

A Pilot Investigation of Antibiotic Susceptibility Profile of *Escherichia coli* in Wastewater from a Boarding High School in Türkiye

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ABSTRACT: This pilot study investigates the microbiological quality and antibiotic resistance profiles of *Escherichia coli* isolates from domestic wastewater at a boarding high school where the population is 1500 people in İstanbul, Türkiye. Samples collected from the main sewer line were subjected to enrichment, isolation on TBX (Tryptone Bile X-Glucuronide) agar, and Kirby-Bauer disk diffusion tests. The sensitivity of the isolates to Ampicillin, Tigecycline, Ciprofloxacin, and Cephalothin was evaluated according to Clinical and Laboratory Standards Institute (CLSI)'s 2024 standards. In this preliminary sample, the two presumptive *E. coli* isolates tested were susceptible to the antibiotics evaluated. These results indicate that the burden of resistant bacteria in healthy and educational populations is lower than in hospital-derived or general municipal wastewaters, reflecting manageable local resistance dynamics.

KEYWORDS: Microbiology, Antibiotic Resistance, *Escherichia coli*, Wastewater, Public Health.

■ Introduction

Antimicrobial resistance is a silent pandemic threatening the protectors of modern medicine and is considered one of the most critical global health challenges of the 21st century.¹ Bacterial resistance mechanisms to antibiotics lead to untreatable infections, increased healthcare costs, and high mortality rates. Data published by the World Health Organization (WHO) underscores the importance of monitoring resistance trends not only in clinical samples but also in environmental reservoirs.²

Wastewater is identified as a "hotspot" for the spread of antibiotics and the transfer of resistance genes between different bacterial species through horizontal gene transfer.^{3,4} Wastewater combines domestic, industrial, and sometimes hospital waste, creating an ideal selective pressure environment for resistant bacteria.⁵ *Escherichia coli* is a globally accepted "indicator organism" for monitoring antimicrobial resistance because it is a natural member of the mammalian gut flora and a primary indicator of fecal contamination.^{6,7}

Antibiotic discs from β -lactam, tetracycline, and fluoroquinolone groups, consisting of Ampicillin, Cephalothin, Tigecycline, and Ciprofloxacin, respectively, were used because they represent different mechanisms of action and antibiotic classes. The increasing rates of bacterial antimicrobial resistance worldwide necessitate the evaluation of susceptibility profiles to different antibiotic groups. According to World Health Organization (WHO) data, resistance rates to β -lactams and fluoroquinolones can exceed 20–50% in many clinical pathogens. In contrast, tigecycline stands out as an alternative agent that largely maintains its efficacy against multidrug-resistant strains. Therefore, the combined study of these antibiotics, representing the β -lactam (ampicillin and cephalothin), fluoro-

quinolone (ciprofloxacin), and tetracycline (tigecycline) groups, allows for a comprehensive evaluation of the susceptibility and resistance characteristics of *E. coli* to antibiotics with different mechanisms of action.²²

This pilot study aims to contribute to local public health data by determining the resistance load of wastewater in our school campus. The antibiotics tested have different mechanisms of action and are agents frequently used in clinical practice.

■ Methods

On February 18th, at 9:30 AM, with prior permission from the school administration, a 500 mL composite sample (2 bottles, 250 mL) was taken from the main sewer outlet of the entire school and boarding sections; sterile bottles were simultaneously used to collect 500 mL of sample from the sewage using the immersion method at one time. These samples were placed in a portbag containing ice packs at +4°C in the wake of collection of sample. The samples were transported to the laboratory immediately under cold chain conditions, as seen in Figures 1 and 2.



Figure 1: The main sewage outlet from where the sample was taken. This sampling point ensures that the collected samples reflect the overall resistance profile of the campus because it represents the combined wastewater of the entire school population.

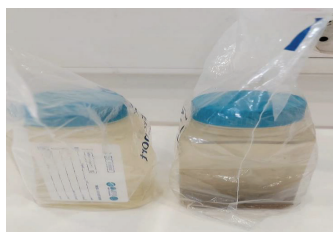


Figure 2: Containers holding samples of wastewater from a high school. Proper collection and transport under controlled conditions preserved sample integrity.

