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Isolation, Identification, and Antibiogram of Colistin-resistant *Acinetobacter baumannii* from Rivers in and around Kathmandu Valley

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Acinetobacter baumannii,* an opportunistic Gram-negative pathogen, poses an escalating threat in clinical settings due to the rise of multidrug-resistant infections. Despite its clinical significance, there exists a considerable gap in understanding its environmental dissemination.
Aims and Objectives: The primary objective is to examine the distribution of *A. baumannii* and its antibiotic resistance in river ecosystems. Specifically, we aim to identify strains resistant to Colistin, a last-resort antibiotic, and elucidate the susceptibility patterns to other antibiotics.
Materials and Methods: Water samples from 10 rivers were collected and subjected to analysis using Leeds *Acinetobacter* Agar Base and a series of biochemical tests. Antibiotic susceptibility testing, focusing on Colistin resistance, was performed using standard procedures.

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Results: Out of the 284 isolated strains, 14 (4.9%) exhibited resistance to Colistin, while demonstrating varying susceptibility patterns to other antibiotics. Notably, Gentamycin showed effectiveness against resistant strains (14.28%), while Ceftazidime resistance was complete. Colistin-sensitive strains displayed high susceptibility to Ciprofloxacin (84.44%) and lower susceptibility to Chloramphenicol (53.33%). Carbapenem susceptibility was observed across all isolates.

Conclusion: The study underscores a concerning environmental presence of multidrug-resistant *A. baumannii* in rivers around Kathmandu Valley, with Sundarijal being the exception. The findings emphasize the necessity of scrutinizing environmental reservoirs for pathogen spread, advocating for heightened awareness of potential health implications beyond clinical settings. Urgent attention is needed to comprehend and counteract the emergence and dissemination of antibiotic resistance, necessitating comprehensive strategies and continued surveillance

Keywords: Acinetobacter baumannii; antibiotic resistance; Colistin; ESKAPE; carbapenem; Kathmandu Valley.

1. INTRODUCTION

Antimicrobial resistance presents a global health challenge, with rising bacterial infections defying conventional antibiotics [1]. Environmental monitoring reveals the pervasive presence of pharmaceuticals in soil and water, raising concerns about the emergence of antibiotic-resistant bacterial strains over time [2]. While antibiotic resistance in clinical settings is acknowledged, the environmental impact has been largely overlooked. The use of antibiotics and the spread of antibiotic resistance in clinical settings is a well-recognized proble000m, but antibiotics and antibiotic resistance as environmental problems and pollutants have largely been overlooked. As a result, the increasing incidence of resistance to a wide range of antibiotic agents by a variety of organisms outside clinical settings is a major concern [3].

Similarly, in the case of *A. baumannii* too, hospital-acquired infections have increased dramatically worldwide [4]. Difficulties caused by this pathogen in the hospital setting are exacerbated by its ability to form biofilms on abiotic or biotic surfaces and to cope with different environmental conditions, including desiccation and disinfectants [5]. Non-susceptibility to commonly used antimicrobials has also been observed, with Carbapenem resistance becoming a global problem since 2000 [4].

A. baumannii, recognized as one of the six 'superbugs' by the Infectious Diseases Society of America (IDSA) [6], is a Gram-negative, non-fermentative coccobacillus within the Moraxellaceae family. Gaining prominence as a nosocomial pathogen in recent years [7], *A. baumannii*'s adaptability to antibacterial agents in its environment is facilitated by mutation, selection, and genetic exchange mechanisms [2]. Studies have demonstrated that wastewater is one of the highly selective environments and that it contributes to the high rates of resistant bacteria that are being discharged in the natural environment, including the river systems [8].

Untreated hospital waste containing residual antibiotics at low concentrations emerges as a significant contributor to antibiotic resistance development. Such locales become pivotal hotspots for horizontal gene transfer (HGT) of antibiotic-resistant genes, fostering the emergence of resistant pathogens [9]. This intricate interplay underscores the urgent need for comprehensive strategies to mitigate the environmental spread of antibiotic resistance associated with *A. baumannii* [6].

