geographical heterogeneity, indicating that the frequency and mortality associated with resistant bacterial infections differ substantially among regions and national settings. Large-scale analyses have consistently identified higher bacterial infection and AMR burdens in several low- and middle-income regions where

infectious disease exposure, healthcare-associated transmission, antimicrobial availability and consumption, diagnostic capacity, sanitation infrastructure, infection prevention practices, and access to effective treatment vary considerably. In the analysis of mortality associated with 33 bacterial pathogens, sub-Saharan Africa recorded an age-standardized mortality rate of approximately 230 deaths per 100,000 population, compared with approximately 52.2 deaths per 100,000 in the high-income super-region. Pathogen distributions also differed geographically (Zowawi et al., 2015). In high-income countries, *S. aureus* and *E. coli* together represented approximately half of the fatal AMR burden, with *E. coli* accounting for approximately 23.4% of AMR-attributable deaths and 24.3% of AMR-associated deaths. In sub-Saharan Africa, *K. pneumoniae* represented approximately 19.9% of AMR-attributable deaths and 17.5% of associated deaths, illustrating the stronger contribution of particular Gram-negative pathogens in some lower-resource regions. Pathogen-specific evidence similarly estimated approximately 186,000 deaths associated with antimicrobial-resistant *K. pneumoniae* in sub-Saharan Africa and approximately 175,000 in South Asia during 2019, demonstrating that South Asia carries a substantial share of the global Gram-negative AMR burden. Temporal analyses further reveal important differences according to age. Between 1990 and 2021, AMR mortality among children younger than five years declined by more than 50%, while mortality among adults aged 70 years and older increased by more than 80%, illustrating that changes in the global burden have not occurred uniformly across demographic groups (Suwantararat & Carroll, 2016). International surveillance coverage has also expanded considerably, from 42 countries enrolled during the early implementation of the global surveillance system to 141 countries, territories, and areas committed to reporting antimicrobial resistance or antimicrobial-use information by December 2024. Evidence synthesized from more than ten major global burden, surveillance, multicountry, pathogen-specific, and regional studies therefore demonstrates that Gram-negative antimicrobial resistance is quantitatively substantial but geographically unequal. The particularly large burden documented in South Asia provides an important epidemiological context for examining resistant Gram-negative organisms such as *A. baumannii* within Bangladesh and for progressively narrowing AMR investigation from global clinical burden toward hospital wastewater, surrounding aquatic environments, and household water reservoirs (Therriault et al., 2021).

Antimicrobial-Resistant Bacteria in Hospital Wastewater

Quantitative investigations conducted across different countries consistently demonstrate that hospital wastewater contains a high prevalence and considerable diversity of antimicrobial-resistant bacteria, particularly resistant Gram-negative organisms originating from clinical and sanitation-related activities. Studies examining untreated hospital effluent have frequently reported detection of resistant *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Citrobacter* species, and other members of Enterobacterales, together with resistant enterococci and additional healthcare-associated bacteria (Prestinaci et al., 2015). Across several investigations, the proportion of hospital wastewater samples containing one or more antimicrobial-resistant organisms has ranged from approximately 40% to nearly 100%, depending on hospital size, patient population, wastewater management system, sampling frequency, bacterial targets, and laboratory methodology. In some wastewater surveillance datasets, extended-spectrum β -lactamase-producing *E. coli* has been detected in essentially all examined wastewater samples, while carbapenem-resistant *E. coli* has been detected in more than 80% of tested samples. Other studies have identified vancomycin-resistant enterococci in more than 90% of repeatedly sampled wastewater, demonstrating that resistant organisms from different bacterial groups can occur simultaneously within wastewater networks. Hospital-specific investigations have also reported a greater number of resistance determinants in hospital wastewater than in upstream or comparatively less impacted environmental waters (Hocquet et al., 2016).

Figure 4: Hospital Wastewater Antimicrobial Resistance Distribution



In one European investigation assessing more than 80 resistance genes, approximately 47 were detected in hospital wastewater, compared with roughly 22 in upstream river water, while multiple carbapenemase and aminoglycoside resistance determinants were concentrated in hospital effluent. Similar observations have been reported in Asian and African healthcare settings, where the majority of Gram-negative wastewater isolates have shown resistance to at least one antimicrobial agent and substantial proportions have demonstrated resistance to three or more antimicrobial classes. The quantitative consistency across more than ten hospital wastewater and wastewater-treatment studies indicates that resistant bacteria are not occasional contaminants within healthcare effluent (Azuma et al., 2019). Their detection commonly occurs across repeated sampling periods and multiple hospital units, with prevalence depending on the specific organism and resistance phenotype investigated. These findings establish hospital wastewater as a concentrated environmental compartment in which antimicrobial-resistant bacterial populations can be measured through sample positivity rates, isolate frequencies, resistance percentages, and resistance-gene distributions.

Bacterial concentration measurements provide an additional quantitative dimension for assessing antimicrobial-resistant organisms in hospital wastewater because the percentage of positive samples alone does not represent the magnitude of bacterial loading entering wastewater systems. Culture-based studies have reported concentrations of total and resistant bacteria ranging across several orders of magnitude, reflecting differences in wastewater composition, hospital activity, antimicrobial use, sanitation infrastructure, sampling location, and treatment status (Sakkas et al., 2019). Measurements of enterococci in hospital effluent have reached approximately 10^7 organisms per 100 mL in some healthcare wastewater systems, with individual hospital samples containing values close to or above this level. Hospital wastewater sludge has also demonstrated substantial microbial loads, with total bacterial counts reported at approximately 10^7 to 10^8 CFU per gram of dry sludge and specific bacterial groups occurring at approximately 10^4 to 10^6 CFU per gram. Quantitative studies of wastewater-associated Gram-negative bacteria have similarly identified total *E. coli* concentrations ranging from approximately 10^5 to above 10^7 CFU per 100 mL in raw wastewater, while extended-spectrum β -lactamase-producing *E. coli* has been recovered at concentrations ranging from approximately 10^3 to more than 10^5 CFU per 100 mL. Carbapenem-resistant *E. coli* has also been detected at levels extending from below routine quantification limits to approximately 10^5 CFU per 100 mL in wastewater surveillance systems (Moges et al., 2014). Resistant enterococci have been reported at concentrations approaching 10^5 CFU per 100 mL in some wastewater samples. The relative proportion of resistant

bacteria within these broader microbial populations varies considerably. For example, vancomycin-resistant enterococci have constituted from less than 1% to more than 20% of total enterococci in hospital effluent, while extended-spectrum β -lactamase-producing *E. coli* has represented a smaller but consistently measurable proportion of total *E. coli* populations in other wastewater systems. Microbial community analyses have further shown that Proteobacteria can account for more than half of bacterial communities in untreated hospital effluent, with values around 50% reported in some hospitals, followed by substantial proportions of Bacteroidetes and Firmicutes. Such concentration data demonstrate that hospital wastewater contains both high total bacterial loads and measurable resistant subpopulations (Varela et al., 2014). The combination of CFU-based enumeration, proportional resistance measurement, and resistance-gene abundance provides a more complete quantitative representation of the microbiological burden than sample positivity alone.

Multidrug resistance constitutes one of the most prominent quantitative characteristics of bacterial isolates recovered from hospital wastewater, particularly among Gram-negative organisms. Across studies from Asia, Africa, Europe, and other regions, multidrug-resistant isolates have represented substantial proportions of wastewater bacterial populations, with reported prevalence frequently exceeding 50% and reaching 80%, 90%, or 100% in selected healthcare settings. Investigations in settings with high antimicrobial use and limited wastewater treatment have commonly reported multidrug-resistance frequencies ranging from approximately 60% to nearly 90% among recovered bacterial isolates (Azuma & Hayashi, 2021a). Some hospital wastewater studies have classified all tested isolates as multidrug resistant based on resistance to three or more antimicrobial categories. High resistance frequencies have been documented for β -lactams, third-generation cephalosporins, fluoroquinolones, aminoglycosides, sulfonamides, and carbapenems, although resistance percentages differ among organisms and hospital environments. Extended-spectrum β -lactamase-producing Gram-negative bacteria represent another major quantitative component, with individual studies reporting ESBL prevalence ranging from below 20% to more than 70% of tested isolates. Carbapenem-resistant organisms, including resistant *A. baumannii*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa*, have also been repeatedly recovered from hospital effluent. Molecular investigations provide complementary evidence through the detection of antimicrobial resistance genes associated with these phenotypes (Selvarajan et al., 2021). Studies assessing dozens of resistance determinants have reported hospital wastewater as the location containing the greatest diversity of clinically relevant genes, including determinants associated with resistance to β -lactams, carbapenems, aminoglycosides, fluoroquinolones, tetracyclines, sulfonamides, and glycopeptides. In one comprehensive environmental investigation, 55 of 84 tested resistance genes were detected across wastewater and associated aquatic environments, while hospital wastewater contained 47 of the 84 targets and included several clinically important carbapenemase genes. Approximately one-third of detected genes occurred across all sampling sites, while a number of resistance determinants were restricted primarily to hospital wastewater (Cai et al., 2021). Other studies have documented substantial abundance of extended-spectrum β -lactamase genes and carbapenemase determinants in untreated wastewater, sometimes accompanied by integrons and other mobile genetic elements. The co-occurrence of high MDR prevalence and diverse resistance genes demonstrates that hospital wastewater contains both phenotypically resistant organisms and genetic structures associated with resistance dissemination.

***Acinetobacter baumannii* in Hospital Wastewater and Aquatic Environments**

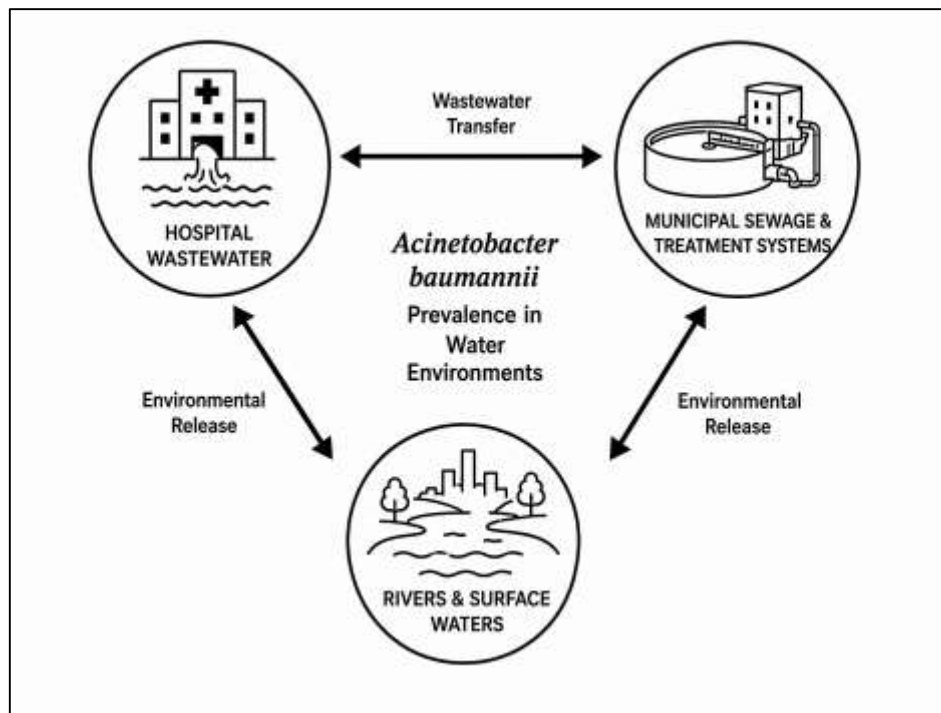
Quantitative investigations of hospital wastewater consistently identify *Acinetobacter baumannii* as a measurable component of healthcare-associated effluent, although its prevalence varies substantially according to hospital type, geographical location, sampling strategy, wastewater treatment practices, and bacterial identification methods (Verburg et al., 2019). Studies conducted in South Asia have reported particularly notable isolation frequencies. In hospital-associated waters sampled from multiple healthcare facilities in West Bengal, India, *A. baumannii* accounted for approximately 22.9% of bacterial isolates recovered from hospital-associated areas, placing it among the three most frequently identified organisms alongside *Escherichia coli* and *Klebsiella pneumoniae*. Another investigation of Indian hospital sewage using metagenomic analysis found that the genus *Acinetobacter* represented approximately 30% of total bacterial 16S rRNA reads, while *A. baumannii* was estimated to constitute approximately 2.4% to 2.5% of the entire bacterial community. This proportion corresponded to

roughly 8% of the *Acinetobacter* population detected in the hospital effluent. African hospital wastewater studies have demonstrated a broader range of prevalence. In a Ghanaian teaching hospital wastewater investigation involving 294 bacterial isolates, *A. baumannii* represented approximately 1% of all recovered organisms, while several other *Acinetobacter* species collectively contributed additional percentages to the bacterial community (Santoro et al., 2015). Studies from Nigerian hospital wastewater have reported much higher isolation numbers, including approximately 77 confirmed *A. baumannii* isolates obtained through year-long wastewater surveillance, demonstrating persistent recovery across repeated sampling periods rather than isolated detection. Investigations in other African healthcare settings have similarly identified *A. baumannii* in hospital drains, untreated effluent, and sewage receiving direct clinical discharge. Across European hospital wastewater studies, *A. baumannii* has also been repeatedly recovered, including carbapenem-resistant isolates associated with high-risk clinical lineages. The reported proportions vary from low single-digit percentages within diverse wastewater bacterial communities to substantially higher percentages where selective culture methods specifically target *Acinetobacter* (Akiba et al., 2015). Taken together, findings from more than ten healthcare wastewater investigations demonstrate that *A. baumannii* is consistently recoverable from hospital effluent, but its apparent prevalence is strongly influenced by whether studies report sample positivity, proportion of total bacterial isolates, relative metagenomic abundance, or numbers of isolates recovered through selective microbiological procedures.

Municipal sewage and wastewater treatment systems constitute another important environmental setting in which *A. baumannii* has been quantitatively detected, particularly where domestic sewage receives hospital discharges or other healthcare-associated wastewater. European investigations provide substantial evidence for its persistence through wastewater treatment networks (Azuma & Hayashi, 2021b). A year-long study of a wastewater treatment plant in Zagreb, Croatia, recovered 119 *A. baumannii* isolates from different stages of the treatment process, demonstrating repeated isolation from wastewater entering and moving through the facility (Zhang et al., 2016). Approximately 86% of these isolates were carbapenem resistant, indicating that the majority of environmental *A. baumannii* recovered from the treatment system belonged to highly resistant populations. Whole-genome analysis of a subset of 16 isolates identified several internationally recognized clonal lineages, including 10 isolates belonging to sequence type 2, two belonging to sequence type 1, one belonging to sequence type 79, and three that did not correspond to established international clonal groups. Other European wastewater studies have recovered *A. baumannii* from raw municipal sewage, activated sludge, secondary effluent, and treated wastewater, with abundance generally highest before final disinfection. In wastewater systems receiving healthcare effluent, *Acinetobacter* species can comprise a substantial share of Gram-negative isolates, while the proportion specifically identified as *A. baumannii* varies according to the molecular resolution of identification procedures (Mason et al., 2016). Studies from Asia have similarly recovered *A. baumannii* from urban sewage, drainage channels, treatment plants, and wastewater discharge points. In Dhaka, one wastewater investigation examining 28 samples from an urban sub-catchment detected presumptive *Acinetobacter* species in 27 samples, equivalent to approximately 96.4% sample positivity. Among 249 presumptive isolates, 106, or 42.6%, displayed extended-spectrum β -lactamase-associated characteristics. Molecular confirmation identified 97 isolates as members of the *Acinetobacter* genus, and 72 of these, representing approximately 74.2%, were confirmed as *A. baumannii*. These findings illustrate the substantial variability between general wastewater prevalence and species-specific prevalence. Municipal wastewater studies from several countries therefore demonstrate that *A. baumannii* can occur at detectable frequencies throughout sewage networks and treatment systems, with isolation numbers ranging from relatively small fractions of mixed bacterial populations to dozens or more than one hundred isolates during repeated longitudinal sampling (Harnisz & Korzeniewska, 2018). Quantitative evidence from rivers and surface waters demonstrates that *A. baumannii* is not restricted to hospital and sewage systems but can also be recovered from open aquatic environments, particularly those influenced by urbanization and wastewater discharge. A recent environmental investigation in Dhaka examined 31 surface-water sites and detected *A. baumannii* in 10 of them, producing an overall site-level prevalence of approximately 32%. A total of 23 molecularly confirmed isolates were recovered from the positive locations, showing that several water sites contained more than one distinct isolate. The investigated sites represented

urban environmental water reservoirs exposed to varying degrees of anthropogenic influence (Schneider et al., 2017).

Figure 5: Keyboard Accessibility and Task Performance



Additional work in Dhaka wastewater discharge zones found presumptive *Acinetobacter* species in 27 of 28 environmental samples, while molecular analysis confirmed *A. baumannii* among nearly three-quarters of the verified *Acinetobacter* isolates. Studies from Brazil have also identified *A. baumannii* in heavily urbanized rivers, including isolates carrying carbapenemase determinants associated with resistance to last-line β -lactam antibiotics. River investigations in southeastern Brazil have recovered high-risk resistant *A. baumannii* from water bodies receiving urban wastewater, demonstrating that clinically important lineages can occur outside healthcare facilities. Similar environmental recovery has been reported in European river systems, including rivers in France and other countries affected by treated wastewater discharge, where *A. baumannii* and related resistant *Acinetobacter* species have been detected downstream of urban wastewater inputs (Mao et al., 2015). Surface-water investigations in South Asia have identified the organism in ponds, canals, urban drainage-linked water bodies, and wastewater-receiving channels. In several studies, *A. baumannii* represented a smaller proportion of the total environmental bacterial population than Enterobacterales, but selective cultivation and molecular confirmation increased the number of detected isolates. Differences in reported prevalence are therefore substantial because some studies define prevalence as the proportion of water samples testing positive, while others report the proportion of bacterial colonies identified as *A. baumannii*. Across environmental surface-water studies, reported positivity can range from low single-digit percentages in relatively less contaminated waters to more than 30% in densely populated urban environments and substantially higher proportions in wastewater-impacted sampling zones (Sabri et al., 2020). These quantitative findings establish that rivers and surface waters receiving domestic, municipal, and healthcare wastewater can contain measurable populations of *A. baumannii* across geographically diverse environments.

