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Evaluating Natural Antibiotic Resistance: Bacterial Isolates from Reynolds Nature Preserve

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Abstract: Clean drinking water is a fundamental human right, yet access remains a global public health challenge due not only to unequal distribution but also to the quality of available water sources. Natural freshwater environments can harbor bacteria with pathogenic potential and intrinsic antimicrobial resistance, posing risks to both human and ecological health. In this study, bacterial isolates collected from a freshwater site at Reynolds Nature Preserve were characterized using classical microbiological techniques, including aseptic sampling, streak plating, Gram staining, and biochemical testing. Antibiotic susceptibility was evaluated using the Kirby–Bauer disk diffusion method, and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Several Gram-negative environmental isolates were identified, exhibiting diverse antimicrobial resistance profiles. These findings suggest that natural freshwater environments may serve as reservoirs of antimicrobial resistance, underscoring the importance of continued environmental surveillance.

1 Introduction

Clean drinking water is essential for human health, yet natural freshwater environments may serve as reservoirs for microorganisms capable of causing disease. Environmental bacteria can also act as sources of antimicrobial resistance (AMR), which is increasingly recognized as one of the world's major public health concerns [1]. The presence of antibiotic-resistant bacteria outside clinical settings suggests that resistance mechanisms are widespread in nature and may be transferred among microbial populations [1].

The objective of this study was to isolate and characterize bacterial species from a freshwater environment at Reynolds Nature Preserve using classical microbiological techniques. Isolates were evaluated through selective culturing, Gram staining, and biochemical testing. Antibiotic susceptibility was further assessed using the Kirby-Bauer disk diffusion method [2] to determine resistance patterns and evaluate potential environmental reservoirs of AMR.

2 Methods

Two environmental water samples were collected from Reynolds Nature Preserve between 08:00 and 08:15 during misty conditions with light rainfall (0.05 in), a dew point of 60°F, and relative humidity of 93%. Samples were collected in a 50mL conical tube containing ~30-40 ml of water. They were serially diluted and plated on MacConkey agar, a selective and differential medium used to isolate Gram-negative bacteria and evaluate lactose fermentation, as well as nutrient agar to assess general bacterial growth. Following incubation at 37°C for 24–48 hours, isolated colonies were subcultured using aseptic streak-plating techniques. Colony morphology was recorded and bacterial cells were examined using simple staining and Gram staining procedures.

Biochemical characterization includes Catalase, Voges-Proskauer (VP), Urease, Methyl Red-Proskauer Broth (MRBP), Triple Sugar Iron (TSI), Citrate, Motility, and Oxidase tests. Results were compared with those of an *Escherichia coli* reference strain to provide a reference for biochemical interpretation.

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method to provide a qualitative assessment of antimicrobial susceptibility [2]. Antibiotics tested include ciprofloxacin, tetracycline, erythromycin, chloramphenicol, cefotaxime-clavulanic acid, gentamicin, trimethoprim-sulfamethoxazole, and amoxicillin-clavulanic acid. Zones of inhibition were measured and interpreted according to CLSI guidelines M100 standard 35th Edition, 2025. [3].

3 Results

3.1 Biochemical Characterization

Biochemical testing revealed distinct profiles among the environmental isolates and the *E. coli* control. Both A1 and A2 were determined to be Gram-negative bacteria. Culture A1 tested positive for catalase, VP, and citrate utilization but negative for oxidase activity and motility. Culture A2 was oxidase-positive but negative for catalase activity, citrate utilization, and motility. The observed biochemical patterns were compared with known bacterial profiles to aid in tentative identification of isolates.

Table 1. Biochemical test results for environmental isolates and the *E. coli* control.

Biochemical Tests	Sample A1	Sample A2	Control EC
Catalase	+	-	+
VP	+	+	-
UREA	-	-	-
MRBP	-	-	-
TSI B/S	Alk/Acid	Alk/Acid	Acid/Acid
Citrate	+	-	-
Motility	-	-	+
Gram	-	-	-
Oxidase	-	+	-

The combined biochemical profile of A1 was most consistent with members of the genus *Klebsiella*, while A2 exhibited characteristics suggestive of a possible *Shigella*-like isolate. These classifications represent provisional identifications based on phenotypic characteristics and should be interpreted as tentative pending molecular confirmation.

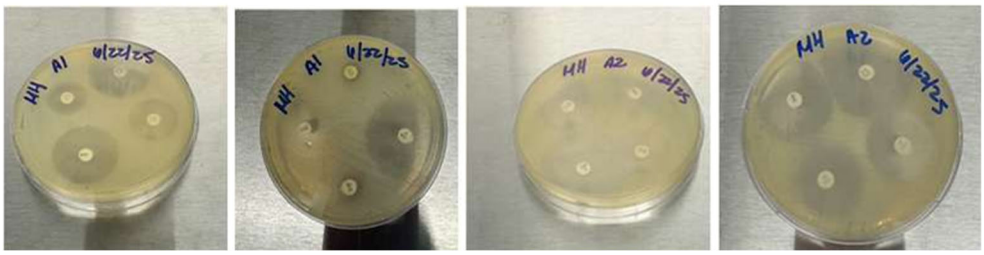

Fig. 1. Representative cultures of isolates A1 and A2 prepared for biochemical characterization and antibiotic susceptibility testing.

