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Detection of Multidrug-Resistant *Acinetobacter Junii* in a Himalayan Freshwater Ecosystem: A Pilot Study from Kumaon Kosi River

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Abstract

Antimicrobial resistance (AMR) is an increasing concern to global health, complicating the treatment of infections and contributing to millions of deaths worldwide. Aquatic ecosystem act as reservoirs for antibiotic resistant bacteria and antibiotic-resistant genes due to discharge of industrial and domestic effluents directly into the waterbodies. While *A. baumannii*, a member of ESKAPE *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter* species) group is a well-known pathogen for hospital-acquired infections (HAIs) but simultaneously other species of *Acinetobacter* such as *A. junii* is also emerging as opportunistic pathogens especially in multi-drug resistant (MDR) infections. In this study, we isolated and identified bacterial isolates from the Kosi River in Uttarakhand, a relatively unpolluted freshwater source compared to urban water bodies. Out of 25 isolates, 24 were identified as *Acinetobacter junii* through biochemical characterization and 16S rRNA gene sequencing. These isolates were screened for multidrug resistance using the disc diffusion method against 13 different antibiotics belonging to six different classes, and broth microdilution assay was performed for selected isolates for determining their susceptibility to Polymyxin B and Colistin. Notably, 100% of the *A. junii* isolates were resistant to Cefepime, 33% to Cefoxitin, and 4% to both Ceftazidime and Amikacin. The detection of MDR *A. junii* in a pristine environment underscores the growing environmental dimension of AMR. This study suggests that natural water bodies may serve as silent reservoirs of resistance genes. The findings reinforce the urgent need for integrated environmental surveillance, stricter control on antibiotic contamination, and global commitment to antimicrobial stewardship to safeguard both environmental and public health.

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Introduction

The presence of residual antibiotic load within aquatic ecosystem has led to development of antimicrobial resistant bacteria (ARBs) which in turn exacerbates the global public health issue of antimicrobial resistance (AMR).[1] Recognized by the World Health Organization (WHO), AMR is one of the most prevailing global health issues and jeopardizes the effective treatment of bacterial infections, contributing to increased mortality, morbidity and cost of healthcare worldwide.[2] In 2019, approximately 4.95 million death and 1.27 million infections associated with antimicrobial resistance (AMR) was reported globally.[3] Gram-negative bacteria, particularly those belonging to the *Acinetobacter* genus, are among the most concerning contributors to this burden due to their ability to persist in diverse environments and rapidly acquire resistance determinants.[4] *Acinetobacter* species are Gram-negative, aerobic, non-motile coccobacilli commonly found in soil, freshwater, and hospital environments.[5,6] Although traditionally considered low-virulence organisms, several species have emerged as opportunistic pathogens, primarily affecting immunocompromised patients and individuals in intensive care settings.[7] Their pathogenicity is largely driven by immune evasion strategies such as capsular polysaccharide production and lipopolysaccharide (LPS)-mediated stimulation of Toll-like receptor 4 (TLR4), leading to systemic inflammation and septic shock.[8,9] The resistance mechanisms employed by *Acinetobacter* spp., are multifactorial, including the production of β-lactamases, reduced outer membrane permeability, active efflux pumps, target site modifications, and robust biofilm formation.[10,11]

Among these species, *Acinetobacter baumannii* has received the most clinical attention as a major cause of nosocomial infections (HAIs), particularly in ICUs.[7,12] However, less-studied species such as *Acinetobacter junii* are now emerging as important players in both clinical and environmental AMR contexts. *A. junii* is one of over 70 recognized species within the *Acinetobacter* genus.[7] Historically under-recognized, recent reports have linked *A. junii* to a variety of opportunistic infections, including neonatal septicaemia, bacteraemia in cancer patients,[13-15] urinary tract infections, and ocular infections such as corneal ulcers and perforation.[16,17] Of growing concern is its ability to harbour and disseminate resistance genes such as NDM-1, OXA-58, and IMP-type carbapenemase that get transferred from one species to another via horizontal gene transfer (HGT).[18] Environmental isolates, particularly from poultry and wastewater sources, have demonstrated resistance to clinically significant antibiotics, suggesting an ecological bridge between environmental, animal, and human health sectors.[5,18]

Large-scale genomic studies have reported limited genetic divergence between clinical and environmental *A. junii* isolates, indicating possible cross-sector transmission routes.[19, 20] Despite this, *A. junii* remains underrepresented in research, with fewer than 170 scientific publications indexed in NCBI as of August 2023.[19] This under-characterization poses a risk, particularly in the context of environmental AMR surveillance and early detection of emerging threats.[19] Moreover, natural water bodies, especially those perceived as pristine, may also serve as silent reservoirs for transmission of antibiotic-resistant bacteria, necessitating their inclusion in AMR surveillance networks. In this context, the present study investigates the infiltration of multidrug-resistant *A. junii* into a relatively unpolluted freshwater ecosystem the Kosi River in Uttarakhand, India. Through systematic isolation, biochemical and molecular identification, and antibiotic susceptibility testing, we highlight the environmental dimension of AMR in environmental isolates of *A. junii*.