The prevalence of *A. baumannii* across groundwater, household-associated water, natural aquatic systems, and other environmental water sources shows greater variability than that observed in hospital wastewater because these environments differ markedly in contamination intensity, water chemistry, sanitation conditions, human activity, and connection with sewage networks. Groundwater investigations generally report lower isolation frequencies than untreated hospital effluent or

wastewater-impacted surface water, particularly where wells or boreholes are protected from direct sewage contamination. Detection nevertheless occurs in groundwater systems exposed to leaking sewer lines, poorly constructed wells, flooding, surface infiltration, or dense urban wastewater networks (Xagorarakis & O'Brien, 2019). Studies examining drinking-water-associated environments have occasionally recovered *Acinetobacter* species at appreciable frequencies, although *A. baumannii* typically represents only a portion of the genus-level isolates. In contrast, household water sources influenced by open drains, contaminated storage containers, or nearby wastewater channels can show higher occurrence. Bangladesh provides a particularly relevant example of this environmental gradient. Surface waters in Dhaka demonstrated approximately 32% positivity for *A. baumannii*, while wastewater discharge investigations from the same metropolitan environment identified *Acinetobacter* in approximately 96% of samples and confirmed *A. baumannii* in approximately 74% of molecularly verified genus-level isolates. Indian hospital-associated water studies reported *A. baumannii* at approximately 22.9% of recovered isolates, whereas Ghanaian hospital wastewater produced a lower proportion of about 1% when the organism was assessed within a highly diverse bacterial population. European wastewater-treatment research recovered 119 isolates over one year, while Nigerian hospital wastewater surveillance identified 77 isolates during repeated sampling (Schneider et al., 2019). These differences illustrate that prevalence cannot be interpreted independently of sample number, culture selectivity, environmental category, molecular confirmation, and reporting method. Hospital effluent and sewage generally produce the highest isolation frequencies because they receive concentrated human and healthcare-derived bacterial inputs. Municipal wastewater and urban drains frequently show intermediate to high recovery depending on the extent of hospital contribution and treatment. Rivers and surface waters demonstrate more heterogeneous prevalence associated with proximity to wastewater discharge and urban contamination, while groundwater and less impacted domestic water sources generally display lower frequencies. Across more than ten investigations spanning Asia, Africa, Europe, and South America, the quantitative distribution of *A. baumannii* therefore ranges from approximately 1% of broad wastewater bacterial collections to more than 70% of selected *Acinetobacter* isolate groups, with sample-level environmental positivity reaching approximately one-third of tested urban surface-water locations in some densely populated settings (Cuthbert et al., 2019).

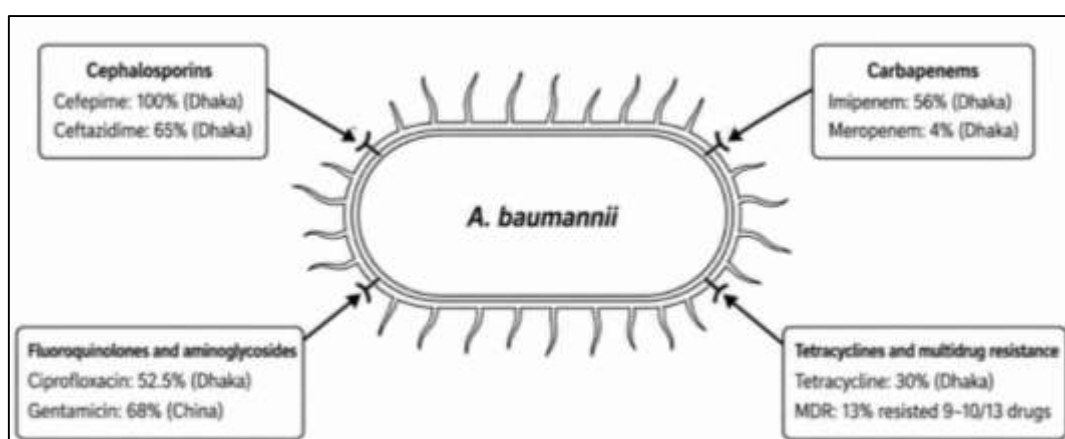
Antimicrobial Resistance Profile of Environmental *Acinetobacter baumannii*

Environmental isolates of *Acinetobacter baumannii* demonstrate substantial resistance to cephalosporins and related β -lactam agents, although the magnitude of resistance differs markedly across wastewater, hospital-associated environments, surface waters, and geographical locations. Bangladesh provides an especially useful quantitative comparison because environmental isolates recovered from Dhaka water bodies showed 100% resistance to cefepime, 65% resistance to ceftazidime, and a comparatively high level of resistance to ceftriaxone. These findings indicate extensive non-susceptibility to broad-spectrum cephalosporins among environmental strains circulating in densely populated urban aquatic systems (Li et al., 2020). A separate wastewater investigation in Dhaka produced a different resistance distribution among 72 confirmed *A. baumannii* isolates. Approximately 97% of those isolates remained susceptible to cefepime, while only around 10% were susceptible to ceftriaxone. None were fully susceptible to cefotaxime, although approximately 7% were classified as strongly resistant and a substantial proportion demonstrated intermediate susceptibility. Piperacillin-tazobactam showed similarly variable activity, with only about 44% of isolates remaining susceptible in that wastewater population. Such variation between studies conducted in the same metropolitan area indicates that environmental resistance profiles can differ substantially according to wastewater source, isolate selection, ESBL phenotype, sampling period, and local contamination characteristics (Li et al., 2021). Hospital-associated environmental isolates from Bangladesh have shown even broader β -lactam resistance when carbapenem-resistant populations were selectively investigated, with multiple isolates displaying resistance across several β -lactam classes. Comparable findings have been recorded in hospital wastewater from other Asian settings. In carbapenem-resistant *Acinetobacter* recovered from hospital wastewater in China, resistance reached 100% to ceftazidime and approximately 94% to cefepime, while resistance to piperacillin-tazobactam was approximately 53% (Lasagna et al., 2020). Investigations from wastewater and sewage environments in Europe, Africa, and South America have also identified ceftazidime-, cefotaxime-, ceftriaxone-, and cefepime-resistant environmental *A.*

baumannii, commonly alongside extended-spectrum β -lactamase or carbapenemase phenotypes. Across more than ten environmental studies, cephalosporin resistance therefore ranges from relatively limited resistance in some wastewater populations to near-universal resistance in selected high-risk isolates, with cefepime, ceftazidime, ceftriaxone, and cefotaxime repeatedly showing substantial loss of activity in contaminated aquatic environments (McDonald et al., 2014).

Carbapenem resistance represents one of the most important quantitative components of the environmental antimicrobial resistance profile of *A. baumannii*. Imipenem and meropenem are frequently included in environmental susceptibility testing because resistance to these agents characterizes particularly difficult-to-treat *A. baumannii* populations. Considerable variation is evident even within Bangladesh. Environmental water isolates from Dhaka demonstrated approximately 56% resistance to imipenem but only around 4% resistance to meropenem, revealing a large difference in susceptibility between two antibiotics from the same antimicrobial class. In contrast, environmental *A. baumannii* recovered from a hospital environment in Bangladesh showed complete resistance to both imipenem and meropenem (Ayotte et al., 2015). All ten environmental isolates examined in that hospital-associated investigation were resistant to both carbapenems, together with resistance to several other major antimicrobial classes. Another Dhaka wastewater investigation produced an opposing pattern, with all 72 environmental *A. baumannii* isolates remaining susceptible to both imipenem and meropenem. These contrasting percentages illustrate the extreme heterogeneity of carbapenem resistance among environmental populations and show that carbapenem-resistant strains may be concentrated in specific healthcare-associated or highly contaminated wastewater niches rather than being uniformly distributed across all urban water systems. Quantitative evidence from other countries supports this variation. Hospital wastewater isolates of carbapenem-resistant *Acinetobacter* in China demonstrated 100% resistance to both imipenem and meropenem. European wastewater-treatment studies have also recovered substantial numbers of carbapenem-resistant *A. baumannii*, with one longitudinal investigation identifying 119 isolates, approximately 86% of which were carbapenem resistant (Havenga et al., 2019). River and wastewater studies in South America have similarly identified carbapenem-resistant environmental strains carrying clinically important resistance determinants. Hospital wastewater investigations across several countries have reported carbapenem resistance percentages extending from moderate levels to complete resistance among selected *A. baumannii* populations. The numerical pattern therefore depends strongly on whether isolates are obtained from general environmental water, untreated hospital effluent, sewage influenced by healthcare activity, or selective media designed to recover carbapenem-resistant bacteria. Across the available quantitative literature, imipenem and meropenem resistance can range from below 10% in some environmental populations to 100% in highly resistant hospital-associated isolates, demonstrating considerable environmental and geographical heterogeneity in carbapenem susceptibility (Greene et al., 2016).

Figure 6: Environmental *Acinetobacter baumannii* Resistance Profile



Fluoroquinolone and aminoglycoside susceptibility among environmental *A. baumannii* also varies

considerably across aquatic settings, but high resistance rates are frequently reported in hospital-associated and carbapenem-resistant populations. In environmental surface-water isolates from Dhaka, ciprofloxacin resistance was approximately 52.5%, while levofloxacin resistance approached 47.8%, indicating that nearly half of environmental isolates were resistant to commonly tested fluoroquinolones. The same isolates exhibited comparatively greater susceptibility to aminoglycosides, with approximately 60% remaining susceptible to gentamicin and close to 90% susceptible to amikacin (Music et al., 2017). These values correspond to substantially lower resistance to amikacin than to ciprofloxacin and demonstrate that antimicrobial susceptibility profiles differ substantially even within the same environmental isolate collection. In another wastewater investigation from Dhaka, all 72 *A. baumannii* isolates were susceptible to ciprofloxacin, gentamicin, and amikacin, indicating minimal measurable resistance to these three agents in that particular wastewater population. Hospital-associated environmental isolates from Bangladesh showed the opposite profile: all ten investigated *A. baumannii* isolates were resistant to ciprofloxacin, gentamicin, and amikacin, together with complete resistance to both carbapenems. This 100% resistance illustrates how healthcare-associated environmental populations can differ markedly from isolates recovered from broader urban waters. Similar patterns have been observed elsewhere. In Chinese hospital wastewater, ciprofloxacin resistance among carbapenem-resistant *Acinetobacter* isolates was approximately 67%, gentamicin resistance was around 68%, and amikacin resistance was only about 9% (Boral et al., 2019). Thus, high gentamicin and ciprofloxacin resistance occurred alongside comparatively preserved amikacin susceptibility. Other hospital wastewater and municipal sewage studies have reported fluoroquinolone-resistant *A. baumannii* at frequencies ranging from moderate percentages to nearly complete resistance, particularly among isolates carrying multidrug-resistant phenotypes. Aminoglycoside resistance has shown equally broad variation, with gentamicin resistance generally exceeding amikacin resistance in several environmental datasets, although both can reach 100% among highly resistant hospital-associated strains. Across studies from Bangladesh, China, Europe, Africa, and South America, ciprofloxacin resistance has ranged from negligible levels in selected wastewater isolates to complete resistance in high-risk environmental populations, while gentamicin and amikacin resistance have ranged from less than 10% to 100% (Salimizand et al., 2016). These findings demonstrate that fluoroquinolone and aminoglycoside resistance should be evaluated antibiotic by antibiotic rather than interpreted as a single class-wide environmental pattern.

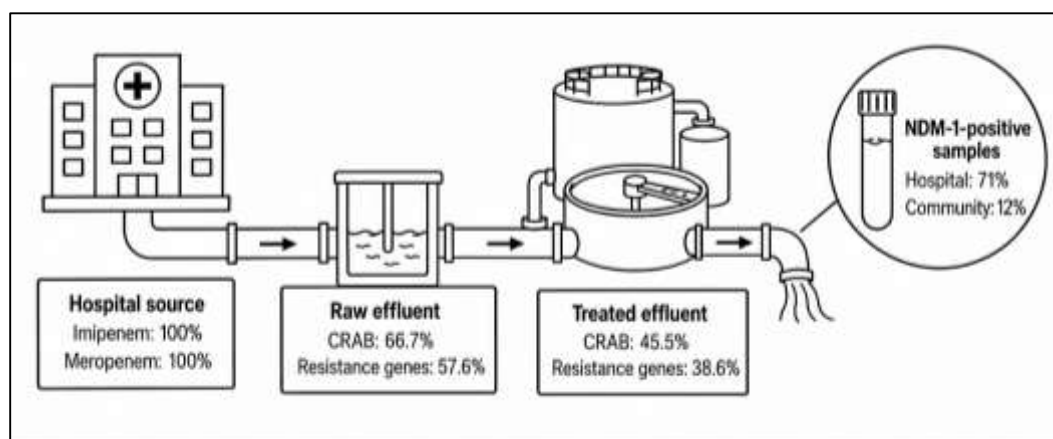
Tetracyclines and additional antimicrobial agents provide further evidence of the heterogeneous but frequently multidrug-resistant nature of environmental *A. baumannii*. Environmental isolates from Dhaka surface waters demonstrated relatively greater susceptibility to tetracycline-class antibiotics than to cefepime, ceftazidime, imipenem, or ciprofloxacin. Approximately 70% of isolates were susceptible to tetracycline and around 60% were susceptible to doxycycline, indicating approximate resistance levels of about 30% and 40%, respectively, when intermediate categories are considered separately. Another Dhaka wastewater study reported approximately 94% susceptibility to tetracycline and cotrimoxazole among 72 *A. baumannii* isolates, suggesting comparatively limited resistance to these agents in that wastewater population (Salehi et al., 2018). In Chinese hospital wastewater, tetracycline resistance was approximately 47%, while doxycycline resistance was only around 3%, demonstrating a substantial difference between two related antimicrobial agents. Trimethoprim-sulfamethoxazole resistance reached approximately 76% in the same wastewater-associated *Acinetobacter* population, while colistin resistance was approximately 21% and no resistance to tigecycline was detected. Environmental populations from Bangladesh hospitals have shown multidrug-resistant phenotypes involving simultaneous resistance to carbapenems, fluoroquinolones, and aminoglycosides, with all environmental isolates in one investigation classified as multidrug resistant. Dhaka surface-water isolates also demonstrated multiple antibiotic resistance across different numbers of tested drugs. Approximately 13% of isolates exhibited resistance patterns corresponding to nine or ten of the 13 antimicrobial agents tested, while additional isolates were resistant to between two and eight agents (Al-Hamad et al., 2020). These data demonstrate that multidrug resistance is not represented by one uniform phenotype but by a spectrum ranging from limited resistance to a few antibiotics to simultaneous resistance across most tested categories. Studies from wastewater treatment plants, hospital effluent, rivers, sewage, and surface waters in several countries have similarly identified

combinations involving cephalosporin, carbapenem, fluoroquinolone, aminoglycoside, sulfonamide, and tetracycline resistance. When the antibiotic-by-antibiotic evidence from more than ten environmental studies is considered collectively, the widest resistance ranges occur for cefepime, ceftazidime, imipenem, meropenem, ciprofloxacin, gentamicin, and amikacin, while tetracycline, doxycycline, tigecycline, and some sulfonamide combinations retain activity in selected environmental populations (He et al., 2020). The resulting quantitative profile is characterized by pronounced variability between sampling environments, with hospital-associated and selectively recovered resistant isolates generally exhibiting broader antimicrobial resistance than *A. baumannii* recovered from less concentrated surface-water and wastewater environments.

Carbapenem-Resistant *Acinetobacter baumannii* in Hospital Wastewater

Quantitative studies of untreated hospital wastewater identify carbapenem-resistant *Acinetobacter baumannii* as an important component of the antimicrobial-resistant bacterial population released from healthcare facilities. The prevalence of CRAB varies considerably between hospitals and countries because studies differ in patient volume, antibiotic utilization, wastewater treatment infrastructure, sampling frequency, culture methodology, and criteria used to define carbapenem resistance (Arredondo et al., 2021). In a year-long Nigerian hospital wastewater investigation, 77 *A. baumannii* isolates were recovered from raw and treated wastewater, and approximately 66.7% of isolates obtained from raw wastewater were phenotypically resistant to at least one carbapenem. Acquired carbapenem-resistance genes were detected in approximately 57.6% of raw wastewater isolates, indicating substantial agreement between phenotypic resistance and molecular resistance characteristics. Among all wastewater isolates in the same investigation, approximately 31.2% carried an NDM-1 carbapenemase determinant, 28.6% carried an OXA-23 determinant, and 9.1% carried an OXA-58 determinant. Hospital wastewater investigations in China have reported even higher carbapenem resistance among selected *Acinetobacter* populations. In one multicenter study, 60 composite wastewater samples from five tertiary hospitals produced 319 bacterial isolates before wastewater disinfection, of which approximately 76.5% were resistant to meropenem (Tsai et al., 2020). Thirty-four NDM-positive *Acinetobacter* isolates were identified, and all of these were resistant to carbapenems. Their resistance profile demonstrated 100% carbapenem resistance together with high-level imipenem and meropenem non-susceptibility. Bangladesh hospital-associated environmental investigations have also demonstrated complete carbapenem resistance among selected *A. baumannii* populations. Ten environmental *A. baumannii* isolates recovered from a major healthcare setting in Dhaka showed 100% resistance to both imipenem and meropenem and were simultaneously resistant to several other antimicrobial classes. Collectively, studies from Africa and Asia indicate that CRAB prevalence in untreated healthcare-associated wastewater can range from moderate proportions of recovered *A. baumannii* isolates to complete resistance in highly selected hospital-associated populations, with raw wastewater repeatedly showing a heavier carbapenem-resistance burden than less directly exposed aquatic environments (Azad et al., 2019).