E. coli isolation was performed according to ISO 7251:2005 (ISO, 2005).²⁰ 5 mL of each sample was homogenized in 225 mL of sterile peptone water. From the homogenized homogenates, 1 mL subsample was taken and added to 9 mL of Tryptone Soy Broth (TSB) containing 20 mg novobiocin per liter, and pre-enriched at 37°C for 24 hours. On Day 2, Thermotolerant coliform selection was then performed using Lauryl Sulphate Tryptone (LST) broth and Brilliant Green Bile Broth (BGBB). Incubation at 44°C for 24 hours during the enrichment and isolation stages was preferred for the selective development of thermotolerant (fecal) coliforms. On Day 3, *E. coli* forms typical colonies at 44°C due to gas formation in the BGBB, as named presumptive *E. coli*. 100µL samples were taken from the cultures and inoculated onto Tryptone Bile X-glucuronide Agar (TBX) using the spread plate method, and then incubated for 48 hours at 44°C. After incubation on Day 4, colonies showing a metallic green reflection on TBX were used to confirm β -glucuronidase activity on presumptive *E. coli* isolates. This temperature suppresses the growth of other mesophilic bacteria that may be present in the medium, facilitating the isolation of the target microorganism, as demonstrated in Figures 3 and 4.⁹ Samples were inoculated onto TBX chromogenic agar and incubated at 44°C for 48 hours. Characteristic blue-green *E. coli* colonies were purified, as seen in Figure 5.^{3,10}

Two suspected *E. coli* were isolated before being passed through TBX the night before, were collected using a loop, and placed into tubes containing Tryptone Broth with 1% tryptophan for the indole test, and into tubes containing methyl red Voges-Proskauer (MR-VP) medium (double tubes) for the methyl red and Voges-Proskauer tests. After incubating the tubes overnight, the formation of a bright red/purple ring at the top of the tube for the indole test was considered positive. For the methyl red test, methyl red indicator was added to the MR-VP tube, and colonies showing a red color in the tubes were considered *E. coli* positive. Barrit's reagent A (0.6 ml of 5% α -naphthol solution in alcohol) and Barrit's reagent B (0.2 ml of 40% KOH solution and 2 drops of 0.5% creatine solution) were added to the other MR-VP tubes, and those that turned red were considered *E. coli* positive.¹⁹



Figure 3: Selection of blue blue-green colonies in TBX agar before using an inoculation loop. The appearance of blue blue-green colonies confirms the successful isolation of *E. coli*.

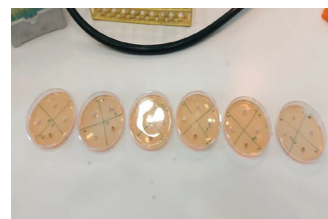


Figure 4: Muller Hinton Agar plates before incubation.

In the wake of biochemical confirmation of *E. coli*, two colonies were taken from the culture and suspended in physiological saline according to the McFarland 0.5 (1×10^8 cfu/ml) opacity standard. Then, 0.1 mL smear plates were made on Mueller-Hinton agar. The plated Petri dishes were kept in a cabinet at room temperature for 5-10 minutes before incubation. After this stage, 4 antibiotic discs, ampicillin (10 µg), ciprofloxacin (5 µg), Tigecycline (15 µg), and cephalothin (30 µg), were placed in one Petri dish. After the inoculation process was completed, 6 Petri dishes were incubated at 37°C for 24 hours, and antibiotic resistance was evaluated according to Clinical and Laboratory Standards Institute (CLSI) 2024 standards by measuring the zone diameters as seen in Table 1. At the end of this period, inhibition zones were formed depending on the susceptibility of the enriched *E. coli* colonies to the four antibiotics. The formation and measurement of inhibition zones are illustrated in Figure 5. Ampicillin, Tigecycline, Ciprofloxacin, and Cephalothin resulted in inhibition zones with average diameters of 26 mm, 20 mm, 28 mm, and 18 mm, respectively, as seen in Table 1. According to the CLSI 2024 Antimicrobial Susceptibility Testing Guidelines, the grown *E. coli* was observed to be susceptible to all four antibiotics. This research comprehensively analyzes the antibiotic susceptibility profiles of *Escherichia coli* strains isolated from wastewater on a boarding school campus, considering global resistance trends, biochemical mechanisms, population dynamics, seasonal factors, and methodological limitations. Our key finding, "almost complete susceptibility," may refute the initial hypothesis that "resistant bacteria will be detected," but provides highly unique and strategic data when compared to existing literature on the microbiological health of school ecosystems.