The basic principle underlying wastewater management is the stringent control of discharging hazardous liquids into sewers without prior treatment, ensuring the prevention of introducing pathogenic organisms into the environment. However, in Nepal, only Kathmandu Valley boasts a sewerage network, accessible to a mere 15% of households [10]. Therefore, even if the hospitals discharge their healthcare liquid waste into the sewerage system, it is mixed with the sewage and gets into surface water without proper treatment [11].

Untreated hospital effluents pose a significant risk, releasing concentrated forms of infectious agents and antibiotic-resistant microbes into communities, leading to waterborne diseases such as cholera, typhoid fever, dysentery, and gastroenteritis. Environmental compartments, including wastewater, surface water,

groundwater, sediments, and soils, have revealed the presence of antibiotics, disinfectants, and bacteria resistant to them [8]. Prolonged exposure of microorganisms to low concentrations of antibiotics in wastewater and surface water enhances the potential for the development of antibiotic resistance in these organisms [11]. Addressing this issue is imperative for safeguarding public health and environmental well-being.

The recent development of antibiotic resistance clearly demonstrates the urgent need for global surveillance data that can inform clinicians, public health experts, policymakers and pharmaceutical companies about the dynamic spread of antibiotic-resistant pathogens in a geographically explicit and timely manner [12]. However, the antibiotic susceptibility pattern of bacterial isolates in much of the developing world is unknown. Susceptibility testing cannot be done readily because equipment, personnel, and consumables are scarce and expensive [13]. Therefore, the purpose of the present study was to examine the presence of MDR *A. baumannii* in river water across Kathmandu Valley.

The escalating challenge of antibiotic resistance underscores the critical need for global surveillance data, essential for guiding clinicians, public health experts, policymakers, and pharmaceutical companies in understanding the dynamic spread of antibiotic-resistant pathogens [14]. Unfortunately, the antibiotic susceptibility pattern of bacterial isolates in much of the developing world is unknown [13]. Considering these challenges, the present study aims to investigate the prevalence of multidrug-resistant *A. baumannii* in river water across the Kathmandu Valley. This research contributes to addressing the information gap in antibiotic resistance patterns in resource-constrained settings.

2. METHODS

2.1 Sampling Site

Ten different sites across Kathmandu Valley (A to J) were selected to carry out this study, namely:

Site A: Chobar (Bagmati)
Site B: Pashupati (Bagmati)
Site C: Balkhu (Balkhu)
Site D: Jadibuti (Hanumante)
Site E: Tilganga (Bagmati)
Site F: Teku (Bishnumati)
Site G: Balkumari (Manohara)
Site H: Chunikhel (Dhobikhola)
Site I: Sundarijal (Bagmati)
Site J: Tripureshor (Tukucha)

These sites were chosen for their geographical significance. Site I is located upstream and offers a baseline for natural water quality before urban influence. Sites C, D, F, G and J are near hospital effluents. Sites A, B, E, and H are influenced by urban runoff.

2.2 Study Period

The study was carried out in the Microbiology Laboratory of St. Xavier's College, Maitighar, Kathmandu from February 2021 to December 2021.

2.3 Sample Size

A total number of 30 water samples were collected from 10 different sites from different rivers around Kathmandu Valley using the grab sample method. The grab sample method involves taking water samples at a specific point in time (7:00 am), providing a representative sample of the water's composition at that moment. This approach is particularly useful when studying short-term variations and assessing the immediate impact of environmental factors on water quality. Each sample was collected weekly over a period of five months from March 2nd to August 16th, 2021, allowing for a comprehensive examination of temporal variations in water quality.

2.4 Sample Collection

River water samples for bacteriological analysis were collected in sterile BOD bottles. During sample collection, the bottle caps were opened aseptically, and the bottles were lowered into the water with their mouth directed against the water current. The water samples were transported in an ice box to the laboratory and processed within two hours of sample collection.

2.5 *A. baumannii* Isolation and Identification

A. baumannii was isolated through the spread plate technique. 0.1 ml of sample was pipetted out from 10^{-3} dilution series onto the centre of the surface of prepared *Acinetobacter* Agar Base (with added Leeds *Acinetobacter* Selective Supplement) from HiMedia Laboratories Pvt. Ltd, Mumbai, India. An L-shaped glass spreader was

dipped into alcohol. The glass spreader was flamed over a Bunsen burner. The sample was spread evenly over the surface of the agar using the sterile glass spreader, carefully rotating the Petri plate underneath at the same time. The plate was incubated at 42°C and growth of the organism was observed after 24 hours.