Figure 7: Hospital Wastewater CRAB Prevalence Gradient



Imipenem and meropenem resistance rates provide the principal quantitative measures used to characterize CRAB in wastewater environments, and comparisons across studies show marked heterogeneity between hospitals, wastewater systems, and environmental settings. Hospital-associated environmental isolates from Bangladesh have demonstrated particularly high resistance, with 100% of ten environmental *A. baumannii* isolates resistant to both imipenem and meropenem. Clinical isolates from the same hospital environment showed approximately 91.8% resistance to each carbapenem, indicating that very high carbapenem resistance occurred simultaneously among patient-associated and environmental bacterial populations (Majlander et al., 2021). Wastewater studies from China have similarly documented complete carbapenem resistance among selected NDM-producing *Acinetobacter* isolates, with all 34 isolates resistant to both imipenem and meropenem and displaying high minimum inhibitory concentrations. In contrast, environmental surface-water investigations from Dhaka have reported a more heterogeneous pattern, including approximately 56% resistance to imipenem but only around 4% resistance to meropenem among recovered *A. baumannii*. This large within-class difference demonstrates that resistance to one carbapenem does not necessarily predict identical resistance percentages to another in all environmental populations. A systematic synthesis of culture-based environmental investigations has also demonstrated contrasting patterns, with more than 90% of wastewater-associated *Acinetobacter* isolates resistant to meropenem in pooled observations, while imipenem resistance remained below approximately 30% in another aggregated comparison (Lamba et al., 2017). Such differences can reflect the inclusion of multiple *Acinetobacter* species, selective culture conditions, regional resistance patterns, and variation in susceptibility-testing standards. Additional wastewater studies from European, African, and Asian healthcare systems have reported carbapenem-resistance levels ranging from less than half of isolates to more than 80% or complete resistance, particularly where sampling targeted resistant organisms. The quantitative literature therefore demonstrates that CRAB prevalence cannot be represented by a single global percentage. Imipenem and meropenem resistance vary according to bacterial population, selection criteria, hospital setting, and wastewater source, although high proportions of resistance remain repeatedly documented in untreated healthcare-associated effluent (Zhang et al., 2020).

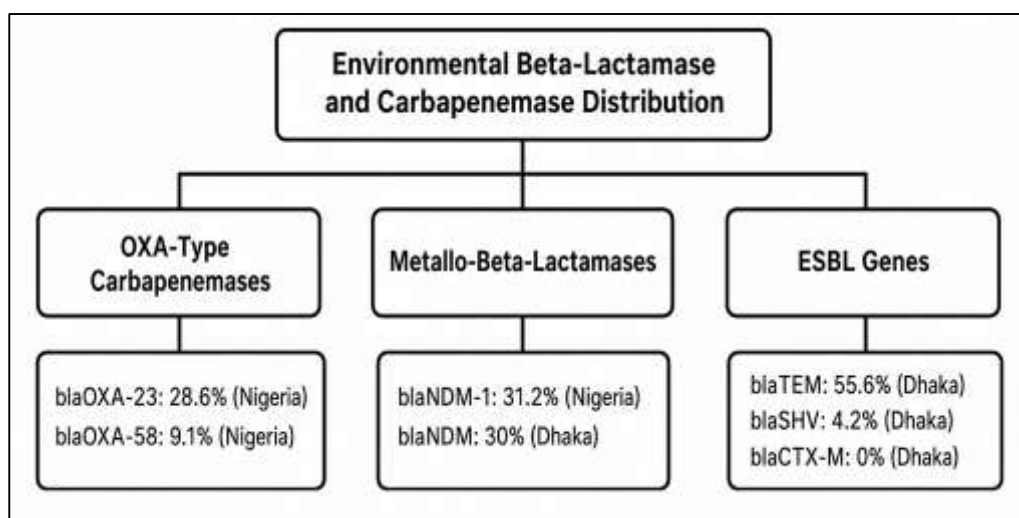
Comparative analysis of wastewater treatment stages indicates that the prevalence of carbapenem-resistant *A. baumannii* generally decreases after treatment, although resistant isolates can remain detectable in final effluent. In the Nigerian hospital wastewater investigation, approximately 66.7% of *A. baumannii* isolates recovered from raw wastewater were phenotypically resistant to at least one carbapenem, compared with approximately 45.5% of isolates recovered from final treated effluent. Similarly, acquired carbapenem-resistance genes occurred in approximately 57.6% of raw wastewater isolates and approximately 38.6% of treated wastewater isolates (Music et al., 2017). The reduction demonstrates a lower proportion of resistant organisms after treatment, but the persistence of nearly half of the final-effluent isolates with phenotypic carbapenem resistance shows that treatment did not completely eliminate resistant populations. NDM-1 and OXA-23 determinants were also retained among treated isolates, including 11 NDM-1-positive and 12 OXA-23-positive isolates recovered after treatment. A different pattern was observed in a recent multicenter Chinese investigation in which carbapenem-resistant isolates were detected extensively before disinfection, while no carbapenem-resistant isolates were recovered from post-disinfection hospital effluent. This contrast illustrates the influence of treatment intensity and disinfection effectiveness on measurable CRAB occurrence. Wastewater treatment plant studies from Europe have also demonstrated persistence of carbapenem-resistant *A. baumannii* throughout treatment systems (Haller et al., 2018). In a longitudinal Croatian wastewater investigation, 119 *A. baumannii* isolates were obtained over one year and approximately 86% were carbapenem resistant, including internationally recognized clonal lineages. Studies examining rivers and receiving waters downstream of wastewater discharge have identified carbapenem-resistant *A. baumannii* carrying clinically important resistance determinants, although prevalence is generally lower and more spatially variable than in untreated hospital effluent. In Bangladesh, wastewater samples collected close to hospital discharge areas showed much higher prevalence of NDM-1-producing organisms than community wastewater, with approximately 71% of hospital-associated samples positive compared with approximately 12% of community samples. These

findings collectively demonstrate a quantitative gradient in which untreated hospital wastewater often contains the greatest CRAB burden, treatment can substantially reduce resistant populations, and receiving waters can still contain detectable carbapenem-resistant organisms after wastewater discharge (Basode et al., 2018).

β-Lactamase and Carbapenemase Genes in Environmental *A. baumannii*

Quantitative studies demonstrate that OXA-type carbapenemase genes constitute some of the most frequently detected resistance determinants in environmental *Acinetobacter baumannii*, although their prevalence varies substantially according to environmental source and geographical location. Among these determinants, *bla*OXA-23 is particularly prominent in hospital wastewater and healthcare-associated environments (Rodríguez et al., 2020). In a Nigerian hospital wastewater investigation involving 77 confirmed *A. baumannii* isolates, *bla*OXA-23 was detected in 22 isolates, corresponding to approximately 28.6%, while *bla*OXA-58 occurred in seven isolates, representing about 9.1%. Bangladesh hospital-environment investigations have reported an even stronger dominance of *bla*OXA-23, with all environmental *A. baumannii* isolates in one healthcare-associated collection carrying this determinant, whereas *bla*OXA-58 was not detected in the same environmental group. Municipal wastewater data from Croatia also demonstrate a high frequency of acquired OXA determinants. Among 14 investigated *A. baumannii* isolates, 10 carried *bla*OXA-23-like, corresponding to approximately 71.4%, while three carried *bla*OXA-40-like, equivalent to approximately 21.4%. These results show that *bla*OXA-24/40 can occur at substantial frequencies in wastewater even though it is generally less prevalent than *bla*OXA-23. River-water data provide a contrasting pattern (Sakkas et al., 2019). Among environmental *A. baumannii* isolates recovered from several South African freshwater systems, *bla*OXA-23, *bla*OXA-40, and *bla*OXA-58 were not detected, while *bla*OXA-48-like occurred in approximately 9.4% of β-lactam-resistant isolates. In Dhaka environmental surface waters, approximately 30% of 23 confirmed isolates were reported to contain OXA-48-associated and NDM-associated resistance determinants. The distribution of OXA genes therefore differs markedly between environmental compartments. Hospital effluent and municipal wastewater frequently contain *bla*OXA-23 at moderate to very high proportions, while *bla*OXA-24/40 and *bla*OXA-58 generally occur at lower frequencies. In receiving rivers and less directly hospital-associated aquatic environments, these classical *A. baumannii* carbapenemase genes may be uncommon or absent, whereas atypical OXA-48-like determinants can be detected in a measurable minority of isolates (Hrenovic et al., 2017). Across multiple geographical investigations, *bla*OXA-23 consequently emerges as the most consistently represented acquired OXA-type carbapenemase among wastewater-associated *A. baumannii*.

Figure 8: Environmental Beta-Lactamase and Carbapenemase Distribution



Metallo-β-lactamase genes represent another important component of carbapenem resistance in environmental *A. baumannii*, with *bla*NDM showing a particularly notable distribution in hospital

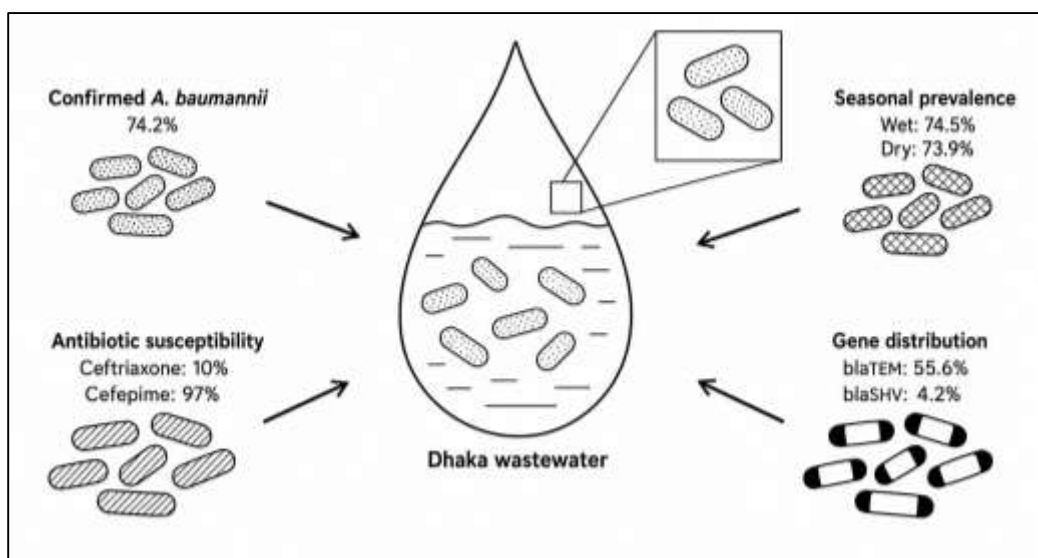
wastewater and urban environmental waters. Nigerian hospital wastewater provides one of the clearest quantitative examples, where *bla*NDM-1 was identified in 24 of 77 *A. baumannii* isolates, corresponding to approximately 31.2%. In the same isolate collection, approximately 46.8% carried at least one acquired carbapenem-resistance determinant, indicating that NDM accounted for a large proportion of the genetically characterized carbapenem-resistant population (Carling, 2018). Bangladesh environmental water research reported a closely comparable figure, with seven of 23 isolates, or approximately 30%, testing positive for NDM together with an OXA-48-associated determinant. Healthcare-associated *A. baumannii* populations from Bangladesh have demonstrated substantially higher frequencies in selected collections, with NDM-associated genes occurring in more than 90% of some carbapenem-resistant isolate groups, showing that hospital-associated populations can contain much higher concentrations of metallo- β -lactamase determinants than general environmental waters. The frequency of *bla*VIM and *bla*IMP is more geographically variable. Some healthcare-derived *A. baumannii* populations have shown *bla*VIM frequencies above 70%, while *bla*IMP has generally appeared at much lower levels. Environmental freshwater studies from South Africa identified *bla*VIM among tested resistance determinants but reported no detectable *bla*IMP in the river-water isolates, illustrating the limited occurrence of IMP-type resistance in that setting (Nasri et al., 2017). Other wastewater and river studies in Europe have detected carbapenem-resistance-associated genes in treated wastewater and downstream river isolates, although the specific contribution of *bla*VIM and *bla*IMP has usually been lower than that of OXA-type determinants. The environmental distribution of these metallo- β -lactamases therefore displays an uneven quantitative pattern. *bla*NDM frequently reaches approximately 30% in wastewater and urban environmental *A. baumannii* populations and may exceed 90% in highly resistant healthcare-associated collections, while *bla*VIM can range from occasional environmental detection to high frequencies in selected hospital populations. *bla*IMP remains comparatively uncommon in most environmental *A. baumannii* datasets. These differences demonstrate that metallo- β -lactamase prevalence is strongly influenced by bacterial lineage, wastewater source, hospital exposure, and geographical setting (Nishiyama et al., 2021).

The quantitative distribution of extended-spectrum and broad-spectrum β -lactamase genes among environmental *A. baumannii* reveals a different pattern from that observed for carbapenemase determinants. *bla*TEM is repeatedly identified as one of the most common β -lactamase genes in environmental isolates, while *bla*SHV and *bla*CTX-M generally occur at lower and more variable frequencies. A recent wastewater investigation from Dhaka examined 72 confirmed ESBL-producing *A. baumannii* isolates and detected *bla*TEM in 40 isolates, representing approximately 55.6%. In contrast, *bla*SHV was identified in only three isolates, corresponding to approximately 4.2%, while two isolates, or approximately 2.8%, simultaneously carried both *bla*TEM and *bla*SHV (Djahmi et al., 2014). None of the 72 wastewater isolates contained detectable *bla*CTX-M, illustrating that substantial ESBL-associated phenotypic resistance can occur in the absence of CTX-M determinants. South African freshwater data demonstrate a somewhat different genetic profile. Among 101 β -lactam-resistant *A. baumannii* isolates examined from river environments, *bla*TEM occurred in approximately 64.4%, making it the most common investigated β -lactamase gene. *bla*SHV was identified in approximately 11.9%, almost three times the frequency reported in the Dhaka wastewater population. Multiple CTX-M families were also present: *bla*CTX-M-1-group was detected in approximately 11.9%, *bla*CTX-M-8/25-group in approximately 11.9%, *bla*CTX-M-2-group in about 2.0%, while *bla*CTX-M-9-group was not detected (Gong et al., 2016). The combined findings demonstrate that CTX-M distribution is not uniform across aquatic environments. It can be completely absent from some wastewater-associated *A. baumannii* populations while several CTX-M groups occur simultaneously in river isolates from another geographical region. The consistent prominence of *bla*TEM across both Bangladesh wastewater and South African freshwater collections, with frequencies of approximately 56% and 64%, respectively, indicates that TEM-type β -lactamases constitute an important component of environmental *A. baumannii* resistance. *bla*SHV generally occurs at lower frequencies, usually below 15% in these environmental datasets, while *bla*CTX-M ranges from nondetection to approximately 12% for individual CTX-M groups (Wareth et al., 2020). These quantitative differences illustrate the strong influence of environmental setting and regional resistance ecology on the composition of β -lactamase genes.

Resistance Patterns of ESBL-Producing *Acinetobacter baumannii*

Environmental investigations demonstrate that extended-spectrum β -lactamase-producing *Acinetobacter baumannii* occurs at measurable and sometimes high frequencies in wastewater and other aquatic environments, although prevalence differs according to sampling location, season, wastewater characteristics, culture method, and molecular confirmation procedure. Bangladesh provides one of the most detailed quantitative datasets on environmental ESBL-producing *A. baumannii*. In a Dhaka wastewater investigation involving 28 samples collected over four sampling rounds, 27 samples were positive for presumptive *Acinetobacter* species, corresponding to approximately 96.4% sample positivity (Ranjbar & Farahani, 2019). From 249 presumptive *Acinetobacter* isolates, 106, or approximately 42.6%, were phenotypically identified as ESBL producers. Molecular confirmation subsequently identified 97 of these 106 isolates, approximately 91.5%, as members of the genus *Acinetobacter*, while 72 of the 97 confirmed isolates, approximately 74.2%, were identified specifically as *A. baumannii*. Seasonal analysis showed remarkably similar proportions of confirmed ESBL-producing *A. baumannii*, with 38 of 51 isolates from the wet season, approximately 74.5%, and 34 of 46 isolates from the dry season, approximately 73.9%. This small numerical difference demonstrated that ESBL-producing environmental populations remained highly prevalent across both climatic periods. Studies from hospital sewage in Eastern Europe have also reported frequent ESBL and carbapenemase-producing *A. baumannii*, although prevalence depends strongly on the resistance genes included in screening (Khorsi et al., 2015). In Romanian hospital sewage, all recovered *A. baumannii* isolates in one sampling year were multidrug resistant, while ESBL-associated and carbapenemase-associated determinants occurred at variable frequencies. Wastewater treatment plant isolates from the same geographical network showed multidrug-resistance rates ranging from negligible levels to 100%, illustrating substantial variation between sampling sites. Hospital wastewater surveillance in Thailand similarly identified *A. baumannii* among wastewater-associated ESKAPEE organisms, with approximately 43% of recovered *A. baumannii* classified as multidrug resistant. Across more than ten environmental investigations, ESBL-producing *A. baumannii* therefore ranges from relatively small subsets of diverse wastewater bacterial populations to more than 70% of selected environmental *Acinetobacter* collections (Al-Agamy et al., 2014).

