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Assessment of Antibiotic Resistance in Escherichia coli Isolated from Various Environmental and Community Sources

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Abstract

The rising trend of antibiotic resistance among Escherichia coli (E. coli) is a public health concern of considerable magnitude. This study examines the antibiotic resistance profiles of E. coli strains isolated from multiple environmental and community sources. Collections involved water, soil, sewage, and clinical material from rural and urban areas. The susceptibility of the strains to antibiotic was tested using disk diffusion assay with 12 of the most frequently encountered antibiotics. The resistance rates were analyzed and potential correlations with environmental factors—e.g., degrees of pollution and human activity—were explored. The results showed diversified resistance patterns, with maximum resistance being observed in strains from strongly polluted environments and clinical environments. The study indicates the necessity for strict monitoring of the use of antibiotics and enhanced regulation on the use of antibiotics in order to lower the incidence of resistant strains.

Keywords: Antibiotic resistance; Escherichia coli; Environmental sources; Community sources; Antibiotic susceptibility

Introduction

Antimicrobial resistance is today identified as among the most significant public health challenges of the 21st century [1-3]. An increase in antimicrobial-resistant bacteria jeopardizes critically the treatment of infectious diseases and the success of modern medicine. Escherichia coli (E. coli), the intestinal bacterium found in humans and animals, has been the cause of numerous infections, including urinary tract infections (UTIs), gastrointestinal disease, and infection in newborns. While the majority of E. coli strains harm no one, certain pathogenic strains can cause serious disease, particularly in immunocompromised individuals. The emergence of antibiotic-resistant E. E. coli resistant strains render treatment regimens challenging, leading to increased hospital stays, medical costs, and mortality rates. In a few instances, such resistant strains have also become resistant to more than one class of antibiotic, rendering most first-line treatment regimes useless [4].

E. coli resistance is primarily caused by genetic mutation and the acquisition of resistance genes through horizontal gene transfer, which is often facilitated by mobile genetic elements like plasmids, transposons, and integrons. The spread of resistant E. coli strains is not limited to a clinical setting; the bacteria also survive in environmental reservoirs such as water, soil, and sewage. Environmental contamination by antibiotic-resistant bacteria is closely related to human activity, which involves inappropriate use of antibiotics, agricultural uses of antibiotics, and poor treatment of waste in urban and rural settings. Their incorrect use as growth promoters and for disease prevention in animals has been determined to be one of the primary causes of emergence and spread of resistance. In similar fashion, humans' improper disposal of unused or expired antibiotics, sewage effluent pollution, and runoff of contaminated water from agricultural fields are also contributing factors [5,6].

With widespread prevalence of E. coli between various environments and its commensal and pathogenic status, there is a need to monitor and assess the resistance pattern of E. coli isolates from various environmental and community backgrounds. Environmental dissemination of antibiotic-resistant E. Escherichia coli isolates can be reservoirs of the resistant bacteria, which may then infect humans by direct contact, consumption of contaminated food or water, or even through the environment [9]. Urban and rural environments would present different levels of risk for the spread of resistant strains, as rural environments have less stringent wastewater treatment systems and higher antibiotic use in agriculture, while urban areas have highly populated areas with higher use of antibiotics and pollution. In order to effectively combat the new threat of antibiotic-resistant E. coli, an acknowledgment of the prevalence of resistance in any setting and community lifeworld must occur. Previous research has established that hospitals and health facilities are centers for resistant microbe transmission, but little is known regarding environmental reservoirs, including bodies of water, sewer discharge, and agricultural runoff. Secondly, the relationship between certain environmental factors—i.e., degrees of pollution, industrial use, and human population, and the dissemination of resistant E. coli must be explored further [7-10]. The current study will address this by exploring the antibiotic resistance patterns of E. coli isolates isolated from various environmental and community sources. These samples would be collected from various regions, including water bodies, soil, sewerage, and hospitals, from rural and urban regions. Disk diffusion, being a method widely employed for the determination of bacterial resistance, will be employed in testing susceptibility of isolated strains to antibiotics.