Microscopic examination was done after the incubation period and the isolated *A. baumannii* were subcultured on MacConkey agar (MA) and Nutrient agar (NA) and incubated at 42°C for 24 hours. Further biochemical tests (Oxidase, Catalase, Methyl red, Voges-Proskaeur test, Citrate utilization test, Indole production test, Triple sugar ion agar test, and Urease test) were performed on all isolates. All the media were) from HiMedia Laboratories Pvt. Ltd, Mumbai, India. The obtained data were interpreted following the Clinical Laboratory Standards Institute (CLSI) [15].

2.6 Screening for Colistin-resistant *A. baumannii*

Colistin (4 mg/ml) infused Nutrient agar plates were prepared and a well diffusion method was used to screen colistin-resistant isolates of *A. baumannii.* 0.1 ml of bacterial inoculum prepared by suspending a single colony from overnight agar plates in nutrient broth to the final turbidity of a 0.5 McFarland standard was added to the well. After diffusion, the plates were incubated at 37°C for 24 hours, and growth was observed.

2.7 Antibiogram of Isolates

Colistin-resistant *A. baumannii* were further tested against a series of antibiotics to study their drug resistance profile. Kirby-Bauer's disc diffusion method was used for the antibiotic susceptibility test.

2.8 Detection of Carbapenemase Production

The modified carbapenem inactivation method was used for phenotypic detection of carbapenemase production in the colistin-resistant *A. baumannii* isolated. The isolates were subcultured on Blood Agar (from HiMedia Laboratories Pvt. Ltd, Mumbai, India) and incubated at 42°C for 24 hours. A single colony from each isolate was emulsified in 2 ml Tryptic Soy Broth (TSB) (from HiMedia Laboratories Pvt. Ltd, Mumbai, India). Meropenem disk was immersed in the suspension and was incubated at 37°C for four hours. A 0.1 ml of *E. coli* ATCC 25922 was then prepared by suspending a single colony from overnight agar plates in nutrient broth to the final turbidity of a 0.5 McFarland standard and was carpet cultured on Mueller Hinton Agar (MHA) plates. Meropenem disks were removed from TSB and placed on the plates. Another fresh meropenem disk was placed as control. The MHA plates were incubated at 37°C for 24 hours and observed for a zone of inhibition.

2.9 Preservation of *A. baumannii*

A. baumannii isolates in pure culture, after performing the biochemical and antimicrobial susceptibility testing, were preserved in Tryptic Soy broth (TSB) containing 20% Glycerol and kept at -70°C until further tests were required.

2.10 Quality Control

Laboratory equipment like incubators, refrigerators, autoclaves, and hot-air ovens were regularly monitored for their efficiency. The temperature of the incubator and refrigerator were monitored twice a day. Reagents and media were regularly monitored for their expiry date and proper storage condition. After media preparation, they were properly labelled with the preparation date. The quality of the media prepared was checked by subjecting one plate of each batch to sterility and performance testing.

A purity plate was used to ensure that the inoculation used for biochemical tests was pure culture and to ensure that the biochemical tests were performed in an aseptic condition. Thus, while performing biochemical tests, the same inoculums were subcultured in the respective medium and incubated. The media were then checked for the appearance of pure growth of organisms. The development of pure culture in the medium would confirm the purity of the inoculum.

Antibiotic susceptibility tests were performed by maintaining the thickness of Mueller Hinton agar at 4mm and pH at 7.2-7.4. Similarly, antibiotic discs containing the correct amount as indicated were used. MHA and the antibiotic discs were checked for their lot numbers, manufacturing dates, expiry dates, and storage conditions. For the standardization of the Kirby Bauer test and for performance testing of antibiotics and MHA, the control strain of *E. coli* ATCC 25922 was tested.

2.11 Statistical Analysis

The data obtained were entered into MS Excel and analyzed using Statistical Package for Social Science (SPSS) software (Version 21.0).