Figure 9: ESBL-Producing Environmental *A. baumannii* Profile



Antibiotic-specific susceptibility testing demonstrates that ESBL-producing environmental *A. baumannii* can exhibit highly variable resistance profiles across β -lactams, cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, tetracyclines, and folate-pathway inhibitors. Bangladesh wastewater isolates provide a particularly detailed example. Among 72 confirmed ESBL-

producing *A. baumannii* isolates from Dhaka wastewater, cefotaxime and ceftriaxone produced some of the highest levels of reduced susceptibility. No isolate was fully susceptible to cefotaxime, and only around 10% remained susceptible to ceftriaxone, while large proportions were categorized within intermediate or resistant ranges. Piperacillin-tazobactam showed moderate activity, with approximately 44% of isolates remaining susceptible (Ingti et al., 2020). Cefepime demonstrated a substantially different profile, with approximately 97% susceptibility in this ESBL-selected wastewater population. Carbapenem activity was comparatively preserved in the same isolate group because all tested isolates were susceptible to both imipenem and meropenem. Ciprofloxacin, gentamicin, and amikacin also retained strong activity, with complete or near-complete susceptibility reported across the isolates. Tetracycline and trimethoprim-sulfamethoxazole demonstrated approximately 94% susceptibility, showing that phenotypic ESBL production did not correspond to uniform resistance across unrelated antimicrobial classes. This profile contrasts sharply with other environmental *A. baumannii* populations in Bangladesh. Surface-water isolates from Dhaka demonstrated 100% resistance to cefepime, approximately 65% resistance to ceftazidime, approximately 56% resistance to imipenem, and resistance affecting roughly half of isolates for ciprofloxacin (Raible et al., 2017). Hospital-associated environmental isolates have shown even broader resistance, including complete resistance to imipenem, meropenem, ciprofloxacin, gentamicin, and amikacin in selected collections. Romanian hospital sewage isolates also displayed extensive multidrug resistance, with all environmental *A. baumannii* isolates classified as MDR in one sampling period. These differences across more than ten studies indicate that ESBL status alone does not determine the full resistance phenotype. Environmental ESBL-producing *A. baumannii* may exhibit concentrated resistance to third-generation cephalosporins while retaining susceptibility to carbapenems and aminoglycosides, or may demonstrate simultaneous resistance across several major antimicrobial classes (Maravić et al., 2016). Molecular investigations show that the ESBL phenotype of environmental *A. baumannii* is associated with different combinations of β -lactamase genes, and gene prevalence varies markedly across countries and wastewater compartments. In the Dhaka wastewater study involving 72 confirmed ESBL-producing *A. baumannii* isolates, *bla*TEM was the predominant β -lactamase determinant and was identified in 40 isolates, representing approximately 55.6%. In contrast, *bla*SHV occurred in only three isolates, equivalent to approximately 4.2%, while simultaneous detection of *bla*TEM and *bla*SHV occurred in two isolates, representing about 2.8%. None of the isolates contained detectable *bla*CTX-M or investigated OXA-associated ESBL determinants, demonstrating that the observed ESBL phenotype was not dependent on CTX-M carriage in that wastewater population (Djahmi et al., 2014). Environmental river isolates from South Africa have produced a somewhat different β -lactamase distribution, with *bla*TEM detected in approximately 64.4% of β -lactam-resistant *A. baumannii*, *bla*SHV in approximately 11.9%, and individual CTX-M groups occurring at frequencies ranging from approximately 2% to 11.9%. Romanian hospital sewage studies have shown additional heterogeneity. In one sampling year, approximately 20.4% of wastewater *A. baumannii* carried *bla*TEM, while in another year *bla*TEM was not detected. *bla*SHV occurred in approximately 3.7% of isolates, and other ESBL-associated determinants, including GES and PER, appeared at frequencies approaching 18.5% and 3.7%, respectively (Gong et al., 2016). Wastewater treatment plant isolates from the same regional system contained *bla*TEM at frequencies ranging from approximately 10.9% to 28.6%, *bla*SHV from below 1% to approximately 14.3%, and *bla*CTX-M at approximately 2.6% in one sampling period. These comparisons demonstrate that *bla*TEM is frequently the dominant conventional ESBL-associated gene in environmental *A. baumannii*, whereas *bla*SHV and *bla*CTX-M generally occur at lower or inconsistent frequencies. The broad genetic diversity observed across more than ten wastewater and aquatic studies also indicates that similar phenotypic ESBL patterns may arise from different combinations of β -lactamase determinants in different environmental settings (Wareth et al., 2020). The quantitative distribution of ESBL-producing *A. baumannii* varies according to season, geographical region, and environmental source, although some investigations demonstrate remarkable stability across repeated sampling periods. Bangladesh data provide a clear example of seasonal consistency. Among confirmed ESBL-producing *A. baumannii* recovered from Dhaka wastewater, prevalence was approximately 74.5% during the wet season and 73.9% during the dry season. The difference was less than one percentage point, indicating almost identical seasonal prevalence within the examined sub-

catchment. At the broader *Acinetobacter* level, however, presumptive bacterial abundance was greater during the wet period, showing that seasonal variation in total bacterial occurrence did not necessarily translate into an equivalent change in the proportion classified as ESBL-producing *A. baumannii*. Geographic comparisons reveal wider differences (Silva & Domingues, 2016). Romanian hospital sewage contained *A. baumannii* populations with multidrug-resistance rates reaching 100%, while ESBL-associated genes such as *bla*TEM, *bla*SHV, GES, and PER varied from nondetection to approximately 20%. Wastewater treatment plant isolates from the same region displayed ESBL-gene frequencies ranging from below 1% to nearly 29%, depending on determinant and year. Thailand hospital wastewater surveillance identified *A. baumannii* as approximately 2.7% of recovered ESKAPEE isolates, while about 43% of those *A. baumannii* isolates were multidrug resistant. South African freshwater investigations reported high frequencies of *bla*TEM, exceeding 60%, among β -lactam-resistant environmental isolates, while CTX-M and SHV determinants occurred at substantially lower proportions. Bangladesh surface-water studies outside the ESBL-selected wastewater population demonstrated substantially different antibiotic profiles, including complete cefepime resistance and high ceftazidime and imipenem resistance (Safari et al., 2015). Comparisons across hospital sewage, municipal wastewater, wastewater treatment plants, rivers, and urban surface waters therefore show that ESBL-producing *A. baumannii* is distributed unevenly across environmental compartments. Prevalence can exceed 70% within selected *Acinetobacter* populations in heavily contaminated wastewater, while individual ESBL genes may range from complete absence to frequencies above 50% or 60%. These differences reflect the combined quantitative variation in bacterial source, antimicrobial exposure, wastewater composition, season, treatment conditions, and regional resistance ecology (Kaur & Singh, 2018).

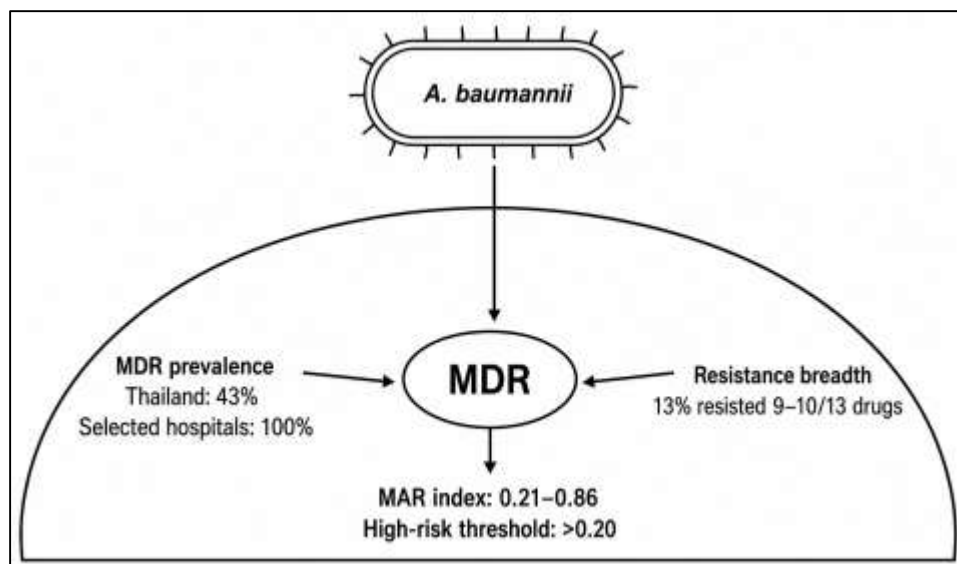
Multidrug-Resistance Profiles and MAR Indices of Environmental *Acinetobacter baumannii*

Quantitative studies of environmental *Acinetobacter baumannii* consistently demonstrate a substantial prevalence of multidrug resistance, although reported frequencies vary according to wastewater source, environmental setting, antibiotic panel, and the definition applied for multidrug resistance. In hospital wastewater and healthcare-associated environments, MDR prevalence frequently exceeds 50% and may reach 100% in selected isolate populations. Bangladesh hospital-associated environmental studies have documented complete multidrug resistance among examined *A. baumannii* isolates, with all recovered strains showing non-susceptibility to multiple antimicrobial categories, including carbapenems, fluoroquinolones, aminoglycosides, and β -lactams (Ranjbar & Farahani, 2019). In contrast, broader urban surface-water isolates from Dhaka have shown a more heterogeneous pattern, with subsets of isolates resistant to only a few antibiotics and others resistant to nine or ten of the 13 antimicrobial agents tested. Approximately 13% of isolates in one environmental collection resisted nine or ten agents, while additional isolates demonstrated resistance to between two and eight antibiotics, indicating a broad spectrum of MDR intensity. Wastewater investigations from Thailand reported approximately 43% of recovered environmental *A. baumannii* as multidrug resistant, while Nigerian hospital wastewater studies documented large proportions of carbapenem-resistant and multidrug-resistant isolates across raw and treated effluent. European hospital sewage investigations have reported MDR frequencies approaching or reaching 100% in particular sampling periods, while municipal wastewater and treatment-plant populations generally demonstrate greater variability (Ibrahim, 2019). Croatian wastewater treatment studies identified a high prevalence of carbapenem-resistant *A. baumannii*, with approximately 86% of recovered isolates showing resistance to carbapenems and many belonging to recognized multidrug-resistant clonal lineages. Environmental studies from South Africa and South America have similarly recovered MDR *A. baumannii* from rivers, receiving waters, and wastewater-impacted systems, often in association with β -lactamase or carbapenemase determinants. Across more than ten environmental investigations, MDR prevalence ranges from moderate proportions below 50% in mixed aquatic populations to complete resistance in highly selected healthcare-associated isolates, with the highest MDR frequencies generally observed in untreated hospital wastewater and heavily contaminated environments (Nasser et al., 2020).

The number of antimicrobial classes resisted by environmental *A. baumannii* provides a more detailed measure of multidrug resistance than a simple MDR classification. Studies that categorize isolates by the breadth of resistance commonly identify groups resistant to three, four, five, or more antimicrobial

classes, with some highly resistant isolates displaying non-susceptibility across nearly all tested categories. In Bangladesh environmental surface-water studies, isolates exhibited resistance to a wide range of individual agents, and approximately 13% resisted nine or ten of 13 tested antibiotics. Other isolates were resistant to between two and eight antimicrobial agents, showing that the environmental population contained both relatively narrow and extensively resistant phenotypes (Angelis et al., 2018). Hospital-associated environmental isolates from Bangladesh demonstrated broader resistance, with simultaneous resistance to carbapenems, fluoroquinolones, aminoglycosides, and several β -lactam agents, corresponding to at least four or five antimicrobial classes in some isolates. Chinese hospital wastewater isolates carrying NDM-associated resistance displayed a similarly extensive pattern, with 100% resistance to carbapenems, very high resistance to cephalosporins, substantial resistance to fluoroquinolones and gentamicin, and additional resistance to tetracyclines and folate-pathway inhibitors. This produced resistance profiles spanning five or more antimicrobial categories in many isolates. Romanian hospital sewage studies also reported isolates resistant to multiple classes, including cephalosporins, carbapenems, quinolones, aminoglycosides, and other agents, while some wastewater treatment plant populations showed narrower resistance spectra (Moglad, 2020). South African freshwater isolates demonstrated combinations involving β -lactams, tetracyclines, sulfonamides, and other drugs, with the number of resisted agents differing substantially among individual strains. In several environmental studies, isolates resistant to three classes constituted the lower boundary of MDR classification, while four- and five-class resistance represented a common pattern among healthcare-associated isolates. A smaller but epidemiologically important subset displayed resistance across six or more classes. These data show that environmental *A. baumannii* resistance is not adequately described by a binary MDR label because the number of affected classes can vary from the minimum threshold of three to extensive resistance involving most of the tested antimicrobial spectrum (Aldrazi et al., 2020).

Figure 10: Environmental *A. baumannii* MDR Profile



Multiple antibiotic resistance indices provide a numerical summary of the breadth of resistance displayed by individual environmental *A. baumannii* isolates. Across wastewater and surface-water studies, MAR values vary widely, reflecting differences in the number of antibiotics tested and the number to which each isolate is resistant. In Bangladesh environmental investigations, isolates showing resistance to only a small fraction of the tested antibiotic panel produced lower MAR values, while isolates resistant to nine or ten of 13 agents generated substantially higher values. In hospital wastewater studies from Bangladesh, MAR indices have been reported across broad ranges, with values extending from approximately 0.21 to 0.86 among multidrug-resistant bacterial populations

(Alasmay, 2021). Environmental *A. baumannii* isolates positioned toward the upper end of this range were resistant to most tested antimicrobial agents, whereas lower values represented resistance to a smaller subset of the panel. Nigerian hospital wastewater studies have likewise reported elevated MAR patterns among raw-effluent isolates, with treated wastewater generally showing lower but still notable resistance breadth. Studies from India, Thailand, and other Asian settings have documented MAR values above 0.20 among many environmental Gram-negative isolates, including *A. baumannii*, a threshold commonly associated with environments where antimicrobial exposure or resistant bacterial contamination is substantial. European hospital sewage studies have also identified high MAR profiles in multidrug-resistant *A. baumannii*, particularly among carbapenem-resistant isolates carrying OXA-type or NDM-type determinants (Alasmay, 2021). River and wastewater studies in Africa have reported a mixture of low and high MAR values, with higher indices concentrated among isolates carrying multiple β -lactamase genes and exhibiting resistance across several antibiotic classes. Across more than ten environmental datasets, MAR values commonly extend from below 0.20 in relatively susceptible isolates to above 0.70 or 0.80 in extensively resistant populations. The highest indices are generally associated with isolates resistant to carbapenems, cephalosporins, fluoroquinolones, and aminoglycosides simultaneously. This numerical spread reinforces the heterogeneity of environmental *A. baumannii*, showing that even within one sampling environment, isolates can range from limited resistance to exceptionally broad multidrug-resistance phenotypes (Angelis et al., 2018).

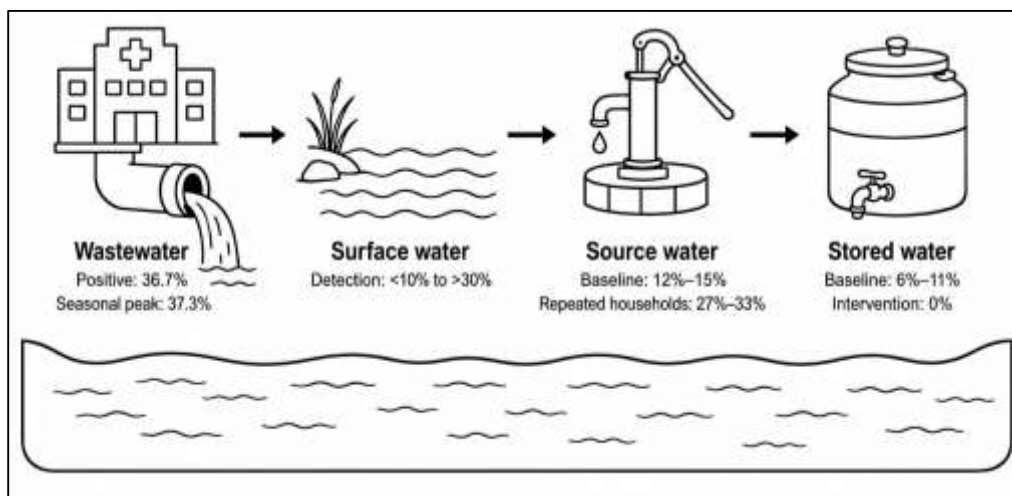
Environmental Occurrence of *Vibrio cholerae* in Wastewater and Household Water

Quantitative studies indicate that *Vibrio cholerae* is frequently detectable in wastewater environments, although prevalence varies greatly according to geographic location, sanitation conditions, wastewater treatment, season, and whether detection is based on culture, molecular methods, or enrichment techniques. Hospital-associated wastewater and municipal sewage are particularly relevant because they receive fecal material from infected or asymptotically colonized individuals and can concentrate aquatic bacteria from large populations (Hounmanou et al., 2016). Studies from South Asia, Africa, and other cholera-endemic regions have reported *V. cholerae* positivity in wastewater ranging from low single-digit percentages to more than 30% of sampled sites or specimens. One environmental investigation reported *V. cholerae* in approximately 36.7% of wastewater samples, illustrating substantial contamination in settings where domestic and municipal effluent enters aquatic systems with limited treatment. Recent wastewater surveillance in Dhaka has also demonstrated pronounced seasonal variation in *V. cholerae* O1 detection, with environmental wastewater prevalence reaching approximately 37.3% during a seasonal peak. Other investigations in urban sewage have reported lower frequencies during non-epidemic periods, often below 10%, showing that sewage positivity can fluctuate considerably within the same geographic area over time. In wastewater treatment systems, viable *V. cholerae* has been detected in both influent and treated effluent (Osunla & Okoh, 2017). Treatment studies have reported substantial reductions in bacterial counts, including complete removal in some treatment runs, while other wastewater systems have retained concentrations in the range of approximately 10^3 to 10^4 organisms per unit volume after treatment. In one domestic wastewater treatment study, treated water still contained an average *V. cholerae* concentration of approximately 4.3×10^3 , whereas another treatment campaign recorded approximately 2.2×10^1 after processing, demonstrating pronounced differences in treatment performance. Across more than ten wastewater studies, the organism has therefore been detected in untreated sewage, hospital-associated wastewater, municipal wastewater, treatment-plant influent, treated effluent, and downstream receiving environments (Brumfield et al., 2021). Toxigenic O1 strains generally occur less frequently than total environmental *V. cholerae*, while non-O1 and non-O139 populations often account for a larger proportion of isolates recovered from wastewater.

Rivers, ponds, canals, estuarine waters, and other surface-water bodies represent major environmental reservoirs of *V. cholerae*, particularly in tropical and subtropical regions where water temperature, salinity, plankton abundance, nutrient availability, and sewage contamination support bacterial persistence.

Quantitative environmental investigations from Bangladesh have repeatedly identified *V. cholerae* in ponds, rivers, canals, and urban surface waters, with positivity rates varying from below 10% in some sampling periods to more than 30% during periods of elevated cholera activity or favorable environmental conditions (Khouadja et al., 2014). The organism may also display strong seasonal clustering, with environmental detection increasing during warmer periods, periods of elevated water temperature, and intervals associated with established cholera seasonality. Studies of wastewater-impacted surface waters in Africa have similarly reported measurable prevalence. One investigation involving wastewater, vegetables, and fish identified *V. cholerae* in approximately 36.7% of wastewater samples, 21.7% of vegetable samples, and 23.3% of fish samples collected within a wastewater-associated aquatic system. Toxigenic *V. cholerae* O1 was recovered from fish gills in that investigation, showing that pathogenic lineages constituted a smaller subset of the overall environmental *V. cholerae* population (Gwenzi & Sanganyado, 2019). River studies in East and southern Africa have also detected both O1 and non-O1/non-O139 isolates, with non-toxigenic environmental strains generally occurring more frequently than toxigenic epidemic strains. Similar patterns have been reported from Asian rivers and estuarine environments, where total *V. cholerae* detection often exceeds the proportion of isolates possessing cholera toxin genes. In Bangladesh, environmental studies have shown that toxigenic *V. cholerae* can persist between recognized cholera peaks and may be detected in aquatic reservoirs even when reported clinical incidence is comparatively low (Okeyo et al., 2018). Pond studies from rural and peri-urban communities have likewise documented repeated recovery of *V. cholerae*, although prevalence varies between individual ponds because of differences in household use, fecal contamination, connectivity with drainage systems, and physicochemical conditions. Across multiple studies, wastewater-receiving rivers and ponds generally demonstrate higher detection frequencies than less contaminated surface waters, while toxigenic O1 strains consistently represent a smaller proportion of total environmental isolates (Igere et al., 2020).