Materials and Methods

Study Area: Water samples were collected from the Kosi River in Nainital (Lohali village) Uttarakhand (29°30'02.5"N, 79°30'17.0"E) on 23 June 2023, as depicted in Figure 1. The Kosi River originates from Dharapani Dhar near Kausani in the Almora district of the Kumaon region and serves as an important tributary of the Ganga River system. Spanning approximately 116.14 km in length, the river plays a vital role in irrigation, hydropower generation, and public water supply. Additionally, it supports rich fish biodiversity and contributes to the local economy through activities such as fishing, sport fishing, and water-tourism.[1,21]

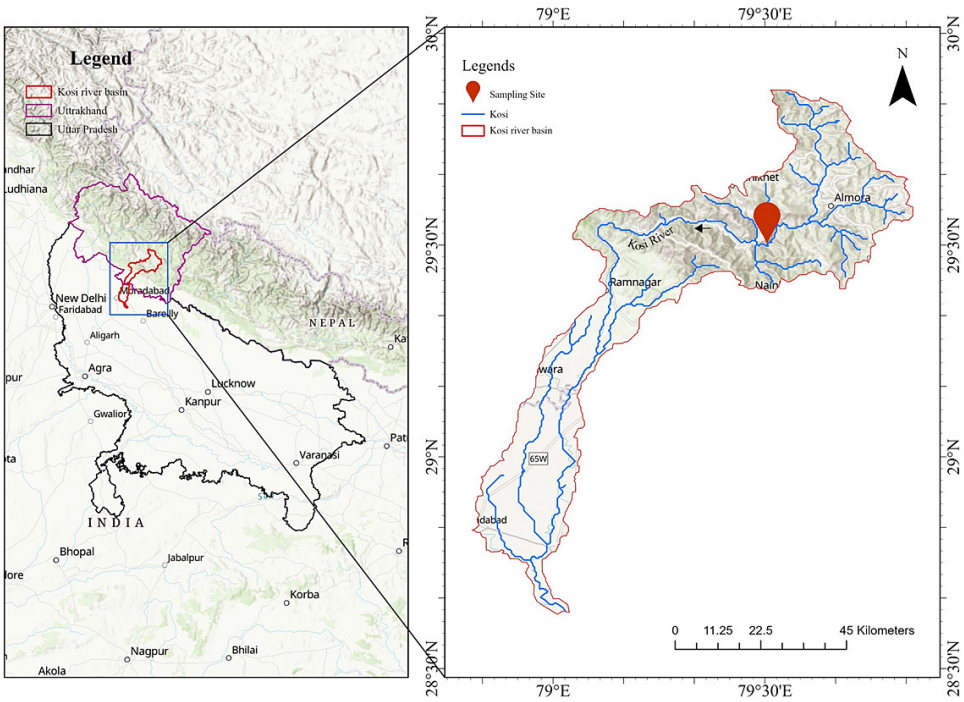

Fig. 1: Study area and location from where the river samples were collected.

Isolation, Identification, Biochemical and Molecular Characterization of Bacterial Isolates

The samples were collected, serially diluted and plated on nutrient rich agar like Brain-Heart Infusion (BHI) as well as selective and differential media like Mannitol Salt Agar (MSA) and Baird-Parker Agar (BPA). The oxacillin susceptibility test involved inoculating bacteria on Brain-Heart Infusion (BHI) supplemented with oxacillin (1 µg/mL); bacterial growth indicates resistance to oxacillin and thus a positive result for resistance.[22] To identify and characterize the bacterial isolates, a series of biochemical tests like catalase, coagulase, oxidase and Gram staining were performed. The catalase test was done to check the production of bubbles indicating a positive result due to the release of oxygen when exposed to hydrogen peroxide (H_2O_2). The coagulase test was performed to detect free coagulase secreted by bacteria, formation of a clot indicates a positive result.[23] The oxidase disc was used to check the cytochrome c oxidase activity which turn the disc blue giving positive result.[24] Finally Gram staining was done to check the morphology of each isolates in order to classify it as Gram positive or Gram negative bacteria.[25] For molecular identification, genomic DNA was extracted from purified colonies, further the quality of DNA was checked on NanoDrop 2000 Spectrophotometer (Thermo Scientific) and 16S rRNA gene amplification was done via Polymerase Chain Reaction (PCR) using universal primers and sequencing were conducted. The bacterial sequences were later identified through NCBI database using BLAST to determine the closest phylogenetic affiliations.