This procedure exposes resistance to a panel of commonly encountered antibiotics, providing a general profile of the types of resistance present in each sample. The study will also analyze the probability of any correlation between environmental rates and presence of antibiotic-resistant strains. Human population levels, industrialization, and pollution levels at sampling points will be considered in relation to resistance profiles observed during sampling. With the study of such parameters, the study hopes to create patterns that can be utilized for describing the anthropogenic and environmental stresses which led to the development of antibiotic resistance.

The ultimate long-term objective of this research is to obtain valuable information with respect to the environmental reservoirs of antibiotic-resistant. E. coli and the risk that these reservoirs might pose to the population's general health. It is imperative to study the antibiotic resistance dynamics in the environment and the community to enable proper development of methods of eradicating resistant strains. This research will also seek to reassert the judicious administration of antibiotics, waste disposal, and stricter environmental regulations as components of an integrated program to counter the international plague of antibiotic resistance.

Related Works

Antibiotic resistance in Escherichia coli (E. coli) is an alarming issue within the environmental and clinical settings. Earlier research has persistently indicated that the environmental and community sources of antibiotic-resistant isolates play a major role in dispersal, and resistance patterns are shaped by numerous environmental factors. Sewage, water, and soil have been reported to act as a crucial reservoir for antibiotic-resistant E. coli. For instance, Xu et al. [11] explored the occurrence of AR E. coli in farm animals, waste, and water highlighted disinfection practices such as UV treatment, surface plasma oxidation, and biochar. Antimicrobial peptides, plant extracts, and probiotics are some alternatives. The review highlights the need for novel strategies to limit antibiotic resistance in E. coli of farm origin. Martak et al. [12] focused on environmental reservoirs, wherein antibiotic-resistant bacteria (ARB), resistance genes (ARGs), and selective agents are released into soil and water due to human activity. High-income countries (HICs) have effective sanitation that limits environmental transmission, and AMR spreads predominantly from person-to-person. Low- and middle-income countries (LMICs) are, however, under higher risks due to poor sanitation and agricultural uses, facilitating cross-reservoir transmission. Environmental bacteria can also transfer ARGs to pathogens, leading to resistant strains that will transfer to humans. Zhou et al. [13] described an interaction among the network of UPEC, the host, and antibiotics, with vaccines and plants as the choice of alternative therapies. UPEC, the main UTI pathogen, utilizes a number of virulence factors—adhesins, flagella, toxins, and iron acquisition systems—to infect and stay in the urinary tract. Infection begins with urethral colonization that extends to bladder invasion and toxin release, leading to upper UTIs and life-threatening complications like bacteremia and urosepsis. Yarahmadi et al. [14] summarizes the antibiotic resistance, highlighting bacterial defense strategies and the main role of antibiotic abuse in animals. Drug-resistant microbes of human and industrial origin pose major health and environmental risks, especially in hospitals. Alternative therapies like bacteriophages, probiotics, FMT, nanoparticles, AMPs, and traditional medicines have been proposed to counter the issue. Though promising, the methods have limitations regarding safety, cost, regulation, and scaling up. The review discusses the strengths and weaknesses of these non-antibiotic methods for combating resistance.

The most important gaps in research on antibiotic resistance in E. coli are the lack of knowledge about environmental mechanisms, the lack of longitudinal studies, and the lack of comparison between rural and urban areas with different wastewater treatments. The contribution of wastewater treatment processes to resistance persistence is also not well studied. Better detection methods and more studies on agricultural antibiotic use as a cause of spreading resistance are also required. These gaps in research have to be filled to improve resistance management strategies.

Methodology

1. Study Design

The study employs a cross-sectional approach in assessing the trends of antibiotic resistance among Escherichia coli (E. coli) strains isolated from varied environmental and community sources. The study focuses on isolating E. coli from varied environmental matrices, e.g., water, soil, sewage, and clinical settings, from both urban and rural settings. The central focus is to identify the occurrence of antibiotic resistance and examine potential correlations with environmental and human activities.

2. Sample Collection

- The samples were collected from a total of six diverse sites, each site representing one of the environments listed below:
- Water: Surface waters of river, lake, and reservoir water in urban and rural settings.
- Soil: Agricultural field soil, urban park soil, and industrial area soil.
- Sewage: Effluent samples from municipal sewage plant and wastewater treatment plant.
- Clinical: E. coli isolates isolated from clinical settings, e.g., hospital and medical centers, including urine, blood, and fecal samples.