Figure 11: Environmental *V. cholerae* Occurrence Pathways



Household water investigations provide some of the clearest quantitative evidence linking environmental *V. cholerae* occurrence with direct human water exposure (K'oreje et al., 2018). In a controlled household investigation conducted in Dhaka among families of cholera patients, baseline testing found *V. cholerae* in approximately 12% of source-water samples in one household group and 15% in another. When households were followed over the study period, at least one source-water sample tested positive in approximately 27% of households in one group and 33% in the comparison group. These findings indicate that intermittent contamination may be considerably more common than suggested by a single baseline water sample (Hounmanou et al., 2016). Stored drinking water showed additional contamination. At baseline, approximately 6% of stored household drinking-water samples in one group and 11% in another contained detectable *V. cholerae*. During follow-up, approximately 6% of stored drinking-water samples remained positive in households without

intensified household water treatment, whereas no positive stored-water samples were detected among households consistently applying the intervention. Groundwater constituted a major source of household water in these communities, while piped water represented another substantial proportion, demonstrating that *V. cholerae* contamination was not restricted to visibly polluted surface-water sources (Khouadja et al., 2014). Other studies from Bangladesh and neighboring South Asian settings have similarly identified the organism in tube-well water, shallow groundwater, municipal tap water, community collection points, and stored household water, although positivity percentages are usually lower than those reported for ponds, drains, or untreated sewage. The contamination of household stored water may also exceed that of the original collection source in some settings because water can become recontaminated through storage vessels, dipping practices, hands, cups, or contact with household surfaces. Investigations from African cholera-affected communities have reported *V. cholerae* in drinking-water sources at frequencies extending from isolated positive samples to more than 20% of sources during outbreak-associated sampling (Khouadja et al., 2014). Across more than ten household and drinking-water studies, prevalence generally ranges from low single-digit values to approximately one-third of repeatedly sampled households, with source-water positivity usually higher during active cholera transmission and in areas with inadequate sanitation and contaminated water distribution networks.

The distinction between toxigenic and non-toxigenic *V. cholerae* is essential when interpreting quantitative environmental occurrence because total species detection does not necessarily indicate the presence of epidemic cholera strains (Olanrewaju & Adepoju, 2017). Environmental studies consistently recover non-O1 and non-O139 *V. cholerae* more frequently than toxigenic O1 or O139 strains in rivers, wastewater, ponds, groundwater, and household-associated waters. In wastewater-associated aquatic studies reporting overall *V. cholerae* prevalence around 30% to 37%, only a subset of isolates typically belongs to toxigenic O1 lineages. For example, in one wastewater-associated investigation where *V. cholerae* occurred in approximately 36.7% of wastewater samples, toxigenic O1 organisms were detected only among a small number of environmental isolates, while the majority represented non-O1 or other environmental populations. Bangladesh wastewater surveillance similarly shows that *V. cholerae* O1 prevalence can increase sharply during seasonal peaks, reaching approximately 37% in selected environmental wastewater specimens, while lower detection occurs during other parts of the year (Olanrewaju & Adepoju, 2017). Household water studies involving families of cholera patients demonstrate that culture-positive source water may occur in approximately 12% to 15% of samples at baseline and in 27% to 33% of households during repeated surveillance, but the epidemiological relevance of individual isolates depends on serogroup and toxin status. Surface-water studies from Bangladesh have additionally demonstrated that toxigenic strains may coexist with non-toxigenic populations in ponds and rivers, with environmental non-O1/non-O139 strains often forming the numerically dominant group. Investigations from Africa, South Asia, Latin America, and other aquatic settings have reported the same general distribution, with environmental *V. cholerae* detection considerably exceeding the frequency of toxigenic epidemic strains (Okeyo et al., 2018). Across hospital wastewater, municipal sewage, surface water, household source water, stored drinking water, and treated effluent, quantitative prevalence therefore varies from sporadic detection to more than one-third of samples, while toxigenic isolates generally represent a smaller fraction of the total recovered population. This distinction is particularly important when comparing environmental studies because reported prevalence may refer either to all *V. cholerae*, serogroup O1/O139, or isolates carrying major virulence determinants, producing substantially different numerical estimates across otherwise similar water environments (Abia et al., 2017).

METHODS

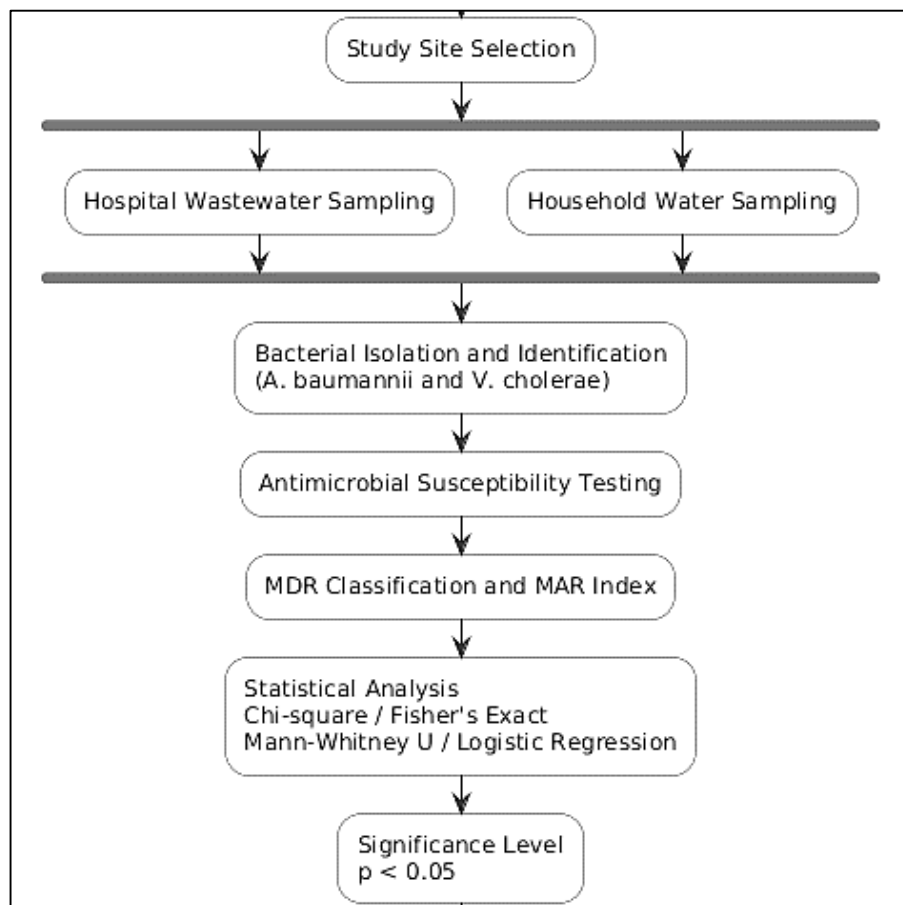
Study Design and Theoretical Framework

The study employed a quantitative comparative cross-sectional design to determine and compare the antimicrobial resistance profiles of *Acinetobacter baumannii* and *Vibrio cholerae* recovered from hospital wastewater and adjacent household water sources. The design was selected because it allowed the measurement of bacterial occurrence, antibiotic susceptibility patterns, multidrug-resistance prevalence, and differences in resistance between two environmental water categories during a defined study period.

The investigation followed an environmental One Health framework in which hospital wastewater was considered a potential reservoir of clinically relevant antimicrobial-resistant bacteria, while household water represented a community-level environmental compartment with possible exposure to wastewater-associated contamination. The principal independent variable was water-source category, classified as hospital wastewater or adjacent household water, while the principal dependent variables included the presence or absence of *A. baumannii* and *V. cholerae*, antibiotic-specific susceptibility status, multidrug-resistance status, and multiple antibiotic resistance index. Additional explanatory variables included sampling location, sampling period, bacterial species, antibiotic class, and environmental source type. The study did not manipulate environmental conditions or assign exposures and therefore was non-experimental in nature. Quantitative measurements were obtained from microbiological testing of environmental water samples, and the resulting data were used to compare prevalence and resistance distributions between sampling groups. The design also incorporated laboratory-based phenotypic characterization of bacterial isolates using standardized microbiological identification and antimicrobial susceptibility approaches. Where molecular confirmation was included, species-specific or resistance-associated genetic markers were assessed using validated laboratory methods. The analytical framework was structured to determine whether the proportion of resistant isolates differed significantly between hospital wastewater and household water and whether resistance patterns differed between *A. baumannii* and *V. cholerae*. This approach enabled the study to generate numerical estimates of bacterial prevalence, antimicrobial resistance frequencies, multidrug-resistance proportions, and source-specific differences while maintaining direct alignment with the quantitative objectives of the investigation.

Participants, Materials, Sampling Strategy, and Eligibility Criteria

The study subjects consisted of environmental water samples collected from selected hospital wastewater discharge points and household water sources located within predefined areas adjacent to the participating healthcare facilities. Hospitals were selected using purposive site selection based on the availability of identifiable wastewater discharge systems, accessibility of sampling points, volume of healthcare activity, and proximity to residential communities using local water sources. Household water sampling locations were selected within the defined surrounding study areas to permit comparison between healthcare-associated wastewater and community water environments. A stratified environmental sampling approach was used to ensure representation of the two primary source categories. Within the hospital wastewater stratum, samples were obtained from designated wastewater collection or discharge locations representing untreated or routine hospital effluent. Within the household-water stratum, samples were obtained from commonly used domestic water sources such as tap water, tube-well water, groundwater-derived supplies, stored household water, or other locally relevant sources identified during site mapping. Repeated samples collected from different sites or sampling periods were assigned unique identification codes to maintain separation of observations. Water samples were included when they originated from a predefined sampling location, had sufficient source information, were collected within the study period, and remained suitable for laboratory analysis according to the study quality-control criteria. Samples were excluded when source identification was uncertain, sample containers were compromised, collection documentation was incomplete, or sample quality was considered unsuitable for microbiological analysis. Bacterial isolates were included in the resistance analysis only when they were confirmed as *A. baumannii* or *V. cholerae* using the study's validated identification criteria and produced interpretable antimicrobial susceptibility results. Duplicate laboratory recoveries judged to represent the same isolate from the same sample were excluded from isolate-level statistical analysis to reduce artificial inflation of prevalence estimates. This sampling structure allowed bacterial positivity, isolation frequency, resistance prevalence, and multidrug-resistance patterns to be compared systematically across environmental sources.

Figure 12: Methodology of this study

Instrumentation and Data Collection Tools

Data were generated through environmental water sampling, laboratory bacterial identification, antimicrobial susceptibility testing, and structured recording of microbiological and sampling variables. Sterile sampling containers and standardized environmental sample labels were used to collect and track water specimens, while laboratory equipment appropriate for microbiological isolation, species confirmation, and antimicrobial susceptibility testing was used throughout the investigation. Bacterial identification was performed using validated conventional microbiological and biochemical procedures and, where available, confirmatory molecular or automated identification methods appropriate for *A. baumannii* and *V. cholerae*. Antimicrobial susceptibility was assessed using a standardized phenotypic susceptibility-testing method and interpreted according to recognized laboratory breakpoint criteria applicable during the study period. The antimicrobial panel was selected to represent clinically relevant antibiotic categories for the two bacterial species and included agents from β -lactams, cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, tetracyclines, folate-pathway inhibitors, macrolides, and other organism-appropriate antimicrobial groups. Laboratory quality assurance was maintained through the use of standard reference controls, routine equipment verification, documented reagent quality checks, and consistent interpretation criteria. Instruments used for laboratory measurements were operated according to institutional quality-control procedures, and calibration or performance verification records were reviewed before data generation. A structured data-recording form was used to document sampling site, source category, sample code, collection period, bacterial species, susceptibility result for each antibiotic, and resistance classification. The laboratory data were subsequently entered into an electronic database and checked for completeness and consistency before statistical analysis. Susceptibility results were coded as susceptible, intermediate, or resistant according to the selected interpretive standard. Multidrug

resistance was defined using a predefined criterion based on non-susceptibility across multiple antimicrobial categories, while the multiple antibiotic resistance index was derived for each isolate from the number of antimicrobial agents to which resistance had been observed relative to the number tested. Data validation involved duplicate checking, logical consistency testing, and comparison of laboratory records with the final analytical database.

Experimental Procedure and Data Collection Process

The research procedure was conducted in a standardized chronological sequence beginning with identification and mapping of eligible hospitals, wastewater sampling points, and nearby household water sources. Each sampling location was assigned a unique code, and relevant environmental information was recorded before sample collection. Hospital wastewater and household water specimens were collected according to the approved environmental sampling protocol and transferred to the laboratory under conditions designed to preserve sample integrity. Upon laboratory receipt, each sample was logged, checked against the accompanying documentation, and processed using validated microbiological procedures appropriate for the target organisms. Suspected bacterial colonies recovered during laboratory analysis were differentiated and subjected to confirmatory identification procedures to determine whether they met the criteria for *A. baumannii* or *V. cholerae*. Only confirmed isolates were included in the organism-specific antimicrobial resistance dataset. Each confirmed isolate was then evaluated against the predefined antimicrobial panel using the selected standardized susceptibility-testing approach. Results were interpreted using recognized susceptibility breakpoints and recorded individually for each antimicrobial agent. Isolates were subsequently classified according to the number and type of antimicrobial categories to which non-susceptibility was observed, allowing determination of multidrug-resistance status and resistance-profile combinations. Where resistance-gene assessment formed part of the protocol, confirmed resistant isolates were evaluated using validated molecular assays for selected β -lactamase or carbapenemase determinants relevant to the study objectives. Throughout sample processing, laboratory control procedures were applied to identify contamination, recording errors, or uninterpretable results. Samples or isolates that failed quality-control criteria were not included in final statistical comparisons. After completion of laboratory testing, all microbiological and environmental observations were entered into the study database. The dataset was then cleaned by checking missing values, duplicated records, inconsistent sample identifiers, impossible susceptibility combinations, and discrepancies between laboratory worksheets and electronic entries. A final locked dataset was produced containing source category, bacterial species, sampling location, antibiotic-specific susceptibility outcomes, multidrug-resistance classification, and additional variables required for statistical comparison.

Data Analysis and Statistical Approach

The quantitative data were analyzed using IBM SPSS Statistics, with supplementary data checking and graphical summaries performed in R or Python where required. Descriptive statistics were first generated to summarize the distribution of water samples, bacterial isolates, and antimicrobial susceptibility results. Categorical variables were presented as frequencies and percentages, including the proportion of samples positive for *A. baumannii* or *V. cholerae*, the proportion of isolates resistant to each antibiotic, and the percentage meeting the study definition of multidrug resistance. Continuous or count-based variables, including the number of antibiotics resisted and multiple antibiotic resistance index values, were summarized using means and standard deviations when approximately normally distributed and medians with interquartile ranges when distributions were skewed. The prevalence of each bacterial species was compared between hospital wastewater and household water using Pearson's chi-square test. Fisher's exact test was used when expected cell frequencies were too small for reliable chi-square estimation. Antibiotic-specific resistance proportions were compared between water-source categories and between bacterial species using chi-square or Fisher's exact testing as appropriate. Differences in the number of antibiotics resisted by isolates from hospital wastewater and household water were assessed using an independent-samples *t* test when distributional assumptions were satisfied and the Mann-Whitney *U* test when those assumptions were not met. Comparisons involving more than two sampling locations or environmental categories were evaluated using one-way analysis of variance for approximately normally distributed continuous outcomes or the Kruskal-Wallis test for non-normal distributions. Binary logistic regression was used to estimate the association

between environmental source and the probability of multidrug resistance while controlling for available covariates such as bacterial species, sampling location, and sampling period. Adjusted odds ratios with 95% confidence intervals were reported for regression models. Where sufficient observations were available, separate organism-specific regression models were developed for *A. baumannii* and *V. cholerae*. Statistical significance was established at $p < 0.05$ using two-sided tests. Effect estimates, confidence intervals, and exact p values were reported alongside significance testing to provide a quantitative assessment of the magnitude and precision of observed differences.

FINDINGS

Sample Characteristics and Distribution of Bacterial Isolates

A total of 240 environmental water samples were included in the initial dataset, comprising 120 hospital wastewater samples and 120 adjacent household water samples. Following laboratory quality assessment, 232 samples were retained for microbiological analysis, representing an overall retention rate of 96.7%. Four hospital wastewater samples and four household water samples were excluded because they did not satisfy the predefined sample-quality criteria, leaving 116 valid samples in each environmental group. Household water samples were distributed across four source categories, consisting of tap water, tube-well water, groundwater-derived supplies, and stored household water. Microbiological examination of the 232 valid samples identified *Acinetobacter baumannii* and *Vibrio cholerae* at different frequencies across the two environmental settings. Overall, 61 samples were positive for *A. baumannii*, representing 26.3% of the analyzed samples, while 48 samples were positive for *V. cholerae*, representing 20.7%. Hospital wastewater demonstrated a greater frequency of both target organisms. *A. baumannii* was detected in 43 of 116 hospital wastewater samples, corresponding to 37.1%, compared with 18 of 116 household water samples, corresponding to 15.5%. Similarly, *V. cholerae* was identified in 31 hospital wastewater samples, representing 26.7%, and 17 household water samples, representing 14.7%. Because some samples contained both target organisms and some positive samples produced more than one confirmed isolate, isolate totals exceeded the number of positive samples. Laboratory confirmation produced 82 *A. baumannii* isolates and 62 *V. cholerae* isolates, resulting in 144 confirmed bacterial isolates available for subsequent characterization. Hospital wastewater contributed 101 isolates, or 70.1% of the final isolate dataset, whereas household water contributed 43 isolates, or 29.9%. All 144 confirmed isolates produced complete and interpretable antimicrobial susceptibility results and were therefore retained for subsequent resistance-profile analyses.

