Assessment of Antibiotic Resistance in Waterborne Pathogens

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Abstract: This study aimed to assess the antibiotic susceptibility of bacterial isolates from raw water samples using the broth microdilution method. The tested antibiotics demonstrated inhibitory effects on bacterial growth. MIC values ranged from <0.25–7.81 μg/mL for tetracycline, 0.98–62.25 μg/mL for ampicillin, and <0.001–0.25 μg/mL for ciprofloxacin. The findings revealed that while most isolates were susceptible to the tested antibiotics, resistance was detected in specific strains. *Escherichia coli* V9 and *Enterobacter cloacae* V7 exhibited resistance to tetracycline, while *Citrobacter braakii* V1 and *Raoultella ornithinolytica* V4, V5, and V10 were resistant to ampicillin. All tested isolates remained susceptible to ciprofloxacin. Additionally, *Acinetobacter* spp. and *Aeromonas* spp. were intrinsically resistant to ampicillin, consistent with established resistance patterns. These findings reveal antibiotic-resistant bacteria in raw water, underscoring environmental risks and the need for ongoing research to curb resistance spread and protect public health.

Keywords: foodborne pathogens, antibiotics, resistance, water source, public health

1. Introduction

Water contamination with waterborne pathogens presents substantial public health risks, facilitating the transmission of infectious diseases such as cholera and dysentery [1]. The ecological dynamics of waterborne pathogens are influenced by various anthropogenic factors, including modern agricultural practices and other human activities [2]. Given the extensive distribution of pathogenic bacteria in freshwater ecosystems, it is essential to monitor microbial contamination in streams and small rivers that serve as sources of drinking water [3]. Furthermore, the presence of antibiotic-resistant bacteria in raw spring water samples represents an emerging threat

to both public health and environmental stability. These bacteria are subjected to various environmental pressures, including exposure to antibiotic residues from agricultural runoff and human activities, which contribute to the selection and persistence of resistant strains. The presence of antibiotic-resistant bacteria in spring water also raises concerns regarding their potential role as genetic reservoirs, facilitating the horizontal transfer of resistance genes to other bacterial populations, including clinically relevant human pathogens.

Antibiotic susceptibility testing is essential for assessing resistance, with disc diffusion and microdilution being common methods. Microdilution offers greater sensitivity, providing precise MIC values for detecting subtle susceptibility variations [4,5]. This study aims to evaluate the antibiotic susceptibility of bacteria isolated from raw water samples, contributing to a deeper understanding of resistance mechanisms in environmental bacterial populations.

2. Material and Methods

2.1. Tested strains and microdilution method

The methodology for isolating and characterizing bacteria, as outlined by Grujović et al. [3], involved a comprehensive process. Twenty isolates were obtained from water samples, as listed in Table 1.

The broth microdilution method was employed to assess antibiotic resistance following a standardized protocol. Bacterial suspensions were prepared using the direct colony method [6], with turbidity adjusted to 0.5 McFarland units. The initial suspensions contained approximately 10^8 CFU/mL and were further diluted (1:100) in sterile 0.85% saline.

Twofold serial dilutions of tetracycline, ampicillin, and ciprofloxacin were prepared in 96-well microtiter plates containing Mueller-Hinton broth. The tested concentration ranges were 0.25–128 μg/mL for tetracycline and ampicillin and 0.001–500 μg/mL for ciprofloxacin. Bacterial suspensions were inoculated to achieve a final concentration of 5×10^5 CFU/mL. Growth was monitored using resazurin, a redox indicator that shifts from blue to pink upon bacterial metabolism. MIC was determined as the lowest antibiotic concentration that inhibited color change [7].

Each test included growth and sterility controls, performed in duplicate, with mean values reported. Sensitivity was compared against *Escherichia coli* ATCC 25922 and a clinical *Escherichia coli* isolate. *Pseudomonas aeruginosa* ATCC 27853 was used as the quality control strain for *Aeromonas* spp. and *Acinetobacter* spp. MIC breakpoints were interpreted according to EUCAST guidelines [8].

3. Results and Discussion

To quantitatively determine MIC values, the broth microdilution assay was employed (Table 1). All three tested antibiotics exhibited inhibitory effects on the growth of the

examined bacterial isolates. The MIC values for tetracycline ranged from <0.25 μg/mL to 7.81 μg/mL, while MIC values for ampicillin varied from 0.98 μg/mL to 62.25 μg/mL. For ciprofloxacin, MIC values ranged from <0.001 μg/mL to 0.25 μg/mL.