3.2 Antibiotic Susceptibility

Antibiotic susceptibility testing demonstrated variation among isolates. Both environmental isolates remained susceptible to ciprofloxacin, chloramphenicol, cefotaxime-clavulanic acid, gentamicin, trimethoprim-sulfamethoxazole, and amoxicillin-clavulanic acid.

Resistance to tetracycline and erythromycin was observed among environmental isolates. Culture A1 exhibited resistance to both antibiotics, whereas A2 exhibited resistance only to erythromycin and remained susceptible to the remaining antibiotics tested, as shown in Table 2.

These findings suggest the presence of naturally occurring antibiotic resistance traits within environmental bacterial populations. Freshwater ecosystems harbour diverse microbial communities that play essential roles in nutrient cycling and ecological balance. However, these environments also serve as interfaces for the accumulation and dissemination of antimicrobial resistance (AMR) genes and resistant organisms [1]. Environmental bacteria exposed to anthropogenic inputs, including agricultural runoff, wastewater effluent, and pharmaceutical residues, can acquire, maintain, and transfer resistance determinants to clinically relevant pathogens through horizontal gene transfer. The identification and characterization of AMR-carrying organisms in natural freshwater systems is therefore critical for understanding the ecology of resistance and anticipating emerging public health threats.

Table 2. Antibiotic susceptibility profiles and zone of inhibition measurements.

Antibiotic	Con. (µg)	Code	*E. coli*	A1	A2
Ciprofloxacin	5	CIP5	34-S	40-S	40-S
Tetracycline	30	TE30	24-I	9-R	24-S
Erythromycin	15	E15	13-R	9-R	14-I
Chloramphenicol	30	C30	33-S	23-S	27-S
Cefotaxime-Clavulanic acid	30	CTX	40-S	32-S	36-S
Gentamicin	10	GM10	27-S	23-S	28-S
Trimethoprim-Sulfamethoxazole	23.75	SXT	33-S	30-S	34-S
Amoxicillin-Clavulanic Acid	30	AMC30	25-S	19-S	26-S

4 Discussion

Freshwater environments can harbour diverse Gram-negative bacteria with varying antibiotic susceptibility profiles [1]. The use of MacConkey agar enabled early differentiation of Gram-negative bacteria based on lactose fermentation and supported subsequent biochemical characterization. Most antibiotics tested remained effective against the isolates; however, resistance to tetracycline and erythromycin was observed. The presence of resistant environmental bacteria is notable, as natural ecosystems may serve as reservoirs for antimicrobial resistance genes that can persist and disseminate within microbial communities [3]. Because this investigation examined environmental isolates rather than clinical specimens, susceptibility testing provided comparative antimicrobial resistance profiles and should not be interpreted as clinical susceptibility determinations.

Biochemical testing suggested that isolate A1 may belong to the genus *Klebsiella*, while A2 exhibited characteristics suggestive of a *Shigella*-like organism or a related environmental variant. However, classical microbiological methods alone are insufficient for definitive species-level identification. While biochemical testing provided sufficient information for tentative identification and comparison of environmental isolates, certain assays, including TSI, are inherently sensitive to inoculation technique and incubation conditions. Consequently, the combined biochemical profile was used to support provisional classification rather than definitive species identification, which would require molecular confirmation.

Overall, the detection of antibiotic-resistant bacteria in a natural freshwater environment highlights the potential role of these ecosystems in antimicrobial resistance dynamics. Environmental bacteria may exchange genetic material through horizontal gene transfer, facilitating the spread of resistance phenotypes among microbial populations [3]. Although the isolates were not clinically confirmed pathogens, their resistance profiles emphasize the importance of including natural environments in antimicrobial resistance surveillance efforts.

5 Conclusion

Environmental water samples collected from Reynolds Nature Preserve contained a diverse community of Gram-negative bacteria, which were isolated and screened for resistance to selected antibiotics. To date, no significant antibiotic resistance has been detected among the isolates examined. These findings underscore the importance of monitoring environmental microbial communities as potential reservoirs of antimicrobial resistance (AMR). Continued surveillance of natural water sources can improve our understanding of environmental contributions to the global AMR crisis and support efforts to safeguard public health.

6 Future Work

Future studies should incorporate molecular identification techniques such as 16S rRNA sequencing to confirm species-level classification. Repeating key biochemical assays would further improve confidence in isolating identification before molecular confirmation. Additional investigations should evaluate plasmid-mediated resistance mechanisms and examine the potential transfer of resistance genes among environmental bacteria. Expanding susceptibility testing to include additional antibiotic classes, including carbapenems, would provide a more comprehensive resistance profile. Long-term monitoring of environmental sites could further clarify the role of natural ecosystems in the emergence and spread of antimicrobial resistance.

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