3. RESULTS

3.1 Culture Result of the Samples

The samples were cultured on Leeds *Acinetobacter* Agar Base at 42°C. Out of the 30 samples, 27 samples showed growth for *A. baumannii*, whereas three samples from Site I showed no growth on the media.

3.2 pH

The pH was noted every time the samples were collected. Each sample's maximum and minimum pH are given below, which was found to be within the range of WHO guidelines and was between 6.1 to 8.4. There were no huge differences in pH among the 10 sites.

Table 1. pH of the river water samples

Site	pH	
	Maximum	Minimum
A	7.1	6.8
B	7.0	6.7
C	6.9	6.8
D	7.1	6.8
E	6.5	6.4
F	8.1	7.8
G	7.1	7.0
H	7.4	7.2
I	7.4	7.2
J	6.6	6.4

3.3 Morphological and Biochemical Characteristics of *A. baumannii*

From the 27 samples, 284 isolates of *A. baumannii* were isolated using the *Acinetobacter* agar base. All the isolates were subjected to Gram staining, followed by biochemical tests. The isolates were Gram-negative, non-motile coccobacillus which showed growth on Simmon's citrate and MacConkey agar at 37 °C.

3.4 Screening of Colistin-Resistant *A. baumannii*

The susceptibility breakpoint of colistin is 4 µg/ml for *A. baumannii* 17978 (CLSI 2022). All 284 isolates of *A. baumannii* were grown on MHA with 4 µg/ml colistin. Out of them, 14 (4.9%) isolates showed growth on the media and 270 (95.1%) showed negative growth. The isolates that grew on MHA incorporated with 4 µg/ml were screened as colistin-resistant.

3.5 Antibiotic Susceptibility Pattern of Colistin-Resistant *A. baumannii*

The 14 colistin-resistant *A. baumannii* were further subjected to different types of antibiotics from different groups, namely chloramphenicol, gentamycin, ceftazidime, nalidixic acid, ciprofloxacin, cefotaxime, by Kirby-Bauer disk diffusion method following CLSI guidelines. The highest susceptibility was found towards gentamycin (14.28%) and the least susceptible towards ceftazidime (0.00%).

3.6 Screening of Carbapenem-Resistant *A. baumannii* Isolates

The 14 colistin-resistant *A. baumannii* were initially subjected to meropenem by Kirby-Bauer disk diffusion methods following CLSI guidelines to screen out meropenem-resistant isolates i.e. those isolates whose zone of inhibition were less than 20 mm. Four of the isolates were resistant to Meropenem.

3.7 Confirmation of Carbapenem-Resistant *A. baumannii* isolates

Four isolates that were screened as carbapenem-resistant were further subjected to MCIM (Modified Carbapenem Inactivation Method--procedure) for confirmation of carbapenem resistance. All four of them were sensitive towards meropenem after the MCIM test.

3.8 Antibiotic Susceptibility Pattern of Colistin-Sensitive *A. baumannii*

The 270 remaining isolates that did not show resistance to colistin were further subjected to different types of antibiotics from different groups, namely chloramphenicol, gentamycin, nalidixic acid, cefotaxime, ciprofloxacin and ceftazidime by Kirby-Bauer disk diffusion method following CLSI guidelines. The highest susceptibility was found towards ciprofloxacin (84.44%) and the least susceptible towards chloramphenicol (53.33%).