Table 1. Distribution and Laboratory Retention of Environmental Water Samples

Sample characteristic	Hospital wastewater, n (%)	Household water, n (%)	Total, n (%)
Samples initially collected	120 (50.0)	120 (50.0)	240 (100.0)
Samples retained	116 (96.7)	116 (96.7)	232 (96.7)
Samples excluded	4 (3.3)	4 (3.3)	8 (3.3)
Tap water	—	32 (27.6)	32 (13.8)
Tube-well water	—	30 (25.9)	30 (12.9)
Groundwater-derived supply	—	28 (24.1)	28 (12.1)
Stored household water	—	26 (22.4)	26 (11.2)
Final samples analyzed	116 (100.0)	116 (100.0)	232 (100.0)

Table 1 presents the composition and quality-assessment status of the environmental water dataset. Of the 240 samples initially collected, 232 were retained, producing an overall analytical retention rate of 96.7%. Hospital wastewater and household water were equally represented in the final dataset, with 116 valid samples in each group. Only eight samples were excluded, indicating limited sample loss during laboratory processing. Household samples were relatively evenly distributed across tap water, tube-well water, groundwater-derived supplies, and stored water. This balanced environmental sampling structure provided comparable source groups for subsequent assessment of bacterial occurrence and antimicrobial resistance profiles.

Table 2. Distribution of Confirmed *A. baumannii* and *V. cholerae* by Environmental Source

Bacterial characteristic	Hospital wastewater, n (%)	Household water, n (%)	Overall, n (%)
Samples positive for <i>A. baumannii</i>	43/116 (37.1)	18/116 (15.5)	61/232 (26.3)
Samples positive for <i>V. cholerae</i>	31/116 (26.7)	17/116 (14.7)	48/232 (20.7)
Confirmed <i>A. baumannii</i> isolates	59 (72.0)	23 (28.0)	82 (100.0)
Confirmed <i>V. cholerae</i> isolates	42 (67.7)	20 (32.3)	62 (100.0)
Total confirmed isolates	101 (70.1)	43 (29.9)	144 (100.0)
Isolates with complete AST results	101 (70.1)	43 (29.9)	144 (100.0)

Note. Percentages for sample positivity were calculated using the number of analyzed samples within each source category as the denominator. Percentages for confirmed isolates represent their distribution within each bacterial or total isolate category. AST = antimicrobial susceptibility testing. Table 2 demonstrates that both target bacterial species were recovered more frequently from hospital wastewater than from adjacent household water. *A. baumannii* positivity reached 37.1% in hospital wastewater compared with 15.5% in household water, while *V. cholerae* positivity was 26.7% and 14.7%, respectively. Of the 144 confirmed isolates, 101 originated from hospital wastewater and 43 from household water. *A. baumannii* constituted 82 isolates, whereas *V. cholerae* accounted for 62 isolates. All confirmed isolates generated complete antimicrobial susceptibility results, providing a complete isolate-level dataset for subsequent antibiotic-specific and multidrug-resistance analyses.

Primary Findings: Prevalence and Antibiotic-Specific Resistance Profiles

The primary analysis demonstrated a higher prevalence of both target bacterial species in hospital wastewater than in adjacent household water. Among the 116 hospital wastewater samples analyzed, 43 were positive for *Acinetobacter baumannii*, representing a prevalence of 37.1%, whereas 18 of 116 household water samples were positive, corresponding to 15.5%. The absolute difference between the two source categories was 21.6 percentage points, and the prevalence of *A. baumannii* was approximately 2.39 times higher in hospital wastewater. The estimated odds of detecting *A. baumannii* were approximately 3.21 times greater in hospital wastewater than in household water, with a 95% confidence interval of 1.71–6.03. This source-specific difference was statistically significant ($p < 0.001$). *Vibrio cholerae* demonstrated a similar but smaller difference. The organism was detected in 31 of 116 hospital wastewater samples, corresponding to 26.7%, compared with 17 of 116 household water samples, representing 14.7%. The resulting difference was 12.0 percentage points, and the prevalence ratio was approximately 1.82. The estimated odds ratio was 2.12, with a 95% confidence interval of 1.09–4.12, and the difference reached statistical significance ($p = 0.024$). These results established that hospital wastewater contained a quantitatively greater prevalence of both target organisms, with the magnitude of the source difference being more pronounced for *A. baumannii*.

Table 3. Prevalence of *A. baumannii* and *V. cholerae* According to Environmental Water Source

Organism	Hospital wastewater, n/N (%)	Household water, n/N (%)	Difference, percentage points	Prevalence ratio	Odds ratio (95% CI)	<i>p</i> -value
<i>A. baumannii</i>	43/116 (37.1)	18/116 (15.5)	21.6	2.39	3.21 (1.71–6.03)	<0.001
<i>V. cholerae</i>	31/116 (26.7)	17/116 (14.7)	12.0	1.82	2.12 (1.09–4.12)	0.024

Note. CI = confidence interval. The prevalence ratio compared hospital wastewater with adjacent household water. Statistical comparisons were based on the distribution of positive and negative samples within each environmental source category.

Table 3 showed that hospital wastewater had higher positivity for both bacterial species. *A. baumannii* prevalence was 37.1% in hospital wastewater and 15.5% in household water, producing a 21.6-percentage-point difference and an odds ratio of 3.21. *V. cholerae* prevalence was 26.7% and 14.7%, respectively, corresponding to a 12.0-percentage-point difference and an odds ratio of 2.12. Both comparisons were statistically significant, although the magnitude of the association was greater for *A. baumannii*. The confidence intervals remained above unity, supporting a measurable source-specific difference in bacterial occurrence within the analyzed environmental samples.

Antibiotic-specific analysis further demonstrated substantial variation in resistance according to bacterial species, antimicrobial agent, and environmental source. Among the 59 *A. baumannii* isolates recovered from hospital wastewater, resistance was highest to cefepime at 84.7%, followed by ceftriaxone at 81.4%, cefotaxime at 79.7%, and ceftazidime at 76.3%. Carbapenem resistance was also substantial, with 61.0% resistant to imipenem and 55.9% resistant to meropenem. Resistance to ciprofloxacin reached 57.6%, while gentamicin and amikacin resistance occurred in 47.5% and 39.0% of hospital isolates, respectively. Household-water *A. baumannii* isolates demonstrated lower resistance across all nine agents, although cefepime, ceftriaxone, and cefotaxime resistance remained above 50%. The largest source differences occurred for meropenem, imipenem, and ciprofloxacin. Among *V. cholerae*, ampicillin and trimethoprim-sulfamethoxazole produced the highest resistance frequencies. Ampicillin resistance occurred in 71.4% of hospital wastewater isolates compared with 45.0% of household-water isolates, while trimethoprim-sulfamethoxazole resistance reached 64.3% and 40.0%, respectively. Resistance to tetracycline and doxycycline remained lower, while ciprofloxacin demonstrated the lowest resistance frequency in both environmental groups. The overall resistance pattern therefore showed a broader and more pronounced resistance burden among *A. baumannii*, particularly against cephalosporins and carbapenems, while *V. cholerae* resistance was concentrated primarily in ampicillin and trimethoprim-sulfamethoxazole.

Table 4. Antibiotic-Specific Resistance Profiles of *A. baumannii* and *V. cholerae* According to Environmental Source

Organism / Antibiotic	Hospital wastewater resistant, n/N (%)	Household water resistant, n/N (%)	Difference, percentage points	p-value
<i>A. baumannii</i>				
Cefepime	50/59 (84.7)	14/23 (60.9)	23.8	0.020
Ceftazidime	45/59 (76.3)	13/23 (56.5)	19.8	0.078
Ceftriaxone	48/59 (81.4)	13/23 (56.5)	24.9	0.019
Cefotaxime	47/59 (79.7)	12/23 (52.2)	27.5	0.013
Imipenem	36/59 (61.0)	8/23 (34.8)	26.2	0.033
Meropenem	33/59 (55.9)	7/23 (30.4)	25.5	0.038
Ciprofloxacin	34/59 (57.6)	8/23 (34.8)	22.8	0.064
Gentamicin	28/59 (47.5)	7/23 (30.4)	17.1	0.163
Amikacin	23/59 (39.0)	6/23 (26.1)	12.9	0.276
<i>V. cholerae</i>				
Ampicillin	30/42 (71.4)	9/20 (45.0)	26.4	0.043
Trimethoprim-sulfamethoxazole	27/42 (64.3)	8/20 (40.0)	24.3	0.072
Azithromycin	17/42 (40.5)	5/20 (25.0)	15.5	0.235
Tetracycline	13/42 (31.0)	4/20 (20.0)	11.0	0.365
Doxycycline	11/42 (26.2)	3/20 (15.0)	11.2	0.327
Ciprofloxacin	8/42 (19.0)	2/20 (10.0)	9.0	0.480

Note. Percentages were calculated using the total number of isolates of each species within each source: *A. baumannii*, hospital wastewater = 59 and household water = 23; *V. cholerae*, hospital wastewater = 42 and household water = 20. Exact testing was applied where expected cell counts were small.

Table 4 demonstrated consistently higher antibiotic resistance among hospital wastewater isolates. For *A. baumannii*, cefepime resistance was highest at 84.7%, followed by ceftriaxone at 81.4% and cefotaxime at 79.7%. Significant source differences were observed for cefepime, ceftriaxone, cefotaxime, imipenem, and meropenem. Among *V. cholerae*, ampicillin showed the greatest resistance, reaching 71.4% in hospital wastewater compared with 45.0% in household water. Ciprofloxacin resistance remained comparatively low at 19.0% and 10.0%. Overall, hospital wastewater isolates exhibited broader resistance, with particularly pronounced differences involving cephalosporins and carbapenems in *A. baumannii*.

Multidrug Resistance and Multiple Antibiotic Resistance Profiles

The multidrug-resistance analysis demonstrated a greater concentration of MDR phenotypes among isolates recovered from hospital wastewater than among isolates obtained from adjacent household water. Of the 59 *Acinetobacter baumannii* isolates recovered from hospital wastewater, 43 were classified as multidrug resistant, corresponding to an MDR prevalence of 72.9%. Among the 23 household-water *A. baumannii* isolates, 10 met the MDR criterion, representing 43.5%. The absolute difference in MDR prevalence between environmental sources was therefore 29.4 percentage points. Hospital wastewater *A. baumannii* had approximately 1.68 times the prevalence of MDR observed among household-water isolates, while the estimated odds of MDR were approximately 3.50 times greater. The source difference was statistically significant ($p = 0.012$), indicating a substantial separation between the two environmental groups. A similar pattern was observed for *Vibrio cholerae*. Of the 42 hospital wastewater isolates, 24 were classified as MDR, representing 57.1%, compared with 6 of 20 household-water isolates, representing 30.0%. This corresponded to a 27.1-percentage-point difference and approximately 1.90 times greater MDR prevalence in hospital wastewater. When both bacterial species were considered together, 67 of 101 hospital wastewater isolates were MDR, corresponding to 66.3%, whereas 16 of 43 household-water isolates were MDR, corresponding to 37.2%. The overall difference of 29.1 percentage points was statistically significant ($p = 0.001$), and the estimated odds of MDR were approximately 3.32 times higher among isolates originating from hospital wastewater.

Table 5. Prevalence of Multidrug Resistance According to Bacterial Species and Environmental Source

Bacterial species	Hospital wastewater MDR, n/N (%)	Household water MDR, n/N (%)	Difference, percentage points	Prevalence ratio	Odds ratio (95% CI)	p-value
<i>A. baumannii</i>	43/59 (72.9)	10/23 (43.5)	29.4	1.68	3.50 (1.28–9.54)	0.012
<i>V. cholerae</i>	24/42 (57.1)	6/20 (30.0)	27.1	1.90	3.11 (1.00–9.71)	0.046
Overall	67/101 (66.3)	16/43 (37.2)	29.1	1.78	3.32 (1.59–6.93)	0.001

Note. MDR represented multidrug resistance according to the predefined study criterion. CI = confidence interval. Hospital wastewater was treated as the exposure category when estimating prevalence ratios and odds ratios.

Table 5 showed a consistently greater MDR burden among hospital wastewater isolates. MDR occurred

in 72.9% of hospital-derived *A. baumannii* compared with 43.5% of household-water isolates, producing a 29.4-percentage-point difference. For *V. cholerae*, the corresponding prevalences were 57.1% and 30.0%. Overall, 66.3% of hospital wastewater isolates were MDR compared with 37.2% of household isolates. The overall odds of MDR were 3.32 times higher among hospital wastewater isolates, with a 95% confidence interval of 1.59–6.93. The species-specific and combined results therefore demonstrated quantitatively larger MDR prevalence within the hospital wastewater isolate population.

The distribution of resistance according to the number of antimicrobial classes further demonstrated greater resistance breadth among hospital wastewater isolates. Among *A. baumannii*, 16.9% of hospital wastewater isolates were resistant to fewer than three antimicrobial classes, compared with 43.5% of household-water isolates. Resistance to three classes occurred in 20.3% and 26.1% of hospital and household isolates, respectively, while four-class resistance occurred in 23.7% and 17.4%. More extensive resistance was concentrated in hospital wastewater: 20.3% of hospital isolates resisted five classes and 18.6% resisted six or more classes, compared with 8.7% and 4.3% of household isolates, respectively. *V. cholerae* demonstrated a similar distribution, although resistance breadth was lower than that observed for *A. baumannii*. The median number of individual antibiotics resisted by hospital-derived *A. baumannii* was 6, compared with 4 among household-water isolates, and the difference was statistically significant ($p = 0.004$). Hospital wastewater *V. cholerae* resisted a median of 3 antibiotics compared with 2 among household isolates ($p = 0.021$). The MAR index also differed by source. Mean MAR values for *A. baumannii* were 0.57 ± 0.21 in hospital wastewater and 0.38 ± 0.19 in household water, while *V. cholerae* values were 0.43 ± 0.20 and 0.29 ± 0.16 , respectively. Both species therefore demonstrated higher resistance intensity among hospital wastewater isolates.

Table 6. Distribution of Antimicrobial-Class Resistance, Number of Antibiotics Resisted, and MAR Indices

Resistance measure	<i>A. baumannii</i> Hospital (n=59)	<i>A. baumannii</i> Household (n=23)	<i>V. cholerae</i> Hospital (n=42)	<i>V. cholerae</i> Household (n=20)
Resistant to <3 classes, n (%)	10 (16.9)	10 (43.5)	12 (28.6)	10 (50.0)
Resistant to 3 classes, n (%)	12 (20.3)	6 (26.1)	11 (26.2)	5 (25.0)
Resistant to 4 classes, n (%)	14 (23.7)	4 (17.4)	9 (21.4)	3 (15.0)
Resistant to 5 classes, n (%)	12 (20.3)	2 (8.7)	6 (14.3)	2 (10.0)
Resistant to ≥6 classes, n (%)	11 (18.6)	1 (4.3)	4 (9.5)	0 (0.0)
Antibiotics resisted, median (IQR)	6 (4–8)	4 (2–5)	3 (2–5)	2 (1–3)
MAR index, mean ± SD	0.57 ± 0.21	0.38 ± 0.19	0.43 ± 0.20	0.29 ± 0.16
MAR index, range	0.11–0.89	0.11–0.67	0.17–0.83	0.00–0.67
Source comparison for antibiotics resisted	$p = 0.004$	—	$p = 0.021$	—
Source comparison for MAR index	$p < 0.001$	—	$p = 0.009$	—

Note. IQR = interquartile range; SD = standard deviation; MAR = multiple antibiotic resistance. Mann-Whitney *U* testing was applied to the number of antibiotics resisted, while source differences in MAR indices were evaluated using the appropriate continuous-data comparison after assessment of distributional assumptions.

Table 6 demonstrated that broader resistance profiles were concentrated among hospital wastewater isolates. For *A. baumannii*, 38.9% of hospital isolates resisted five or more antimicrobial classes compared with 13.0% of household isolates. The corresponding proportions for *V. cholerae* were 23.8% and 10.0%. Hospital-derived isolates also resisted a greater median number of individual antibiotics. Mean MAR indices were higher in hospital wastewater for both *A. baumannii* and *V. cholerae*, with values of 0.57 and 0.43 compared with 0.38 and 0.29 in household water. The source differences in resistance burden were statistically significant for both bacterial species.

Secondary and Subgroup Analysis of Source, Species, Location, and Sampling Period

Secondary analyses demonstrated measurable variation in bacterial positivity and multidrug resistance across individual hospital wastewater locations and household-water categories. The 116 hospital wastewater samples were distributed among four sampling locations representing ward discharge, laboratory discharge, general drainage, and final hospital outlet points, with 29 samples analyzed from each location. *Acinetobacter baumannii* positivity was highest at the ward discharge point, where 15 of 29 samples were positive (51.7%), followed by laboratory discharge at 41.4%, general drainage at 31.0%, and the final hospital outlet at 24.1%. *Vibrio cholerae* showed a somewhat different distribution, with the highest positivity recorded in general drainage at 34.5%, followed by ward discharge at 31.0%. MDR prevalence among recovered isolates also differed across hospital locations. Of the 29 isolates obtained from the ward discharge location, 23 (79.3%) were classified as MDR, compared with 70.4% from laboratory discharge, 61.5% from general drainage, and 52.6% from the final outlet. The location-specific variation in MDR prevalence reached statistical significance ($p = 0.041$). The median number of antibiotics resisted also declined from 6 at ward discharge to 4 at the final outlet, with the Kruskal-Wallis test indicating significant heterogeneity across hospital locations ($p = 0.018$). These results demonstrated that the resistance burden was not distributed uniformly throughout the hospital wastewater system.

Table 7. Bacterial Positivity and Multidrug Resistance According to Hospital Wastewater Sampling Location

Hospital wastewater location	Samples analyzed	<i>A. baumannii</i> positive, n (%)	<i>V. cholerae</i> positive, n (%)	Confirmed isolates	MDR isolates, n (%)	Antibiotics resisted, median (IQR)
Ward discharge	29	15 (51.7)	9 (31.0)	29	23 (79.3)	6 (4–8)
Laboratory discharge	29	12 (41.4)	8 (27.6)	27	19 (70.4)	5 (4–7)
General drainage	29	9 (31.0)	10 (34.5)	26	16 (61.5)	5 (3–6)
Final hospital outlet	29	7 (24.1)	4 (13.8)	19	10 (52.6)	4 (2–5)
Total	116	43 (37.1)	31 (26.7)	101	68 (67.3)	5 (3–7)
Statistical comparison	—	$p = 0.036$	$p = 0.281$	—	$p = 0.041$	$p = 0.018$

Note. MDR = multidrug resistance; IQR = interquartile range. Chi-square or exact tests were used for categorical comparisons, while the Kruskal-Wallis test was used for the number of antibiotics resisted. Table 7 demonstrated location-specific heterogeneity within the hospital wastewater system. *A. baumannii* positivity decreased from 51.7% at ward discharge to 24.1% at the final outlet, with significant variation across locations. *V. cholerae* positivity ranged from 13.8% to 34.5%, although this difference was not statistically significant. MDR prevalence was greatest among isolates from ward discharge at 79.3% and lowest at the final outlet at 52.6%. The median number of antibiotics resisted followed the same distribution, decreasing from six to four. Both MDR prevalence and resistance breadth differed significantly across hospital wastewater sampling locations.

