Original Research Article

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

Abstract

Acinetobacter baumannii, an opportunistic Gram-negative pathogen, is increasingly causing multidrug-resistant infections and becoming a significant health concern in clinical settings. This study investigated the presence and antibiotic resistance of *A. baumannii* in 10 different rivers across Kathmandu Valley, Nepal. Water samples were collected and tested using Leeds Acinetobacter Agar Base and biochemical tests, leading to the isolation of 284 *A. baumannii* strains. Among these, 14 (4.9%) demonstrated resistance to Colistin, a last-resort antibiotic. These Colistin-resistant strains showed the highest susceptibility to Gentamycin (14.28%) and complete resistance to Ceftazidime. The remaining Colistin-sensitive strains exhibited high susceptibility to Ciprofloxacin (84.44%) and lowest to Chloramphenicol (53.33%). All 284 isolates were susceptible to Carbapenem. The study found that all rivers, except Sundarijal, contained multidrug-resistant *A. baumannii*, raising health concerns for humans and animals interacting with these water bodies. The findings underscore the importance of examining possible environmental reservoirs for pathogen spread and the potential health implications.

Keywords: *Acinetobacter baumannii*, antibiotic resistance, Colistin, ESKAPE, Carbapenem, Kathmandu Valley.

Introduction

Antimicrobial resistance is a prevalent challenge to global public health as the rapid rise in severe bacterial infections with antibiotic resistance might render only a few antibiotics (Lim et al, 2007). The occurrence of antibiotics and other pharmaceuticals in the environment has become an increasing public concern as recent environmental monitoring activities reveal the presence of a broad range of persistent pharmaceuticals in soil and water which may lead to the emergence of resistant bacterial strains against antibiotics or therapeutics over a period of time (Magiorakos et al, 2012). 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 (Aslam et al, 2018).

Similarly, in the case of *Acinetobacter baumannii* too, hospital-acquired infections have increased dramatically worldwide (Howard et al, 2012). 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 (Espinal et al, 2012). Non-susceptibility to commonly used antimicrobials has also been observed, with Carbapenem resistance becoming a global problem since 2000 (Howard et al, 2012).

A. baumannii is one of the six 'superbugs' identified by the Infectious Diseases Society of America (IDSA) (Goff and Kaye, 2014). It is a Gram-negative, non-fermentative, coccobacillus belonging to the family Moraxellaceae and has in recent years gained increasing notoriety as a nosocomial pathogen (Lopes, 2011). Mutation and selection, together with the mechanisms of genetic exchange, enabled many bacterial species to adapt quickly to the environment where antibacterial agents are present (Navon-Venezia, 2007). 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 (Iversen et al, 2002). As demonstrated by Colomer-Lluch et al in 2006, the occurrence of bacteriophages from samples of animals' fecal wastes can be environmental vectors for the

horizontal transfer of antibiotic resistance genes. Untreated hospital waste disposals containing un-metabolized antibiotics in a low concentration contribute largely to the development of antibiotic resistance. Consequently, those places can work as hotspots for horizontal gene transfer (HGT) of antibiotic-resistant genes, resulting in resistant pathogens (Moura et al, 2012).

The basic principle of underlying wastewater management is the strict limit on the discharge of hazardous liquids into sewers without prior treatment so that living pathogenic organisms are not introduced into the environment (WHO, 2018). However, in Nepal, only the towns in Kathmandu Valley have a sewerage network system and the sewerage facility is provided to 15% of the houses only (Sharma et al, 2010). 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 (Verlicchi et al, 2012). If the hospital effluents are not treated, concentrated forms of infectious agents and antibiotic-resistant microbes are shed into communities resulting in waterborne diseases such as cholera, typhoid fever, dysentery and gastroenteritis. Antibiotics, disinfectants and bacteria resistant to them have been detected in environmental compartments such as wastewater, surface water, groundwater, sediments and soils (Goic-Barisic et al, 2009). Long-term exposure of microorganisms to low concentrations of antibiotics in wastewater and surface water has the potential for the development of antibiotic resistance in these organisms (Iversen et al, 2002).