Antibiotic Susceptibility Test (AST)

Kirby-Bauer disk diffusion method was used to test antibiotic susceptibility profile by following Clinical & Laboratory Standards Institute (CLSI 2024) guidelines. All *Acinetobacter junii* isolates and the control strain (*Acinetobacter baumannii*) were tested against a panel of antibiotics: amikacin, ampicillin, cefepime, cefuroxime, ceftazidime, ciprofloxacin, cefoxitin, gentamicin, doripenem, meropenem, imipenem, streptomycin and tigecycline. The inhibition zones were measured and compared with CLSI guidelines to classify them as sensitive, intermediate, or resistant isolates.

Minimum Inhibitory Concentration (MIC) Assay

The minimum inhibitory concentration (MIC) was determined for polymyxin B and colistin using broth microdilution methods, given their relevance as last-resort antibiotics against Gram-negative multidrug-resistant bacteria as per CLSI guidelines.[25] Selected environmental *A. junii* isolates, GRS 3 and GRS 5 and control strain *A. baumannii* were studied. In brief, a sterile 96-well microtiter plate was prepared with two-fold serial dilutions of the antibiotic in Mueller Hinton II Broth Cation adjusted (MHB) across columns 1 to 10, concentration ranging from 256 µg/ml to 0.5 µg/ml. Column 11 served as a positive growth control (media + bacteria), and column 12 as a negative control (media only).

Next, bacterial cultures were adjusted to OD_{600} of 0.3–0.4 (~10^8 CFU/ml) and diluted to 10^6 CFU/ml. Then, 50 µl of this suspension was added to wells from columns 1–11 containing antibiotic. The plate was incubated at 37°C without shaking for overnight growth. Bacterial growth was assessed the next day in each column based on visible turbidity.

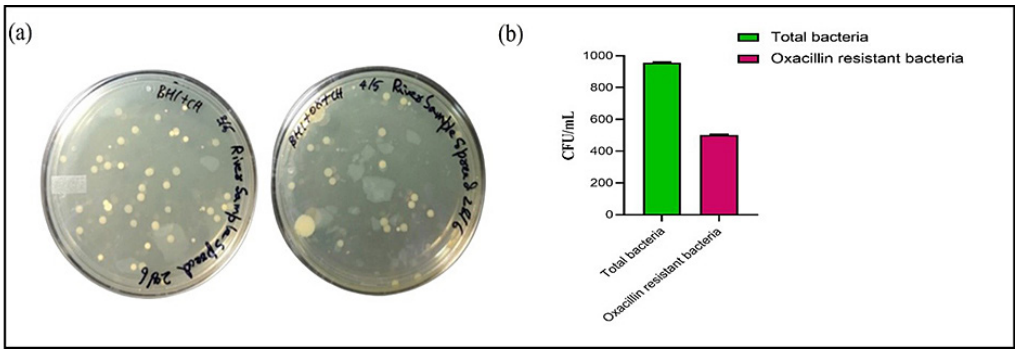

Fig. 2: Microbiological load in Kosi River: (a) shows the total bacterial load and total oxacillin resistant bacterial load on BHI plates while the figure (b) shows the graphical representation of the same.

Results

Isolation and Identification of Bacterial Isolates from Kosi River Water Sample

The collected water sample was brought to the laboratory and 50 µL of it was spread on BHI Agar with and without 1µg/ml oxacillin for total bacterial load and presumptive oxacillin resistant bacteria respectively. After an overnight incubation at 37°C it was observed that the BHI media without oxacillin had 48 colonies and the BHI media containing oxacillin had 25 colonies. Therefore, the total load of bacteria in Kosi River sample was 960 CFU/mL, and oxacillin resistant bacterial load was 500 CFU/mL as shown in Figure 2 (a and b). Further, glycerol stocks were prepared from the primary culture of these bacterial isolates and stored at -80°C for future use. The isolation from the Kosi River water samples indicated a substantial bacterial load (960 CFU/mL) reflecting active microbial presence, possibly due to anthropogenic influences.[26] The Oxacillin supplemented BHI plates gave 500 CFU/mL count, highlighting 50% of total culturable bacterial population showing presumptive resistant to β-lactam antibiotics. This finding raises concern regarding the dissemination of antibiotic resistance in freshwater sources, suggesting discharge of untreated sewages from hospital, domestic and agricultural runoff.[27]