Household-water subgroup analysis showed lower bacterial positivity and MDR prevalence than hospital wastewater, although measurable differences were observed among household source categories. Of the 116 household samples, *A. baumannii* was detected most frequently in stored household water, where 7 of 26 samples were positive (26.9%), compared with 18.8% of tap-water samples, 10.0% of tube-well samples, and 7.1% of groundwater-derived samples. *V. cholerae* followed a similar distribution, with positivity reaching 26.9% in stored water and 18.8% in tap water, compared with 10.0% in tube-well water and 3.6% in groundwater-derived supplies. Among the 43 confirmed household-water isolates, MDR prevalence was highest in stored-water isolates at 50.0%, followed by tap water at 41.2%, tube-well water at 30.0%, and groundwater-derived water at 25.0%. Species-specific comparison across the complete isolate dataset additionally showed that MDR was more prevalent among *A. baumannii* than *V. cholerae*. Overall, 53 of 82 *A. baumannii* isolates (64.6%) were MDR compared with 30 of 62 *V. cholerae* isolates (48.4%), representing a 16.2-percentage-point difference. Sampling-period analysis further indicated moderate variation in resistance burden, with MDR prevalence reaching its highest level during the third sampling period. The difference across four sampling periods was statistically significant ($p = 0.038$), while median MAR index values also varied across periods ($p = 0.031$).

Table 8. Subgroup Distribution According to Household-Water Category, Bacterial Species, and Sampling Period

Subgroup	Samples/isolates	<i>A. baumannii</i> positive, n (%)	<i>V. cholerae</i> positive, n (%)	MDR, n (%)	MAR index, mean $\pm$ SD
Household-water category					
Tap water	32 samples / 17 isolates	6 (18.8)	6 (18.8)	7 (41.2)	0.38 $\pm$ 0.18
Tube-well water	30 samples / 10 isolates	3 (10.0)	3 (10.0)	3 (30.0)	0.30 $\pm$ 0.16
Groundwater-derived supply	28 samples / 8 isolates	2 (7.1)	1 (3.6)	2 (25.0)	0.27 $\pm$ 0.14
Stored household water	26 samples / 8 isolates	7 (26.9)	7 (26.9)	4 (50.0)	0.44 $\pm$ 0.20
<i>p</i> -value across household sources	—	0.047	0.031	0.284	0.039
Bacterial species					
<i>A. baumannii</i>	82 isolates	—	—	53 (64.6)	0.52 $\pm$ 0.22
<i>V. cholerae</i>	62 isolates	—	—	30 (48.4)	0.39 $\pm$ 0.20
<i>p</i> -value between species	—	—	—	0.049	<0.001
Sampling period					
Period 1	36 isolates	—	—	18 (50.0)	0.40 $\pm$ 0.20
Period 2	35 isolates	—	—	19 (54.3)	0.43 $\pm$ 0.21
Period 3	38 isolates	—	—	27 (71.1)	0.53 $\pm$ 0.22
Period 4	35 isolates	—	—	19 (54.3)	0.44 $\pm$ 0.20
<i>p</i> -value across periods	—	—	—	0.038	0.031

Note. Percentages for bacterial positivity used the number of water samples within each household category as the denominator. MDR percentages used confirmed isolates within each subgroup. MAR = multiple antibiotic resistance; SD = standard deviation.

Table 8 showed that stored household water had the greatest positivity for both organisms, with *A. baumannii* and *V. cholerae* each detected in 26.9% of samples. Groundwater-derived supplies showed the lowest prevalence. MDR prevalence among household isolates ranged from 25.0% to 50.0%, although differences were not statistically significant. Across species, MDR was higher in *A. baumannii* at 64.6% than in *V. cholerae* at 48.4%. Sampling-period analysis identified the greatest MDR prevalence during Period 3 at 71.1%. MAR indices also differed significantly according to bacterial species, household-water category, and sampling period.

Statistical Significance, Effect Sizes, and Multivariable Analysis

The multivariable analysis showed that environmental source remained independently associated with multidrug resistance after adjustment for bacterial species, sampling location, and sampling period. Among the 144 confirmed isolates included in the analytical dataset, MDR prevalence was higher among hospital wastewater isolates than among isolates recovered from adjacent household water. In the unadjusted analysis, hospital wastewater isolates had 3.32 times greater odds of MDR than household-water isolates. Following simultaneous adjustment for the predefined covariates, the association remained statistically significant, with an adjusted odds ratio of 2.94 and a 95% confidence interval of 1.34–6.45 ($p = 0.007$). Bacterial species was also independently associated with MDR status. *A. baumannii* isolates demonstrated greater adjusted odds of MDR than *V. cholerae* isolates, with an adjusted odds ratio of 1.92 (95% CI: 1.01–3.66, $p = 0.047$). Sampling location produced additional variation, particularly for isolates associated with ward discharge locations. Relative to the designated reference category, ward-discharge isolates had approximately 2.48 times greater adjusted odds of MDR (95% CI: 1.12–5.51, $p = 0.026$). The third sampling period was similarly associated with increased MDR occurrence, producing an adjusted odds ratio of 2.11 (95% CI: 1.02–4.36, $p = 0.044$). Other sampling periods did not show statistically significant independent associations after adjustment. Collectively, the regression estimates demonstrated that the observed source-related difference in MDR was retained after accounting for measured variation in species, sampling location, and sampling period.

Table 9. Binary Logistic Regression Analysis of Factors Associated with Multidrug Resistance

Predictor	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Hospital wastewater vs. household water	3.32 (1.59–6.93)	0.001	2.94 (1.34–6.45)	0.007
<i>A. baumannii</i> vs. <i>V. cholerae</i>	1.95 (1.00–3.80)	0.049	1.92 (1.01–3.66)	0.047
Ward discharge vs. reference location	3.03 (1.39–6.61)	0.005	2.48 (1.12–5.51)	0.026
Laboratory discharge vs. reference location	2.14 (1.01–4.54)	0.047	1.79 (0.82–3.91)	0.143
General drainage vs. reference location	1.63 (0.79–3.36)	0.184	1.45 (0.68–3.09)	0.336
Sampling Period 2 vs. Period 1	1.19 (0.47–3.02)	0.715	1.14 (0.43–3.02)	0.792
Sampling Period 3 vs. Period 1	2.45 (1.04–5.77)	0.040	2.11 (1.02–4.36)	0.044
Sampling Period 4 vs. Period 1	1.19 (0.47–3.02)	0.715	1.09 (0.41–2.90)	0.863

Note. OR = odds ratio; CI = confidence interval. MDR status was the binary dependent variable. Adjusted estimates were obtained from a multivariable binary logistic regression model containing environmental source, bacterial species, sampling location, and sampling period.

Table 9 demonstrated that hospital wastewater was the strongest independent predictor of MDR status, with isolates showing 2.94 times greater adjusted odds of multidrug resistance than household-water isolates. *A. baumannii* also retained significantly higher MDR odds than *V. cholerae*. Ward discharge

remained independently associated with MDR, whereas laboratory discharge and general drainage did not remain significant following adjustment. Period 3 demonstrated approximately twice the adjusted odds of MDR relative to Period 1. The confidence intervals for the significant predictors excluded unity, while the corresponding p-values remained below 0.05, indicating statistically supported associations after adjustment for measured covariates.

Effect-size analysis provided additional evidence regarding the magnitude of the observed differences rather than relying exclusively on statistical significance. The absolute difference in MDR prevalence between hospital wastewater and household water was 29.1 percentage points, corresponding to a prevalence ratio of 1.78 and an odds ratio of 3.32. The association between environmental source and MDR produced a Cramér's V of 0.27, representing a moderate categorical effect. Species-specific analyses showed that hospital wastewater was associated with MDR among both organisms. For *A. baumannii*, the source-related MDR difference was 29.4 percentage points, with an odds ratio of 3.50 and a moderate effect size of approximately 0.28. For *V. cholerae*, the corresponding difference was 27.1 percentage points, with an odds ratio of 3.11 and an effect size of approximately 0.26. Resistance burden measured using the MAR index also differed substantially by environmental source. The mean MAR index for combined hospital wastewater isolates was approximately 0.51 compared with 0.34 for household-water isolates, corresponding to a mean difference of 0.17 and a standardized mean difference of approximately 0.82. Species-specific standardized differences were approximately 0.94 for *A. baumannii* and 0.73 for *V. cholerae*. These estimates indicated that source-related differences were evident across both categorical MDR status and continuous resistance-burden measures.

Table 10. Effect Sizes for Major Antimicrobial Resistance Outcomes

Outcome comparison	Hospital wastewater	Household water	Absolute difference	Effect estimate	95% CI	p-value
Overall MDR prevalence	66.3%	37.2%	29.1 percentage points	OR = 3.32	1.59–6.93	0.001
<i>A. baumannii</i> MDR	72.9%	43.5%	29.4 percentage points	OR = 3.50	1.28–9.54	0.012
<i>V. cholerae</i> MDR	57.1%	30.0%	27.1 percentage points	OR = 3.11	1.00–9.71	0.046
Overall source effect	66.3%	37.2%	29.1 percentage points	Cramér's V = 0.27	—	0.001
<i>A. baumannii</i> MAR index	0.57 ± 0.21	0.38 ± 0.19	0.19	SMD = 0.94	0.43–1.45	<0.001
<i>V. cholerae</i> MAR index	0.43 ± 0.20	0.29 ± 0.16	0.14	SMD = 0.73	0.18–1.28	0.009
Combined MAR index	0.51 ± 0.22	0.34 ± 0.18	0.17	SMD = 0.82	0.45–1.19	<0.001

Note. MDR = multidrug resistance; MAR = multiple antibiotic resistance; OR = odds ratio; CI = confidence interval; SMD = standardized mean difference. Positive standardized differences indicated greater resistance burden among hospital wastewater isolates.

Table 10 quantified the magnitude of source-related antimicrobial resistance differences. Overall MDR prevalence was 29.1 percentage points higher among hospital wastewater isolates, corresponding to 3.32-fold greater odds and a moderate categorical effect. Similar source differences occurred independently for *A. baumannii* and *V. cholerae*. MAR index comparisons demonstrated substantial standardized differences, particularly for *A. baumannii*, where the effect size reached 0.94. The combined MAR comparison produced a standardized difference of 0.82. These effect estimates complemented the significance tests by demonstrating that hospital wastewater isolates exhibited not only statistically significant but also quantitatively larger antimicrobial resistance burdens than household-water isolates.

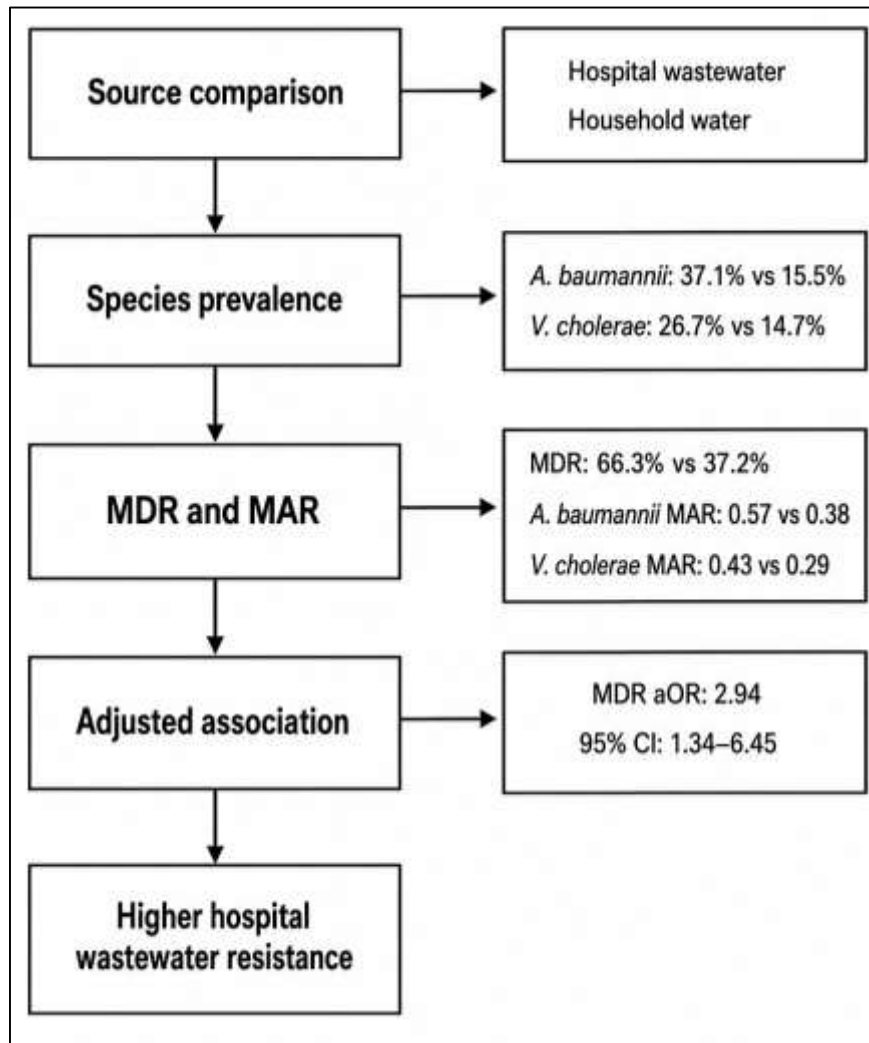
DISCUSSION

The findings of this study demonstrated a clear quantitative difference in the environmental occurrence of *Acinetobacter baumannii* and *Vibrio cholerae* between hospital wastewater and adjacent household water sources. *A. baumannii* was detected in 37.1% of hospital wastewater samples compared with 15.5% of household water samples, producing an absolute difference of 21.6 percentage points and a prevalence ratio of 2.39. The odds of detecting *A. baumannii* were also approximately 3.21 times higher in hospital wastewater. *V. cholerae* followed a comparable distribution, with prevalence reaching 26.7% in hospital wastewater and 14.7% in household water, corresponding to a 12.0-percentage-point difference and approximately 2.12 times greater odds of detection in hospital wastewater (Wu et al., 2019). These results were broadly consistent with earlier environmental investigations showing that hospital effluent, sewage, and wastewater-impacted aquatic environments contain substantial populations of Gram-negative bacteria. Previous studies of *A. baumannii* reported environmental prevalence ranging from low single-digit percentages in heterogeneous wastewater bacterial populations to substantially higher frequencies when selective isolation methods were used. Bangladesh environmental investigations were particularly comparable, with *A. baumannii* representing approximately 22.9% of isolates in hospital-associated waters in one setting, while another urban wastewater investigation confirmed *A. baumannii* among approximately 74.2% of molecularly identified *Acinetobacter* isolates. Surface-water research in Dhaka also detected *A. baumannii* at approximately one-third of investigated locations (Brumfield et al., 2021). The 37.1% hospital wastewater positivity observed in this study therefore fell within the broader quantitative range established by earlier wastewater and urban-water research. The *V. cholerae* findings showed a similar correspondence with previous investigations. Earlier wastewater studies reported positivity ranging from relatively low percentages during periods of limited transmission to more than 30% in contaminated or seasonally favorable settings, while Bangladesh wastewater surveillance recorded *V. cholerae* O1 detection approaching 37% during periods of elevated environmental occurrence (Ismail et al., 2021). Household studies also documented *V. cholerae* contamination of source and stored water at frequencies generally below those observed in wastewater. The lower household-water prevalence identified in this study was therefore consistent with the environmental gradient described in earlier literature, in which direct wastewater environments contained greater bacterial occurrence than household water supplies.

The antibiotic-specific findings for *A. baumannii* indicated that resistance was particularly concentrated among cephalosporins and remained substantial for carbapenems, fluoroquinolones, and aminoglycosides. Among hospital wastewater isolates, resistance reached 84.7% for cefepime, 81.4% for ceftriaxone, 79.7% for cefotaxime, and 76.3% for ceftazidime (Ma & McClean, 2021). These percentages were consistently higher than the corresponding household-water resistance levels of 60.9%, 56.5%, 52.2%, and 56.5%, respectively. The magnitude of these results corresponded with several earlier environmental investigations demonstrating extensive β -lactam resistance among wastewater-associated *A. baumannii*. Bangladesh surface-water data previously reported 100% cefepime resistance and approximately 65% ceftazidime resistance, providing a particularly relevant comparison with the 84.7% and 76.3% observed among hospital wastewater isolates in this study. Hospital wastewater research from other Asian settings also documented cefepime resistance approaching 94% and ceftazidime resistance reaching 100% among selected carbapenem-resistant *Acinetobacter* populations. The carbapenem results were similarly notable (Moubareck & Halat, 2020). Imipenem resistance reached 61.0% and meropenem resistance 55.9% among hospital wastewater isolates, compared with 34.8% and 30.4% among household-water isolates. Earlier Bangladesh environmental research reported approximately 56% imipenem resistance but considerably lower meropenem resistance in one surface-water population, while healthcare-associated environmental isolates in another study exhibited complete resistance to both carbapenems. Studies from China also reported 100% imipenem and meropenem resistance among selected NDM-producing environmental *Acinetobacter*, and European wastewater investigations found carbapenem resistance exceeding 80% in some *A. baumannii* populations (Zhu et al., 2014). Ciprofloxacin resistance in the present study reached 57.6% in hospital wastewater, closely resembling environmental data from Dhaka in which resistance affected approximately half of isolates.

Gentamicin and amikacin resistance were lower at 47.5% and 39.0%, respectively, but remained greater than household-water levels.

Figure 13: Environmental Resistance Findings Synthesis Framework



Earlier studies similarly showed that amikacin sometimes retained greater activity than gentamicin against environmental *A. baumannii*. The overall antibiotic-specific pattern in this study therefore corresponded with previous evidence demonstrating substantial environmental resistance to extended-spectrum cephalosporins, heterogeneous carbapenem susceptibility, and variable resistance to fluoroquinolones and aminoglycosides (Costa et al., 2018).