For quality control purposes in antibiotic susceptibility testing, the *Escherichia coli* ATCC 25922 reference strain was used. The control strain was tested under the same conditions (0.5 McFarland suspension, same medium, and incubation

conditions) on each test day to verify that the inhibition zone diameters were within the range specified in the CLSI 2024 standards. The test results showed that the zone diameters of the control strain were within acceptable limits; thus, the reliability of the findings obtained for the isolates was confirmed. The Kirby-Bauer disk diffusion method was applied. Inhibition zones were measured and interpreted according to the CLSI 2024 standards.²¹

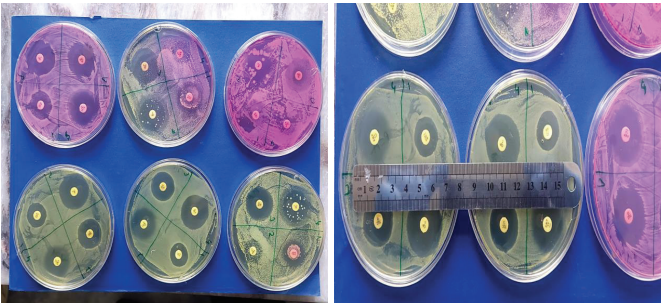


Figure 5: A ruler was placed on two Muller-Hinton Agar plates after 24 hours of incubation at 37°C to measure the diameters of the inhibition zones. Clearly visible inhibition zones show antibiotic activity against the isolates, supporting the finding of full susceptibility across every antibiotic that has been tested.

■ Results and Discussion

Results:

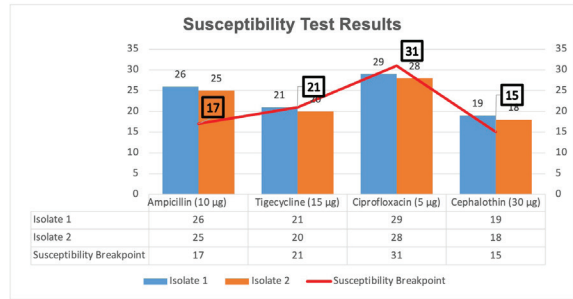
Samples were collected on February 18th at 9:30 AM and immediately taken to the laboratory for analysis. After the *E. coli* identification analysis, at the end of the sixth day, two presumptive strains were confirmed as *E. coli* strains and were transferred to Muller-Hinton Agar, and four antibiotic discs were placed to observe the antibiotic reactions against the *E. coli* strains; the resulting diameters were measured with a ruler.

The data obtained from the analyses showed that the isolated *E. coli* strains were susceptible and intermediately susceptible to all four different classes of antibiotics tested.

Table 1: Inhibition zone diameters of isolates (mm). All measured inhibition zones exceed the CLSI susceptibility breakpoints (mm).

Isolates #	Antibiotics															
	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm.(CLSI,2024)															
	Ampicillin (10 µg)				Tigecycline (15 µg)				Ciprofloxacin (5 µg)				Cephalothin (30 µg)			
	S	SSD	I	R	S	SSD	I	R	S	SSD	I	R	S	SSD	I	R
#1 (mm)	26 (S)				21 (S)				29 (I)				19 (S)			
#2 (mm)	25 (S)				20 (I)				28 (I)				18 (S)			

S (Susceptible), SSD (Susceptible-Dose Dependent), I (Intermediate), R (Resistant).



Discussion:

Our susceptibility rate contrasts sharply with a vast body of literature arguing that urban wastewater is a primary reservoir for resistant pathogens. To understand the reasons for this, comparison with studies in geographies where resistance is much higher is essential. Research in densely populated urban areas like Dhaka, Bangladesh, where antibiotic use is uncontrolled, shows that wastewater acts like a "resistance factory." A study by Siddique *et al.* found that over 90% of *E. coli* strains isolated from urban wastewater showed resistance to at least three different classes of antibiotics (MultiDrug Resistance MDR).⁵ In the case of Dhaka, resistance to ampicillin and ciprofloxacin, in particular, has reached endemic levels. The detection of 0% resistance to these antibiotics in our study data demonstrates the extent to which the settlement is protected from urban pollution pressures and the introduction of resistant strains. Our results are remarkable even when compared to data from developed countries. Studies conducted in 17 municipal wastewater treatment plants in Oregon, USA, found ampicillin resistance to be in the 30-40% range and ciprofloxacin resistance in the 10-15% range.³ The absence of this baseline resistance level, seen even in regions with well-organized healthcare systems like Oregon, on our high school campus can be attributed to both the limited size of the population and the high level of awareness among campus residents regarding antibiotic use. The prevalence of resistant bacteria is directly related to the volume and quality of antibiotic consumption in that community.² The 0% resistance rate in our study indicates that the gut flora (microbiome) of individuals within the campus is dominated by "wild-type" susceptible bacteria.¹¹ Furthermore, urban wastewater systems often combine hospital, industrial, and domestic waste. The isolation of our school's sewage system from high-resistance foci such as the hospital eliminates the risk of "cross-contamination."¹⁵ This isolated micro-ecosystem lacks the biochemical pressure necessary for the establishment and spread of resistant strains. The fact that the study was conducted on February 18th, during the winter period, plays a central role in interpreting the results. Temperature is a variable that directly affects bacterial metabolic rate, reproduction rate, and, most importantly, horizontal gene transfer (conjugation).¹² In a study conducted on municipal wastewater in Poland, it was found that resistance rates in *E. coli* strains isolated during the winter months were significantly lower than those isolated during the summer months.¹³ Low temperatures suppress the transfer of plasmids carrying resistance genes between bacteria (conjugation frequency). The low temperatures in February may have minimized the rate of bacterial genetic exchange in the sewer line. In addition, the flow rate of water in the sewer system increases with precipitation during the winter months, which dilutes the bacterial concentration (dilution effect).³ This dilution could technically reduce the likelihood of detecting resistant bacteria. If this pilot study were repeated during the summer months, a low level of resistance might be detected due to accelerated horizontal gene transfer resulting from increased temperatures and decreased water flow. Therefore, the

detected full susceptibility result may reflect the period when the population is seasonally at its "cleanest".

Modern public health understanding is based on the "One Health" approach, which argues that human health and environmental health are inseparable.¹⁴ When wastewater is discharged into natural water sources without treatment, it carries resistance elements into the environment.^{4,12} The fact that our high school's wastewater would be clean in terms of resistance means that resistant pathogens are not being pumped into the local ecosystem. Environmental bacteria (aquatic or soil bacteria) can become "genetic reservoirs" by incorporating resistance genes from wastewater.¹⁵ Our study may prove that the school campus does not negatively contribute to this global resistance chain. This may be an indicator of the success of educational institutions in terms of environmental sustainability.¹⁶

Wastewater-based surveillance is like a massive biobank where the biological data of thousands of individuals are collected in a single sample.¹⁷ A sample taken from the school's sewage outlet provided a summary of the common "gut health" of all individuals living on that campus. This approach is the most effective way to monitor a community's resistance profile when individual testing is costly. A study conducted in Sweden by Hutinel *et al.* demonstrated that wastewater analyses showed more than 80% agreement with clinical resistance data in that area.⁶ The exact sensitivity in our high school would likely parallel infection data and antibiotic prescribing rates in the school's infirmary records. This provides school administrations with a scientific basis for developing public health policies. The results of this pilot study can be considered a concrete reflection of the health education and awareness of rational drug use provided at the school. The absence of resistant bacteria is also indirect evidence that hygiene standards and food safety (cafeteria, etc.) within the school are high. This is because resistant bacteria usually infiltrate the community through contaminated food or inadequate sanitation.¹⁰

■ Conclusion

In conclusion, this pilot study, conducted on February 18, 2026, demonstrated that wastewater on our high school campus currently exhibits a low-risk profile in terms of antibiotic resistance. The susceptibility of the isolated *Escherichia coli* strains to Ampicillin, Tigecycline, Ciprofloxacin, and Cephalothin antibiotics was confirmed by the inhibition zones being well above the CLSI 2024 standards. The rejection of the initial hypothesis that "resistant bacteria will be detected" reveals that the school community may have a healthy microbial flora and that the campus ecosystem may not yet be severely impacted by the global antimicrobial resistance crisis. However, given the dynamic nature of antimicrobial resistance, maintaining this positive picture requires the continued commitment to periodic surveillance studies, rational antibiotic use policies, and "One Health" focused environmental monitoring activities.^{14,18}

This research is a successful pilot study aimed at raising environmental and public health awareness in our school. Furthermore, it is believed that future studies in this area would

benefit from collecting samples at different times and from different depths to detect seasonal fluctuations and conducting genetic research to identify potential resistant microorganism genes.

■ Acknowledgments

I would like to express my sincere gratitude to my project advisor and AP Biology teacher, Dünya Önen. Without her continuous support, guidance, and invaluable feedback, this research would not have been possible. I would also like to thank the school authorities for their support and for facilitating my access to the wastewater samples from the sewage system.

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