Simultaneously, the isolates with different morphology were selectively isolated from oxacillin supplemented BHI plates and restreaked multiple times for purification. After purification the isolates were restreaked on MSA, and BPA plates. On MSA, none of the 25 isolates demonstrated growth, indicating a negative result and suggesting the absence of *Staphylococcus* spp. Similarly, no growth on BPA was observed, whereas only GRS13, which developed grey-black colonies, while the remaining isolates showed no growth, ruling out presumptive *Staphylococcus* identification for most of the isolates as shown in Figure 3.

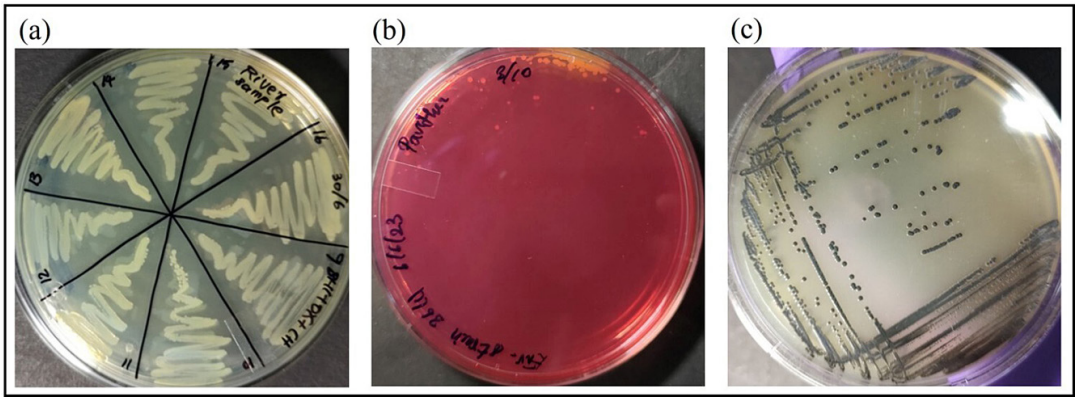

Fig. 3: The representative plates for selective isolation of bacteria: (a) Oxacillin supplemented BHI plates (b) Mannitol Salt Agar (MSA) showing negative growth of environmental isolates (c) Baird-Parker Agar (BPA) showing greyish black colony of environmental isolate (GRS 13).

Subsequently, biochemical tests like catalase, coagulase, oxidase and Gram-staining tests were performed to classify the isolated strains. Out of 25, only 24 isolates were catalase positive, coagulase negative, oxidase negative with no blue colouration on disc and Gram staining revealed the coccobacillus shape suggesting the isolates to be Gram-negative bacteria as shown in Figure 4 respectively.

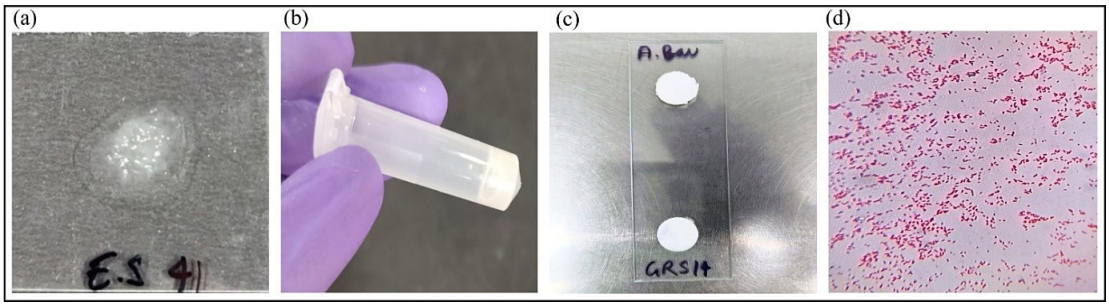

Fig. 4: Biochemical characterization of *Acinetobacter* isolates: (a) Positive catalase test indicated by bubble formation; (b) Negative coagulase test showing no clot formation; (c) Negative test for oxidase (no blue coloration); (d) Gram staining shows Gram-negative coccobacilli shaped bacterial isolate

All the 24 presumptive isolates were identified using 16s rRNA gene sequencing and it confirmed to be *A. junii* which were carried forward for subsequent analysis. This predominant presence of *A. junii* in river system, warrants further investigation into its environmental reservoirs and transmission pathways.