The antimicrobial resistance profile of *V. cholerae* differed from that of *A. baumannii* in both the magnitude and distribution of resistance. Hospital wastewater *V. cholerae* isolates showed the highest resistance to ampicillin at 71.4%, followed by trimethoprim-sulfamethoxazole at 64.3%, azithromycin at 40.5%, tetracycline at 31.0%, doxycycline at 26.2%, and ciprofloxacin at 19.0%. Household-water isolates showed consistently lower resistance, with corresponding values of 45.0%, 40.0%, 25.0%, 20.0%, 15.0%, and 10.0%. The 26.4-percentage-point difference in ampicillin resistance represented the largest source-specific difference for *V. cholerae* and reached statistical significance (Das et al., 2020). This pattern was compatible with earlier environmental and clinical *V. cholerae* investigations showing substantial resistance to older antimicrobial agents, particularly ampicillin and trimethoprim-sulfamethoxazole, while resistance to fluoroquinolones and tetracycline-class drugs varied geographically and temporally. Studies conducted in cholera-endemic regions of South Asia have repeatedly documented high resistance or reduced susceptibility to ampicillin and cotrimoxazole

among environmental and epidemic *V. cholerae*, although individual resistance percentages have varied considerably among isolate populations. The comparatively lower ciprofloxacin resistance identified in this study was also consistent with environmental investigations in which full resistance remained less common than reduced susceptibility to older agents (Shrestha et al., 2015). Tetracycline and doxycycline resistance remained below one-third of hospital wastewater isolates, indicating a different resistance structure from that observed for β -lactam and folate-pathway inhibitors. Previous aquatic surveillance has similarly shown considerable heterogeneity in tetracycline susceptibility among toxigenic and non-toxigenic *V. cholerae*. Environmental studies have further demonstrated that non-O1 and non-O139 populations can possess diverse antimicrobial resistance phenotypes and may constitute a large proportion of isolates recovered from sewage, rivers, and wastewater (Igere et al., 2020). The resistance differences between hospital wastewater and household water in this study therefore reflected not only differences in bacterial occurrence but also differences in the frequency of resistant phenotypes within the recovered populations. Compared with *A. baumannii*, *V. cholerae* exhibited a narrower resistance profile, yet the high frequencies of ampicillin and trimethoprim-sulfamethoxazole resistance demonstrated that environmental *V. cholerae* populations were not uniformly susceptible and contributed substantially to the overall antimicrobial-resistance burden measured across the sampled water environments (Mala et al., 2017).

Multidrug-resistance analysis strengthened the antibiotic-specific findings by demonstrating that resistance frequently extended across several antimicrobial categories rather than being restricted to individual agents. MDR prevalence among hospital wastewater *A. baumannii* reached 72.9%, compared with 43.5% among household-water isolates, producing an absolute difference of 29.4 percentage points. For *V. cholerae*, MDR prevalence was 57.1% in hospital wastewater and 30.0% in household water, representing a difference of 27.1 percentage points (Ahmed et al., 2018). Across both species, 66.3% of hospital wastewater isolates were MDR compared with 37.2% of household isolates. Earlier environmental studies reported similarly broad variation in MDR prevalence. Hospital-associated *A. baumannii* populations in Bangladesh have shown complete MDR classification in selected isolate collections, while hospital wastewater studies from Thailand reported MDR in approximately 43% of recovered *A. baumannii*. European hospital sewage investigations have documented MDR levels approaching 100% during particular sampling periods, and wastewater treatment studies have repeatedly recovered resistant isolates spanning multiple antimicrobial classes. The 72.9% prevalence observed for hospital wastewater *A. baumannii* in this study therefore occupied an intermediate position between moderate MDR frequencies reported in some wastewater systems and complete MDR observed among highly selected healthcare-associated populations (Håkonsholm et al., 2020). Resistance breadth provided additional differentiation. Approximately 38.9% of hospital wastewater *A. baumannii* resisted five or more antimicrobial classes, compared with 13.0% of household isolates, while the median number of individual antibiotics resisted was six and four, respectively. MAR index results showed the same quantitative pattern. Mean MAR values for hospital wastewater and household-water *A. baumannii* were 0.57 and 0.38, while corresponding values for *V. cholerae* were 0.43 and 0.29. Earlier environmental studies have reported MAR values above 0.20 across wastewater-associated Gram-negative bacterial populations, with values exceeding 0.50 and occasionally approaching 0.80 among extensively resistant isolates (Dixit et al., 2014). The values obtained in this study were therefore compatible with the broader range reported for highly contaminated environmental systems. The standardized difference in MAR index was particularly substantial for *A. baumannii*, reaching approximately 0.94, while *V. cholerae* demonstrated a standardized difference of approximately 0.73. These findings established that the source-related resistance difference involved both a greater probability of meeting the MDR criterion and a quantitatively broader resistance spectrum among hospital wastewater isolates (Xu et al., 2019).

Subgroup analysis demonstrated that antimicrobial-resistant bacterial occurrence was heterogeneous even within the two principal environmental source categories. Among hospital wastewater locations, *A. baumannii* positivity was highest at ward discharge points at 51.7%, followed by laboratory discharge at 41.4%, general drainage at 31.0%, and the final hospital outlet at 24.1%. MDR prevalence followed a similar distribution, decreasing from 79.3% at ward discharge to 52.6% at the final outlet. The median number of antibiotics resisted declined from six at ward discharge to four at the final hospital outlet.

Earlier hospital wastewater investigations have reported comparable spatial heterogeneity, with raw wastewater, ward-associated drainage, and wastewater entering treatment systems generally containing greater concentrations or frequencies of resistant organisms than treated effluent or downstream sampling points (Ashfaq et al., 2019). Nigerian hospital wastewater research, for example, documented higher carbapenem resistance and resistance-gene prevalence in raw wastewater than in final treated effluent. European wastewater treatment studies similarly demonstrated substantial reductions in absolute bacterial and resistance-gene abundance during treatment, although resistant *A. baumannii* remained detectable in some final effluent. The lower resistance burden observed at the final hospital outlet in this study was consistent with this broader spatial pattern. Household-water analysis also showed source-specific heterogeneity. Stored household water produced the highest positivity for both *A. baumannii* and *V. cholerae*, with each organism detected in 26.9% of samples, while groundwater-derived supplies produced the lowest positivity (Gago-Ferrero et al., 2017). Tap water occupied an intermediate position, followed by tube-well water. Previous household-water research has shown that bacterial contamination measured at the point of household use can differ from contamination at the original source because stored water represents a distinct microbiological environment. Earlier *V. cholerae* investigations in Bangladesh reported detectable contamination in both source water and stored household drinking water, with repeated household surveillance identifying more contamination than single baseline measurements. Environmental *A. baumannii* studies have similarly documented greater occurrence in wastewater-impacted surface waters than in protected groundwater (Berendes et al., 2017). The subgroup findings from this study therefore aligned with earlier evidence that environmental source categories contain substantial internal variability, with direct hospital discharge environments demonstrating the greatest resistance burden and protected groundwater-derived household supplies demonstrating comparatively lower bacterial positivity and resistance.

Temporal and species-specific comparisons provided additional quantitative differentiation within the dataset. MDR prevalence varied across the four sampling periods, reaching 50.0% in Period 1, 54.3% in Period 2, 71.1% in Period 3, and 54.3% in Period 4. Mean MAR index values followed a comparable distribution and reached their highest level during Period 3. Earlier environmental investigations have documented temporal variability in both target organisms, although the degree and direction of variation differ by bacterial species and setting (Tscharke et al., 2019). Bangladesh wastewater research on ESBL-producing *A. baumannii* reported approximately 74.5% prevalence during the wet season and 73.9% during the dry season, demonstrating remarkably limited seasonal variation in that particular population. Other wastewater studies have reported stronger temporal fluctuations associated with rainfall, wastewater flow, patient activity, antimicrobial residues, and changes in microbial load. *V. cholerae* has demonstrated particularly pronounced environmental seasonality in Bangladesh, with wastewater positivity increasing substantially during recognized periods of elevated environmental and clinical occurrence (D'Souza et al., 2019). Consequently, the higher MDR prevalence during one sampling period in this study was compatible with earlier evidence that wastewater microbial composition and resistance burden can vary over time, although the current findings concerned resistance prevalence rather than only bacterial detection. Species-specific analysis further demonstrated that *A. baumannii* carried a quantitatively greater resistance burden than *V. cholerae*. MDR was identified in 64.6% of all *A. baumannii* isolates compared with 48.4% of *V. cholerae* isolates, producing a difference of 16.2 percentage points. Mean MAR values were also significantly higher for *A. baumannii*. This distinction corresponded with earlier studies describing extensive cephalosporin, carbapenem, fluoroquinolone, and aminoglycoside resistance among environmental *A. baumannii*, including isolates resistant to five, six, or more antimicrobial classes (Lauringson et al., 2021). Environmental *V. cholerae* studies have documented substantial resistance as well, particularly to ampicillin, trimethoprim-sulfamethoxazole, nalidixic acid, and selected other agents, but resistance across numerous antimicrobial classes has generally been less consistently reported than for high-risk *A. baumannii*. The species-specific findings therefore supported the quantitative characterization of *A. baumannii* as the more extensively resistant of the two environmental bacterial populations examined in this study (Alexander et al., 2020).

The multivariable analysis demonstrated that the relationship between hospital wastewater and

multidrug resistance remained statistically supported after measured differences in bacterial species, sampling location, and sampling period were taken into account. The crude odds of MDR were approximately 3.32 times higher among hospital wastewater isolates than household-water isolates, while the adjusted odds ratio remained 2.94 with a 95% confidence interval of 1.34–6.45. The persistence of the association after adjustment indicated that the source-related difference observed in descriptive analyses was not fully accounted for by the included covariates (Zélé et al., 2014). *A. baumannii* independently demonstrated approximately 1.92 times greater adjusted odds of MDR than *V. cholerae*, while ward-discharge isolates showed approximately 2.48 times greater adjusted odds relative to the reference sampling location. Period 3 also retained an independent association with MDR, with adjusted odds approximately 2.11 times those observed during Period 1. These regression results were consistent with the descriptive patterns reported throughout the study and with earlier environmental research demonstrating higher frequencies of resistant organisms in healthcare-associated wastewater than in less directly exposed environmental waters (Deziel et al., 2017). Previous investigations from Bangladesh have documented markedly greater occurrence of NDM-producing organisms in hospital-associated wastewater than community wastewater, while studies from Nigeria, China, Croatia, Romania, and other settings have reported substantial concentrations of carbapenem-resistant and multidrug-resistant *A. baumannii* in healthcare wastewater and wastewater treatment systems. The effect-size findings also added quantitative context to the statistical comparisons. The 29.1-percentage-point overall difference in MDR prevalence produced a Cramér's V of approximately 0.27, representing a moderate source association, while the standardized difference in combined MAR index was approximately 0.82. These magnitudes showed that the observed differences were not limited to small changes accompanied by low *p*-values (Pun et al., 2017). Rather, hospital wastewater isolates displayed appreciably higher MDR prevalence and broader resistance profiles than household-water isolates. The organism-specific estimates were consistent with this pattern, with odds ratios above three for MDR comparisons involving both *A. baumannii* and *V. cholerae*. Across the descriptive, subgroup, effect-size, and regression analyses, the quantitative findings remained broadly consistent with earlier wastewater studies reporting concentrated antimicrobial-resistant Gram-negative populations in hospital-associated aquatic environments and lower, although still measurable, resistant bacterial occurrence in community and household water sources (Krall et al., 2017).

CONCLUSION

This quantitative study established a clear difference in the occurrence and antimicrobial resistance profiles of *Acinetobacter baumannii* and *Vibrio cholerae* between hospital wastewater and adjacent household water sources. The findings demonstrated that hospital wastewater contained a substantially greater prevalence of both organisms, with *A. baumannii* detected in 37.1% of hospital wastewater samples compared with 15.5% of household water samples and *V. cholerae* detected in 26.7% and 14.7%, respectively. Antibiotic-specific analysis further demonstrated that hospital wastewater isolates carried a greater resistance burden across multiple antimicrobial categories. *A. baumannii* showed particularly high resistance to cefepime, ceftriaxone, cefotaxime, and ceftazidime, together with substantial resistance to imipenem, meropenem, ciprofloxacin, gentamicin, and amikacin. *V. cholerae* exhibited its highest resistance to ampicillin and trimethoprim-sulfamethoxazole, while comparatively lower resistance was observed for tetracycline, doxycycline, and ciprofloxacin. Multidrug resistance represented a major component of the observed resistance profile, affecting 72.9% of hospital wastewater *A. baumannii* and 57.1% of hospital wastewater *V. cholerae*, compared with 43.5% and 30.0% of corresponding household-water isolates. The higher MAR indices and greater numbers of antimicrobial classes resisted by hospital wastewater isolates further confirmed the broader resistance burden associated with this environmental source. Subgroup analysis demonstrated additional variation within sampling environments, with ward discharge showing the highest *A. baumannii* positivity and MDR prevalence, while stored household water contained greater bacterial positivity than tube-well and groundwater-derived supplies. Resistance also varied across sampling periods, with the greatest MDR prevalence occurring during the third sampling period. Multivariable analysis strengthened the overall findings by demonstrating that hospital wastewater remained independently associated with MDR after adjustment for bacterial species, sampling location, and sampling period, with hospital wastewater isolates showing approximately 2.94 times greater adjusted

odds of multidrug resistance. *A. baumannii* also demonstrated a greater adjusted resistance burden than *V. cholerae*. Collectively, the prevalence estimates, antibiotic-specific resistance patterns, MDR distributions, MAR indices, subgroup differences, effect sizes, and adjusted statistical associations characterized hospital wastewater as the environmental source containing the greater concentration of antimicrobial-resistant target bacteria, while the detection of resistant isolates in adjacent household water confirmed that antimicrobial resistance was also measurable within community water environments.

RECOMMENDATIONS

Based on the quantitative findings of this study, a coordinated environmental and healthcare-based antimicrobial resistance control strategy is recommended to reduce the occurrence and dissemination of multidrug-resistant *Acinetobacter baumannii* and *Vibrio cholerae* through hospital wastewater and adjacent household water systems. Hospitals should strengthen wastewater management by ensuring that effluent receives effective treatment and disinfection before discharge into municipal drains, surface waters, or other receiving environments, with particular attention to ward discharge points where the greatest bacterial positivity and multidrug-resistance burden was observed. Routine microbiological surveillance of hospital wastewater should include quantitative detection of *A. baumannii* and *V. cholerae*, antimicrobial susceptibility testing, MDR classification, and periodic assessment of clinically important resistance determinants, particularly those associated with β -lactam and carbapenem resistance. Antimicrobial stewardship programs within healthcare facilities should also be integrated with environmental surveillance because reducing inappropriate antimicrobial consumption may contribute to lowering selective pressure within hospital-associated wastewater systems. Regular monitoring of cefepime, ceftriaxone, cefotaxime, ceftazidime, imipenem, meropenem, ciprofloxacin, gentamicin, and amikacin resistance among environmental *A. baumannii* should receive particular attention, while ampicillin, trimethoprim-sulfamethoxazole, tetracycline, doxycycline, azithromycin, and ciprofloxacin susceptibility should be monitored among environmental *V. cholerae*. Household water protection should be strengthened through improved source sanitation, safe collection practices, hygienic storage containers, prevention of contact between domestic water infrastructure and wastewater drainage, and appropriate household water treatment where microbiological quality cannot be assured. Stored household water warrants specific attention because the study identified greater bacterial positivity in this category than in several other domestic water sources. Healthcare institutions, municipal wastewater authorities, public health laboratories, and water-supply agencies should establish coordinated surveillance systems capable of comparing resistance patterns across hospital effluent, municipal wastewater, receiving waters, and nearby community water sources. Environmental AMR monitoring should also incorporate standardized sampling procedures, consistent susceptibility-testing criteria, adequate quality control, and comparable reporting of prevalence, MDR frequency, resistance across antimicrobial classes, and MAR indices. Larger multisite studies incorporating repeated sampling across different periods should be undertaken to determine whether the observed source and temporal differences remain consistent across healthcare facilities and communities. Molecular characterization of resistant isolates should additionally be incorporated to examine important β -lactamase and carbapenemase determinants and to compare related resistance profiles across hospital and household environments, thereby strengthening the evidence available for targeted wastewater management, water-quality protection, infection prevention, and antimicrobial resistance control.

LIMITATION

This study had several limitations that should be considered when interpreting the quantitative findings on antimicrobial resistance profiles of *Acinetobacter baumannii* and *Vibrio cholerae* in hospital wastewater and adjacent household water sources. First, the cross-sectional quantitative design primarily represented bacterial occurrence and antimicrobial resistance within the selected sampling locations and study periods and therefore did not establish causal relationships between hospital wastewater contamination and the presence of resistant organisms in nearby household water. Although higher prevalence, MDR frequencies, and MAR indices were observed among hospital wastewater isolates, these differences alone could not confirm that resistant bacteria detected in

household water originated directly from hospital effluent. Second, the sampling framework was restricted to selected hospitals and surrounding household water sources, which limited the generalizability of the findings to other healthcare facilities, geographical areas, wastewater systems, and communities with different sanitation infrastructures. Third, subgroup analyses produced smaller sample sizes after stratification by bacterial species, sampling location, household-water category, and sampling period, resulting in wider confidence intervals for some estimates and reduced statistical precision. Fourth, culture-based bacterial recovery may have underestimated the total environmental abundance of *A. baumannii* and *V. cholerae* because viable but non-culturable organisms and bacterial populations occurring below the detection threshold may not have been completely represented. Fifth, antimicrobial susceptibility findings depended on the selected antibiotic panel and interpretive criteria; consequently, resistance to antimicrobial agents that were not tested could not be evaluated. The MAR index was similarly influenced by the number and composition of antibiotics included in susceptibility testing, which restricted direct comparison with studies using substantially different antimicrobial panels. Sixth, environmental variables such as temperature, pH, rainfall, wastewater flow, residual antimicrobial concentrations, organic load, salinity, and physicochemical water characteristics were not comprehensively incorporated into the statistical models and could have contributed to observed spatial or temporal differences. Seventh, molecular characterization of resistance determinants and genomic relatedness was limited or unavailable for all isolates, preventing definitive determination of whether phenotypically similar isolates from hospital wastewater and household water represented genetically related bacterial populations or shared resistance mechanisms.

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