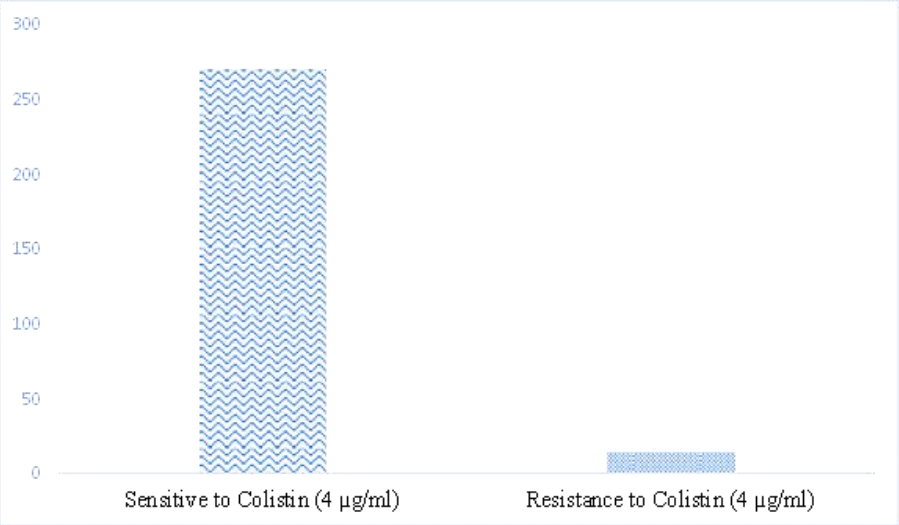

Fig. 1. Colistin-resistance in *A. baumannii* isolates

Table 2. Antibiotic susceptibility pattern of in *A. baumannii* isolates

Antibiotics	Disc content (µg)	Sensitive	Intermediate	Resistance	Total isolates
Cefotaxime (CTX)	30	1 (7.14%)	0 (0.00%)	13 (92.86%)	14
Ceftazidime (CAZ)	30	0 (0.00%)	0 (0.00%)	100 (100.00%)	14
Chloramphenicol (C)	30	1 (7.14%)	0 (0.00%)	13 (92.86%)	14
Ciprofloxacin (CIP)	5	1 (7.14%)	0 (0.00%)	13 (92.86%)	14
Gentamycin (GEN)	10	2 (14.28%)	1 (7.14%)	11 (78.57%)	14
Nalidixic Acid (NA)	30	1 (7.14%)	NA	13 (92.86%)	14

Table 3. Screening of Carbapenem resistance in Colistin-resistant *A. baumannii* isolates

Antibiotics	Total isolates	Sensitive	Resistance
Meropenem (MRP)	14	10 (71.43%)	4 (28.57%)

Table 4. Antibiotic susceptibility pattern of Colistin-sensitive *A. baumannii* isolates

Antibiotics	Disc content (µg)	Sensitive	Intermediate	Resistance	Total isolates
Cefotaxime (CTX)	30	174 (64.44%)	42 (15.56%)	54 (20.00%)	270
Ceftazidime (CAZ)	30	210 (77.78%)	48 (17.79%)	12 (4.42%)	270
Chloramphenicol (C)	30	144 (53.33%)	54 (20.00%)	72 (26.67%)	270
Ciprofloxacin (CIP)	5	228 (84.44%)	24 (8.89%)	18 (6.67%)	270
Gentamycin (GEN)	10	225 (83.31%)	24 (8.89%)	21 (7.80%)	270
Nalidixic Acid (NA)	30	210 (77.78%)	NA	60 (22.22%)	270

Table 5. Relation between MDR and colistin-resistant *A. baumannii* isolates

MDR	Susceptibility to Colistin		Total	p-value
	Colistin resistant	Colistin sensitive		
Positive	14	144	158	
Negative	0	126	126	<0.05
Total	14	270	284	

3.9 Screening of Carbapenem Resistance among the *A. baumannii* colistin-Sensitive Isolates

The 270 colistin-sensitive *A. baumannii* were initially subjected to Meropenem by the Kirby-Bauer disk diffusion method following CLSI guidelines to screen out those isolates whose zone of inhibition was less than 20 mm. All the isolates were sensitive to Meropenem.

3.10 The Multidrug Resistance Profile of *A. baumannii* Isolates

Multidrug-resistant *A. baumannii* is defined as resistant to at least three classes of antimicrobial agents—penicillins, cephalosporins, fluoroquinolones, and aminoglycosides. Of the 284 A. baumannii isolates, 158 (57.04%) were found to be multidrug resistant. 73 were resistant to penicillins + quinolones + cephalosporins, 53 were resistant to penicillins + quinolones + aminoglycosides, 32 were resistant to penicillins + quinolones + aminoglycosides + cephalosporins, and none were resistant to penicillins + quinolones + aminoglycosides + cephalosporins + carbapenem.