Antibiotic Resistance Profiles of *A. Junii* Isolates from Kosi River

All twenty-four *Acinetobacter* isolates were subjected to the Kirby-Bauer disc diffusion susceptibility test for amikacin, ampicillin, cefepime, cefuroxime, ceftazidime, ciprofloxacin, cefoxitin, doripenem, gentamicin, imipenem, meropenem, streptomycin and tigecycline. The control *Acinetobacter* baumannii, is an MDR strain which we used as a control strain for Kirby-Bauer Test. The antibiotic susceptibility profile of all environmental isolates reveals predominantly moderate to high sensitivity for all the antibiotics. While, the reference strain *A. baumannii* showed multi-drug resistance phenotype, especially against beta-lactams class of antibiotics.

All isolates were shown to be resistant to cefepime, the isolate (GRS5) (Figure 5) and isolate (GRS3) exhibited resistance and intermediate resistance to ceftazidime, whereas, 8 isolates (GRS3, GRS5, GRS18, GRS19, GRS20, GRS21, GRS22 and GRS24) showed intermediate resistance to cefoxitin (2^{nd} generation cephalosporins) (Table 1). Compared to MDR phenotype of *Acinetobacter* baumannii, all the *A. junii* showed higher susceptibility towards most antibiotics primarily due to isolation from pristine environment.

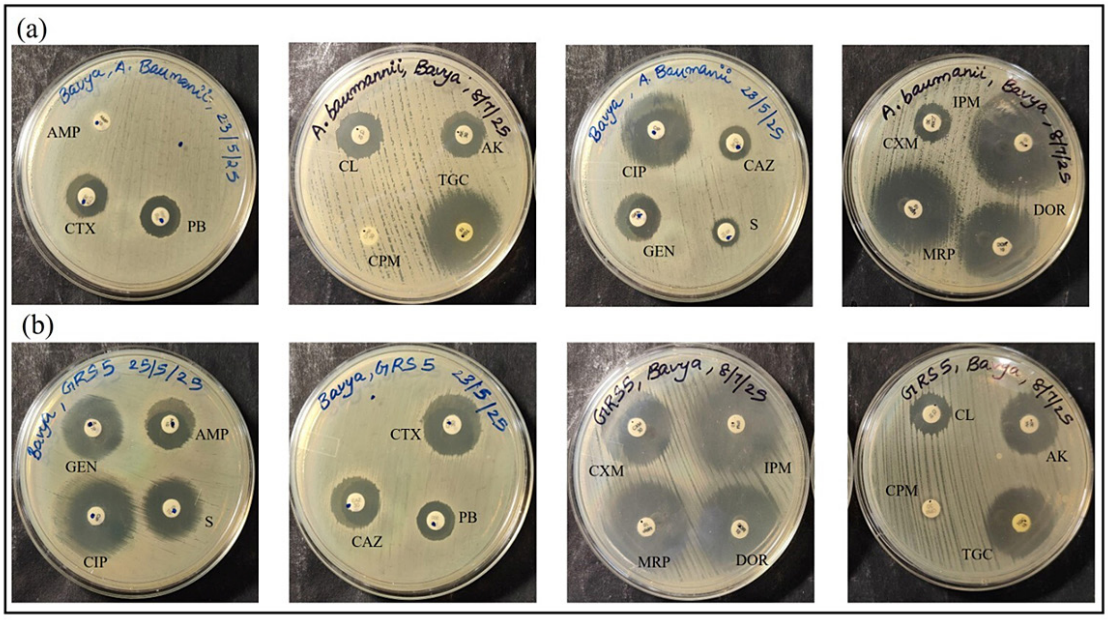

Fig. 5: The representative picture of disk diffusion test against amikacin (AK), ampicillin (AMP), cefepime (CPM), cefuroxime (CXM), ceftazidime (CAZ), ciprofloxacin (CIP), cefoxitin (CTX), gentamicin (GEN), doripenem (DOR), meropenem (MRP), imipenem (IPM), streptomycin (S) and tigecycline (TGC) antibiotics (a) *Acinetobacter baumannii* and (b) environmental isolates (GRS 5) respectively.