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 (Meier et al, 2011). 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 (Magiorakos et al, 2012). Therefore, the purpose of the present study was to examine the presence of MDR *A. baumannii* in river water across Kathmandu Valley.

Methods

Sampling site

10 different sites across Kathmandu Valley (AJ) 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)

Study period

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

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, 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, allowing for a comprehensive examination of temporal variations in water quality.

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.

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). 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.

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.

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.

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 and incubated at 42°C for 24 hours. A single colony from each isolate was emulsified in 2 ml Tryptic Soy Broth (TSB). 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 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.

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.

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 subculture 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.

Statistical analysis

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

Result

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.

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.

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

Table 1: pH of the river water samples

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.

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.

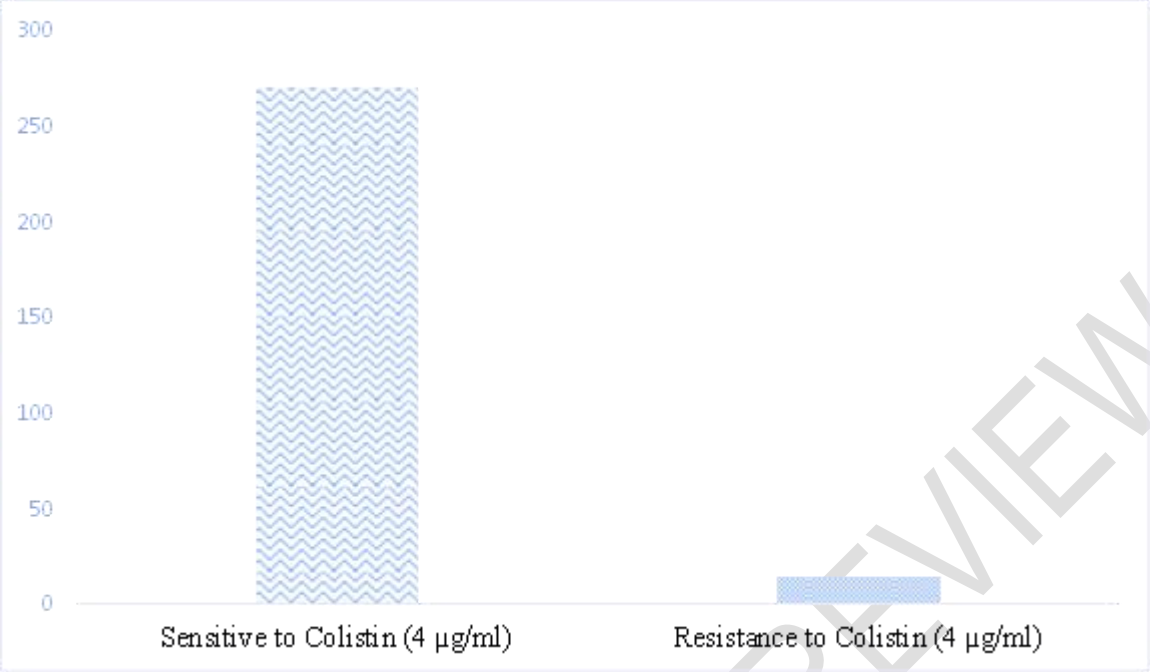

Figure 1: Colistin-resistance in *A. baumannii* isolates

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%).

Antibiotics	Disc content (µg)	Total isolates	Sensitive	Intermediate	Resistance
Cefotaxime (CTX)	30		1 (7.14%)	0 (0.00%)	13 (92.86%)
Ceftazidime (CAZ)	30		0 (0.00%)	0 (0.00%)	100 (100.00%)
Chloramphenicol	30		1	0	13

(C)			(7.14%)	(0.00%)	(92.86%)
Ciprofloxacin (CIP)	5	14	1 (7.14%)	0 (0.00%)	13 (92.86%)
Gentamycin (GEN)	10		2 (14.28%)	1 (7.14%)	11 (78.57%)
Nalidixic Acid (NA)	30		1 (7.14%)	NA	13 (92.86%)

Table 2: Antibiotic susceptibility pattern of colistin-resistant in *A. baumannii* isolates

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.