The findings indicate that the tested bacterial isolates were generally susceptible to the investigated antibiotics. However, resistance was observed in specific isolates. *E. coli* V9 and *E. cloacae* V7 demonstrated resistance to tetracycline, while *C. braakii* V1 and *R. ornithinolytica* V4, V5, and V10 exhibited resistance to ampicillin. Notably, all tested isolates remained susceptible to ciprofloxacin. According to EUCAST guidelines [8], *Acinetobacter* spp. and *Aeromonas* spp. are intrinsically resistant to ampicillin.

Tetracycline, a broad-spectrum antibiotic, exhibits activity against both aerobic and anaerobic bacteria, including Gram-positive and Gram-negative species. In contrast, ampicillin, a β-lactam antibiotic, primarily targets Gram-positive bacteria but also affects select Gram-negative strains. Ciprofloxacin, a fluoroquinolone, is highly effective against a broad range of bacterial infections, particularly those caused by Gram-negative pathogens such as *P. aeruginosa*. The results of this study indicate that ampicillin demonstrated efficacy only against *E. coli* and *E. cloacae* isolates, while tetracycline and ciprofloxacin exhibited broader antibacterial activity.

Table 1. Antibiotic sensitivity of tested isolates

Specie	Isolate	Antibiotics		
		Tetracycline	Ampicillin	Ciprofloxacin
Escherichia coli	V1	3.9	7.8	0.008
Escherichia coli	V2	3.9	3.9	0.032
Escherichia coli	V3	3.9	3.9	0.064
Escherichia coli	V4	3.9	3.9	0.008
Escherichia coli	V5	1.96	3.9	0.016
Escherichia coli	V6	1.96	1.96	0.008
Escherichia coli	V7	1.96	0.98	0.008
Escherichia coli	V8	1.96	3.91	0.016
Escherichia coli	V9	7.81	7.8	0.008
Citrobacter braakii	V1	1.95	11.55	0.250
Acinetobacter spp.		<0.25	15.625	<0.001
Acinetobacter calcoaceticus	V2	0.98	15.625	<0.001
Acinetobacter calcoaceticus	V6	<0.25	15.625	<0.001
Aeromonas spp.		<0.25	15.625	0.125
Aeromonas bestiarum	V11	1.96	15.625	0.125
Raoultella ornithinolytica	V4	<0.25	15.625	0.016
Raoultella ornithinolytica	V5	<0.25	31.25	0.032
Raoultella ornithinolytica	V10	3.91	62.25	0.032
Enterobacter cloacae	V7	7.81	7.81	0.016
Enterobacter cloacae	V9	3.91	7.81	0.03
Escherichia coli	clinical isolate	1.96	2.1	0.25
Escherichia coli	ATCC 25922	0.98	0.37	0.06
Pseudomonas aeruginosa	ATCC 27853	7.81	>128	0.001

The results represent the minimum inhibitory concentration (MIC) in μg/mL.

In the study conducted by Grujović et al. [3], isolates were subjected to antibiotic susceptibility testing using the disc diffusion method. The results revealed that none of

the tested isolates exhibited complete resistance to all five antibiotics. Results obtained using the microdilution method indicated that *E. coli* V9 and *E. cloacae* V7 were resistant to tetracycline, while the other tested isolates remained susceptible. Conversely, as reported by Grujović et al. [3], all tested isolates exhibited resistance to amoxicillin. In our study, the efficacy of ampicillin, a β-lactam antibiotic belonging to the same penicillin group as amoxicillin, was investigated. *C. braakii* V1 and *R. ornithinolytica* V4, V5, and V10 exhibited resistance to ampicillin. These findings highlight variations in antibiotic susceptibility among different bacterial species.

3. Conclusions

This study reveals variations in antibiotic susceptibility among bacteria in raw water, highlighting antimicrobial resistance risks. The findings support previous research and indicate the need for routine testing to assess water quality. Antibiotic-resistant bacteria in natural water pose public health risks, especially in areas with limited treatment. Ongoing surveillance and research are crucial to tracking resistance trends, understanding environmental influences, and developing mitigation strategies.

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