The intermediate resistance to cefoxitin by 8 isolates also points towards the possible development of resistance in process. These findings reinforce aquatic environment as a potential reservoir of AMR particularly where animal and human interface overlap posing serious concerns for public health. Nonetheless, the antibiotic susceptibility test using disc diffusion test revealed an alarming trend: all *A. junii* isolates were found to be resistant to cefepime, a fourth-generation cephalosporin commonly used in clinical settings. Although bacterial isolates showed intermediate resistance to ceftazidime, cefoxitin and resistance to cefepime, suggesting either a β-lactamase resistance especially the Extended Spectrum Beta-lactamase (ESBL) and AmpC activity. Overall, the environmental isolates showed higher susceptibility to tigecycline and carbapenems indicate the retained activity of carbapenemase activity, highlighting their continued efficacy as last-resort drug. The percentage of antibiotic susceptibility of *A. junii* isolates showing high level of susceptibility (100%) against ampicillin, ciprofloxacin, gentamicin, streptomycin, doripenem, imipenem, meropenem, cefuroxime and tigecycline as shown in Figure 6 and Table 1.

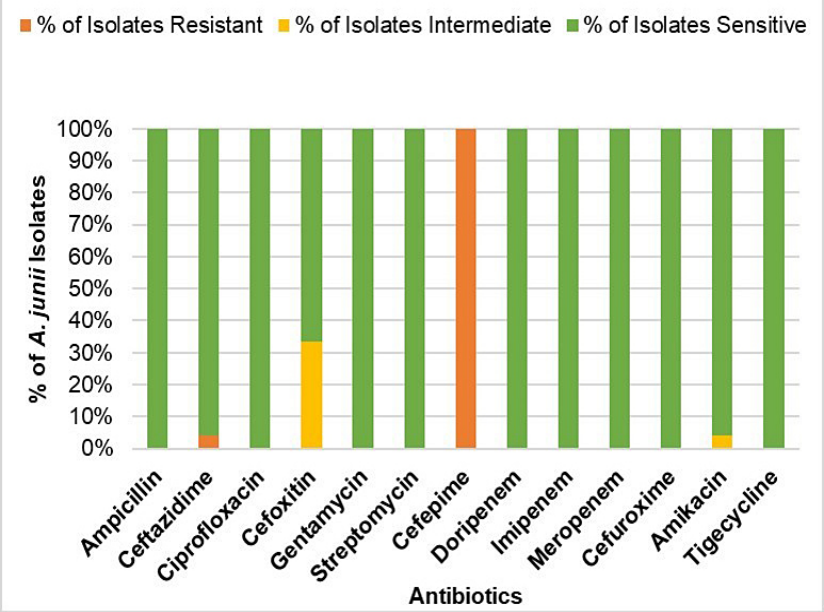

Fig. 6: The antibiotic susceptibility profile of *A. junii* isolates against 13 different antibiotics using disk diffusion assay.

Further the MIC values of selected isolates that were multi-drug resistant was determined against polymixin B and colistin. The MIC is defined as the minimum concentration of antibiotic at which no bacterial growth is visible, indicating complete inhibition of bacterial proliferation, as mentioned in Table 1. The MIC assay results provide a quatitative assessment of antibiotic resistance against polymyxin B and colistin in GRS 3 and GRS 5 particularly important in treatment of Gram-negative infections, helping us assess the antibiotic selective pressure on environmental isolates. The MIC assay revealed the isolate GRS5 to have relatively higher MIC value (≤2 µg/ml) as compared to GRS3 (≤0.5) but still susceptible to the polymixin B and colistin suggesting the retained efficacy of last-resort drugs. Nonetheless, the colistin and polymixin B remains effective against environemntal isolates but routine surveillance of antibiotic susceptibility patterns in environmental settings for AMR of both pathogenic and non-pathogenic isolate is needed.

Discussion

Antimicrobial resistance (AMR) is the ability of microbe to resist the antibiotic and survive in their presence.[28] AMR has become a global public health crisis now due to constant cycling of AMR bacteria between human, animals and environment.[29] Earlier it was reported mostly from clinical samples and from clinical settings only.[25] However, the presence of antimicrobial resistant bacteria and antibiotic resistance related genetic determinants are ubiquitously present in environmental settings including air, water and soil samples due to contamination of these samples with residual amount of antibiotics allowing bacteria to evolve with molecular mechanisms of AMR.[30]

Table 1: The summary of the disc diffusion results: Green ■ is for susceptible, Yellow ■ is Intermediate and Red ■ is for resistant isolates. Note: Ampicillin (AMP), Ceftazidime (CAZ), Ciprofloxacin (CIP), Cefoxitin (CTX), Gentamycin (GEN), Streptomycin (S), Cefepime (CPM), Doripenem (DOR), Imipenem (IMP), Meropenem (MRP), Cefuroxime (CXM), Amikacin (AK), Tigecycline (TGC), Polymyxin B (PB) and Colistin (CL). The entry "NA" indicates as not tested for MIC of that specific isolate.