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

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

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.

Antibiotic susceptibility pattern of carbapenem-resistant 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%).

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

Table 4: Antibiotic susceptibility pattern of carbapenem-resistant *A. baumannii* isolates

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.

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.

Relation between MDR and colistin-resistant isolates

All 14 of the initially screened colistin-resistant *A. baumannii* were found to be MDR isolates as well. The distribution of MDR among colistin-resistant was found to be statistically significant (p-value<0.05).

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

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

Discussion

Over the past decade, antibiotic resistance shown by *A. baumannii* has emerged as a major cause of nosocomial infections associated with significant morbidity and mortality (Antunes 2014). *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.

In the past few years, many studies, including those in South Asian countries have proved the presence of multidrug-resistant bacteria in river water. Lamba et al, in 2017, studied the release of carbapenem-resistant pathogens in Delhi, India. Similarly, the same year, Islam et al studied the environmental spread of ESBL-producing multidrug resistance bacteria in Dhaka, Bangladesh. Both studies concluded the presence of harmful antibiotic-resistant bacteria, especially Enterobacteriaceae, in wastewater produced by the hospitals. A similar study was also done in Kathmandu by Thakali et al in 2021, where the team studied the release of antibiotic-resistance genes from hospitals.

The samples for this study were collected three times from 10 different sites specified [Chobar (Bagmati), Pashupati (Bagmati), Balkhu (Balkhu), Jadibuti (Hanumante), Tilganga (Bagmati), Teku (Bishnumati), Balkumari (Manohara), Chunikhel (Dhobikhola), Sundarijal (Bagmati), Tripureshor (Tukucha)] over a period of three months. The locations were selected in order to cover different corners of the Valley, most of which were adjacent to major hospitals. Before the samples were subjected to microbiological analysis, their pH was also noted. The pH of natural river waterfalls is between 6.5 and 8.5, with the optimum pH being around 7.4 (Temocin et al 2015).

The pH of the samples in this study was found to be in the range of 6.4 to 8.1. There were no considerable differences in pH among the 30 samples. Out of the 30 samples, 27 showed positive growth on Leeds Acinetobacter Agar Base at 42°C for *A. baumannii*. From those 27 samples, 284 isolates of *A. baumannii* were isolated for this study. Even though the bacteria were isolated using selective media, further tests were done to confirm the isolates. All 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. Furthermore, the isolates were found to be non-fermenter, catalase-positive, and oxidase-negative and did not reduce nitrate. 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 observed a similar result in Zagreb in March 2021, 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. 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. 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 (Goic-Barisic et al, 2009).

The remaining 270 isolates that were susceptible to colistin were further tested against different classes of antibiotics to understand their antibiograms. The antibiotics used were 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 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 in 2003 by Reinthaler et al, significantly higher percentages of MDR *A. baumannii* were seen in the inlet water of a treatment plant receiving hospital waste than in two other treatment plants. In another study conducted at a university hospital in Kathmandu, 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 (Shrestha et al, 2015). In this study, 158 (57.04%) isolates were found to be multidrug-resistant, and 14 were colistin-resistant, despite them being isolated from river water and not from patients. This clearly indicates that the problem of antibiotic resistance is increasing by the day. 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 (Zhang et al, 2013).

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 (Knapp, 2006). 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 (Moura et al, 2011). 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 (Thakali et al, 2021). This can also be backed by the fact that no *A. baumannii* was 44 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 (Amarasiri, 2022). 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. 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.

It is known that the transfer of mobile genetic elements between bacteria is an essential factor in causing the global antibiotic resistance crisis (Andersson and Hughes, 2017). Indeed, HGT allows bacteria to acquire new resistance traits from both members of the same species and more distantly related bacteria, which can carry a vast pool of antibiotic resistance genes together. Of particular concern for general public health, bacterial pathogens can become multidrug-resistant following the acquisition of plasmids carrying multiple antibiotic-resistance genes, including those not previously found in pathogens. The same can be said for the *A. baumannii* isolated in this study.

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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