3.11 Relation between MDR and Colistin-Resistant Isolates

All 14 initially screened colistin-resistant *A. baumannii* isolates were found to be multidrug-resistant (MDR) as well. The chi-square test for independence was conducted to examine the association between multidrug resistance (MDR) and colistin resistance in the *A. baumannii* isolates. The contingency table presented a chi-square statistic of $\chi2=90.03$ with 1 degree of freedom. The test revealed a statistically significant association between MDR and colistin resistance ($\chi2(1) = 90.03$, $p<0.05$). The observed distribution significantly deviated from what would be expected under the assumption of independence. This suggests that the likelihood of being colistin resistant is associated with the presence of multidrug resistance in the studied *A. baumannii* isolates.

4. DISCUSSION

A. baumannii in hospital settings is not a new finding; however, the propagation of viable *A. baumannii* in the natural environment is a pressing public health challenge. From soil to water, the antibiotic-resistant bacteria may also transmit and colonize a new home/habitat elsewhere.

We undertook this research due to the growing evidence of multidrug-resistant bacteria in river water, as demonstrated by numerous studies, including those conducted in South Asian countries. For instance, Lamba et al., in 2017, investigated the release of carbapenem-resistant pathogens in Delhi, India [16]. Additionally, a parallel study in Kathmandu by Thakali et al. [17] focused on the release of antibiotic-resistance genes from hospitals. The existing body of research highlighted the urgent need to further explore and understand the dynamics of antibiotic resistance in river water, particularly in the context of the Kathmandu Valley.

In this study, river water samples (pH 6.4-8.1) yielded 284 *A. baumannii* isolates as they showed positive growth at 42°C on *Acinetobacter* Agar Base (with added Leeds *Acinetobacter* Selective Supplement). Confirmatory tests identified isolates as Gram-negative, non-motile coccobacilli, with specific growth characteristics. Because the study focused on isolating colistin-resistant *A. baumannii* and further investigating their antimicrobial patterns, all 284 isolates were subjected to 4 µg/ml Colistin. Out of them, 14 isolates showed positive growth against it. Jovcic et al, in March 2021, observed a similar result in Zagreb, where clinically relevant isolates of *A. baumannii* were recovered from hospital wastewater and wastewater treatment plants, seven of which were found to be colistin-resistant [18]. The 14 colistin-resistant isolates of *A. baumannii* also showed similarity to the clinical isolate with which it was compared in the pattern of susceptibility to different antibiotics, namely chloramphenicol, gentamicin, ceftazidime, nalidixic acid, ciprofloxacin, cefotaxime, ceftazidime/clavulanic acid, and

cefotaxime/clavulanic acid. The highest susceptibility was found towards gentamycin (14.28%). The βlactam antibiotic, cefotaxime, used in combination with clavulanic acid, showed the least sensitivity (0.00%) towards all 14 isolates.

According to a 2012 study done by Espinal et al, the β-lactam antibiotic is the recommended antibiotic treatment for *Acinetobacter* infections [5]. However, it did not show promising results against the isolated *A. baumannii* in this study. This is a point to be noted that within a decade, the bacteria have evolved to show resistance towards even the most recommended antibiotics as well. Also, a combination of antibiotic-resistant mechanisms could be one of the likely reasons for a wide range of resistance to different classes of antibiotics by *A. baumannii*. The 14 isolates were subjected to meropenem too. First, the screening process was carried out where the isolates were tested against meropenem by the Kirby-Bauer disk diffusion method following CLSI guidelines to screen out those isolates whose zone of inhibition was less than 20 mm. From this process, four isolates were initially screened out. These four isolates were further subjected to MCIM (Modified Carbapenem Inactivation Method) out of which all were susceptible to Carbapenem (meropenem), which is not 42 unexpected as the prevalence of carbapenem-resistant *A. baumannii* is highly unlikely in environment settings [19].

The remaining 270 isolates that were susceptible to colistin were further tested against different classes of antibiotics to understand their antibiograms. The highest susceptibility was found towards ciprofloxacin (84.44%) and least susceptible towards chloramphenicol (53.33%). After this, the same 270 Colistin-sensitive A. baumannii were initially subjected to meropenem by the Kirby-Bauer disk diffusion method following CLSI guidelines to screen out those isolates whose zone of inhibition was less than 20 mm. All 270 of the isolates showed susceptibility towards meropenem, so further MCIM test was not carried out. The colistin-resistant and susceptible isolates were further studied to understand their multidrug-resistant patterns.