Bacterial Isolates	Zone of Inhibition (mm)													MIC (µg/ml)	
	AMP (10µg)	CAZ (30 µg)	CIP (5 µg)	CTX (30 µg)	GEN (10 µg)	S (10 µg)	CPM (30 µg)	DOR (10 µg)	IMP (10 µg)	MRP (10 µg)	CXM (30 µg)	AK (30µg)	TGC (30 µg)	PB	CL
A. baumannii	0	11	25	14	16	9	0	30	37	31	14	16	28	<0.5	< 0.5
A. junii GRS 1	23	20	30	26	26	25	7	30	35	32	28	22	25	NA	NA
A. junii GRS 2	22	21	25	24	22	21	11	32	36	33	29	24	27	NA	NA
A. junii GRS 3	16	17	24	20	21	20	0	30	33	30	25	23	26	< 0.5	< 0.5
A. junii GRS 5	17	13	31	20	21	21	0	31	35	33	26	22	26	≤2	≤2
A. junii GRS 6	23	22	29	29	24	22	13	35	38	34	28	25	28	NA	NA
A. junii GRS 7	25	22	30	28	25	25	9	33	37	33	27	24	28	NA	NA
A. junii GRS 8	25	24	30	29	25	25	9	35	37	33	28	23	25	NA	NA
A. junii GRS 9	24	22	30	29	25	25	11	31	35	32	26	23	27	NA	NA
A. junii GRS 10	20	20	33	23	24	24	0	32	37	32	25	23	27	NA	NA
A. junii GRS 11	20	21	32	23	24	23	0	33	36	33	27	23	23	NA	NA
A. junii GRS 12	25	20	33	25	24	25	9	33	37	32	28	23	28	NA	NA
A. junii GRS 13	22	21	34	24	24	23	9	35	35	31	25	23	23	NA	NA
A. junii GRS 14	23	20	34	24	23	24	9	31	36	32	24	23	26	NA	NA
A. junii GRS 15	24	22	34	25	23	25	8	36	40	36	28	24	18	NA	NA
A. junii GRS 16	23	20	35	24	24	24	9	33	36	30	26	23	27	NA	NA
A. junii GRS 17	22	21	32	23	23	23	9	33	38	32	26	20	24	NA	NA
A. junii GRS 18	23	18	35	22	23	23	0	31	31	30	25	21	25	NA	NA
A. junii GRS 19	20	20	30	21	24	22	0	28	30	28	23	22	26	NA	NA
A. junii GRS 20	20	20	33	22	23	23	0	31	35	33	26	23	28	NA	NA
A. junii GRS 21	20	20	30	21	22	22	0	31	32	31	24	21	25	NA	NA
A. junii GRS 22	19	19	31	22	22	21	0	30	32	32	24	23	26	NA	NA
A. junii GRS 23	21	20	33	23	23	23	10	32	34	30	25	24	28	NA	NA
A. junii GRS 24	20	21	31	22	22	21	0	32	35	31	25	23	27	NA	NA
A. junii GRS 26	24	20	32	25	24	26	8	32	37	37	26	22	25	NA	NA

This study focuses on analyzing the total bacterial load and oxacillin resistant bacterial load. We found 960 and 500 CFU/mL of total bacterial load and total oxacillin resistant bacterial load respectively in the Kosi River water samples. All the 24 bacterial isolates growing on the oxacillin plate were identified as *A. junii* in the river ecosystem using 16S rRNA gene sequencing. Further, multidrug resistance (MDR) profiling was performed on the *A. junii* isolates, this analysis provides insights into the resistance potential and associated public health risks. The isolates in this study confirms the presence of Gram-negative bacteria, even in pristine water bodies which were 100% resistant to cefepime (a 4th generation cephalosporin) while 8 isolates (33%) were intermediate resistant to cefoxitin (a 2nd generation cephalosporin). This may be due to extensive use of the latest drug i.e., cefepime against Gram-negative bacteria as compared to cefoxitin due to reduced usage of conventional antibiotics rendering less selective pressure on bacteria to evolve resistance. Notably, isolates GRS 3 and GRS 5 exhibited multidrug resistance against cephalosporin class of antibiotics, which is particularly worrying from a public health perspective. Additionally, the emergence of intermediate resistance against polymyxin B and colistin (the last-resort antibiotics against Gram-negative bacteria) further underscores the need to perform such monitoring studies to estimate the AMR burden in environmental ecosystem. While extensive research has focused on *A. baumannii*, comparatively limited work has been conducted on *A. junii*.[31] Even though *A. junii* is considered less pathogenic than *A. baumannii*, its ability to harbour AMR determinants makes it an emerging threat that cannot be overlooked.[32]