Multidrug-resistant *A. baumannii* are those that are resistant to at least three classes of antimicrobial agents—penicillins, cephalosporins, fluoroquinolones, and aminoglycosides. Of the 284 *A. baumannii* isolates, 158 (57.04%) were multidrug resistant. 73 isolates were resistant to penicillins, quinolones and cephalosporins, 53 were resistant to penicillins, quinolones and aminoglycosides, 32 were resistant to penicillins, quinolones, aminoglycosides and cephalosporins. All 14 colistin-resistant *A. baumannii* that were initially screened were also found to be multidrug-resistant and thus were classified as extensively drug-resistant (XDR). A similar study was done at a university hospital in Kathmandu, where 122 (49.6%) *A. baumannii* isolates out of 246 were MDR *A. baumannii*, with the majority being resistant to fluoroquinolones, aminoglycosides and carbapenems. However, they were not resistant to colistin [20]. In a similar study in China, nine MDR *A. baumannii* were recovered from wastewater followed by disinfection (chlorination), afterwards out of 9 only one remained MDR but those strains were not related to clinical isolates [21].

Another study conducted in Croatia also recovered viable MDR *A. baumannii* which was also not related to clinical isolates from municipal wastewater in Zagreb, Croatia, both before and after passage through the secondary wastewater treatment process [22]. However, despite the above observations concerning the ubiquity of *A. baumannii,* there is a lack of clear evidence about the relationship between hospital settings and the natural environment in the propagation of this increasingly important pathogen [9]. In this study, *A. baumannii* showed excellent survival in river water. These isolates disseminated in the environment could represent the source of serious community-acquired infections. The fact that *A. baumannii* is responsible for uncountable hospital-acquired infections worldwide and has recently become one of the top most important healthcare-associated infections in hospitals is a matter of discussion, but their presence in the environment, that too in river water indicates the occurrence of horizontal gene transfer (HGT).

In the rivers in Kathmandu, especially in central city areas where wastewater is directly dumped into the rivers, human-associated and environmental bacteria are mixed together and exposed to many substances, including various antimicrobial compounds, which in turn increase the HGT [17]. This can also be backed by the fact that no *A. baumannii* was isolated from the water collected from Sundarijal, which is considered the starting point of the Bagmati River. The river's downstream and midstream areas, however, flow through settlement areas where hospitals are present. Not only that, the

only functioning wastewater treatment plant in Kathmandu Valley also is in the midstream site. With more than a hundred hospitals and a treatment plant that lacks a chlorine disinfection phase, which is a critical step in removing antimicrobial-resistant genes in bacteria, it is inevitable for multidrug-resistant bacteria, even those found mainly in the hospital environment, to be present in river water [23]. However, a study conducted in Spain by Rodriguez-Mozaz et al in 2015 showed that even after hospital wastewater treatment in proper wastewater treatment plants, antimicrobial-resistant genes still spread in the receiving river sources [14]. This further backs the claim that hospitals and even wastewater treatment plants are potential sources of multidrug-resistant *A. baumannii* in the rivers in Kathmandu Valley.

The findings of this study highlight the presence of colistin-resistant *A. baumannii* in the environmental reservoirs of Kathmandu Valley, underscoring a critical aspect of Nepal's environmental and public health landscape. This discovery fills a notable gap in the current understanding of antibiotic resistance dynamics in the region and emphasizes the need for a holistic approach to combatting the spread of multidrug-resistant pathogens. The study also highlights the potential risks associated with the environmental presence of colistin-resistant *A. baumannii*, particularly in a country with limited healthcare resources. The elevated prevalence of antibiotic resistance in environmental water sources raises concerns about its transmission to humans and animals, posing significant challenges for public health.

To address these concerns, we propose a series of measures for monitoring, control, and prevention. Enhanced surveillance of water sources, coupled with the development of antimicrobial stewardship programs, can play a pivotal role in curbing the dissemination of resistant strains. Moreover, public awareness campaigns and educational initiatives are crucial in fostering a proactive community response to mitigate the risks associated with environmental antibiotic resistance.

5. CONCLUSION

The findings from this study strengthen the urgent need not just to develop new classes of antibiotics against highly resistant bacteria like *A. baumannii*, but also to strictly monitor the haphazard use of antibiotics.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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