This study is limited by random sampling and restricted sampling locations. To obtain a clearer and more representative picture of AMR dissemination in the Kosi River, wider surveillance across the entire river stretch is required, along with long-term sampling at multiple time intervals. Additionally, molecular investigations are essential to bridge the current knowledge gap and to better understand the genetic basis of resistance and potential transmission pathways. The co-selective pressure of heavy metals is precursor to spreading and persistence of ARGs in the environmental settings due to the genetic involvement of metal resistance genes to ARGs. Antimicrobial resistance is being accelerated by co-resistance, cross-resistance, and co-regulation in addition to horizontal gene transfer and mobile genetic elements.[33] For instance, previously Talat, et al., study shows heavy metal resistance genes such as mer operon and CopR were cooccurring with many ARGs alongside hospital waste water suggest co-selection, in which metal contamination may indirectly promote antibiotic resistance.[34] Despite these limitations, the detection of AMR-carrying *A. junii* suggests that the river may serve as an AMR reservoir, with the potential to contaminate connected water bodies and contribute to the spread of resistance on a larger scale.

More specifically, β-lactam antibiotic resistance in freshwater sources can reflect potential inputs from hospital discharge, human encroachment, and agricultural runoff.[35] Antibiotic susceptibility testing using the disc diffusion method suggests that this river may act as a reservoir of antimicrobial resistance, reinforcing the importance of interpreting these findings through a One Health approach to better understand and manage the issue.[36] Further, quantitative assessment through MIC assays for polymyxin B and colistin demonstrates the clinical relevance of resistance patterns even in environmental isolates. These findings are alarming and emphasize the need for routine monitoring of MIC trends in environmental bacteria, particularly those with pathogenic potential. Although the Kosi River, Uttarakhand is generally considered a relatively pristine river system and not heavily contaminated overall, the presence of resistant *A. junii* isolates due to contaminated runoff remains a significant concern. The detection of *Acinetobacter junii* in the Kosi River system raises serious concerns and highlights the need to identify and trace pollution sources in future investigations to block or minimize the entry of these pollutants into the river.

Conclusion

The present study provides crucial insights into the emergence and antimicrobial resistance (AMR) profiling of *Acinetobacter junii* isolated from the Kosi River, a relatively pristine freshwater ecosystem in Uttarakhand. Among the 25 bacterial isolates, 24

were identified as *A. junii* through morphological, biochemical, and molecular characterization using 16S rRNA gene sequencing. The consistent results from catalase, coagulase, and oxacillin susceptibility tests supported their identification and differentiation from other genera. Antibiotic susceptibility testing revealed significant resistance patterns. All 24 *A. junii* isolates exhibited resistance to Cefepime, while only one strain was resistant to Ceftazidime. A subset of isolates showed intermediate resistance to Cefoxitin. Minimum Inhibitory Concentration (MIC) assays further confirmed resistance to last-resort antibiotics like Polymyxin B and Colistin in some environmental isolates. These results are alarming, considering that such resistance was detected in bacteria from a non-clinical, pristine aquatic environment.

The study supports the need for comprehensive AMR monitoring beyond hospitals, incorporating environmental and agricultural sources under the One Health framework. It also emphasizes the importance of regulating antibiotic disposal, improving sanitation infrastructure, and implementing stewardship programs at a broader scale. In conclusion, this research underscores that AMR is not confined to healthcare settings alone and highlights the urgent necessity for integrated environmental surveillance systems. Without immediate action, these environmental reservoirs may contribute significantly to the global AMR burden, compromising the efficacy of existing antibiotics and threatening public health.

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Conflict of Interest
The authors declare no conflict of interest.

Data Availability Statement
This statement does not apply to this article.

Ethics Statement
This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement
This study did not involve human participants and therefore, informed consent was not required.

Permission to reproduce material from other sources
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Author Contributions
- **Madhuri Singh:** Concept Development, Collected Samples, Experiments, Analysis, Manuscript Review and Editing.
- **Manohar Kumar:** Experiment and Manuscript Draft Preparation, Data Curation.
- **Shivangi Singh:** Manuscript Writing and Review Data Curation.
- **Maneet Kumar Chakrawarti:** Data curation, Analysis and Manuscript Review
- **Bavya Krishna :** Performed Part of Experiments**.**
- **Pavithra Babu:** Performed Part of Experiments.
- **Kasturi Mukhopadhyay:** Manuscript Review, Editing and Overall Supervision.

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