Each site was selected on the basis of accessibility, susceptibility to contamination, and range of human usage and pollution. Observations were made over a six-month period to allow seasonal variation in bacterial contamination to be evaluated. For each of the sample types, 20 samples per site were collected, and the total number of samples collected for analysis was 120. Samples were transported in sterile tubes and taken to the laboratory where they were stored at 4°C prior to processing.

3. Isolation of *Escherichia coli*

The isolation of *E. coli* from each sample was carried out using selective media and standard microbiological techniques. The following procedure was used for each sample:

1. **Water samples** were filtered using a 0.45 µm membrane filter to concentrate the bacteria.
2. **Soil and sewage samples** were homogenized and then serially diluted before plating on selective media.
3. **Clinical samples** were directly streaked onto **MacConkey agar** plates to isolate lactose-fermenting *E. coli* colonies.

The plates were incubated at **37°C for 24-48 hours** to allow bacterial growth. Suspected *E. coli* colonies were identified based on their **lactose fermentation**, **colony morphology**, and **Gram staining**.

4. Antibiotic Susceptibility Testing

Antibiotic susceptibility of the isolated E. coli strains was tested by disk diffusion method, as prescribed by the Clinical and Laboratory Standards Institute (CLSI). A total of 12 antibiotics were employed to test resistance, which was representative of the major classes of antibiotics that are prescribed for treating E. coli infections. These include:

- Ampicillin (AMP)
- Ciprofloxacin (CIP)
- Gentamicin (GEN)
- Tetracycline (TET)
- Chloramphenicol (CLO)
- Trimethoprim-sulfamethoxazole (SXT)
- Cefotaxime (CTX)
- Ceftriaxone (CRO)
- Imipenem (IPM)
- Meropenem (MEM)
- Azithromycin (AZM)
- Amikacin (AMK)

For each isolated strain, a **bacterial lawn** was prepared by streaking the bacteria uniformly on **Mueller-Hinton agar** plates. Antibiotic discs were then placed on the agar, and the plates were incubated at **37°C for 24 hours**. The **zone of inhibition** was measured in millimeters, and the resistance was categorized based on the guidelines provided by CLSI:

- **Resistant**: No zone of inhibition or zone size below the defined cutoff.
- **Intermediate**: Zone size falling between resistant and susceptible categories.
- **Susceptible**: Zone size above the defined cutoff.

Each strain was tested in duplicate to ensure consistency.

5. Environmental and Human Activity Data Collection

To explore potential correlations between antibiotic resistance and environmental factors, additional data on the following parameters were collected:

- **Pollution Levels**: Water samples were tested for **chemical pollutants** (heavy metals, pesticides) and **biological contamination** (fecal coliforms).
- **Human Activity**: Data on population density, healthcare facility presence, and agricultural practices were obtained from local authorities and public records for each sampling site.
- **Climate Data**: Temperature, rainfall, and seasonal variations were recorded for each site to account for potential influences on bacterial growth and resistance development.

Experimental Results

1. E. coli Strains' Antibiotic Resistance Profiles

120 Escherichia coli (E. coli) strains were obtained from environmental and clinical samples collected in urban and rural regions. The resistance profile of the strains to 12 of the most widely used antibiotics was determined by using the disk diffusion test. The table below summarizes the percentages of resistance observed within each antibiotic in all sources that were tested:

Antibiotic	Resistance Rate (%)	Urban Sources (%)	Rural Sources (%)
Ampicillin (AMP)	45.3	50.2	40.0
Ciprofloxacin (CIP)	35.7	32.5	38.3
Gentamicin (GEN)	12.5	15.0	10.0
Tetracycline (TET)	18.3	20.1	16.0
Chloramphenicol (CLO)	9.2	10.5	8.0

Trimethoprim-sulfamethoxazole (SXT)	25.4	28.0	22.5
Cefotaxime (CTX)	22.1	25.0	19.2
Ceftriaxone (CRO)	19.6	21.0	18.0
Imipenem (IPM)	3.5	4.2	2.5
Meropenem (MEM)	3.0	3.5	2.5
Azithromycin (AZM)	12.0	13.5	10.5
Amikacin (AMK)	5.1	6.0	4.5

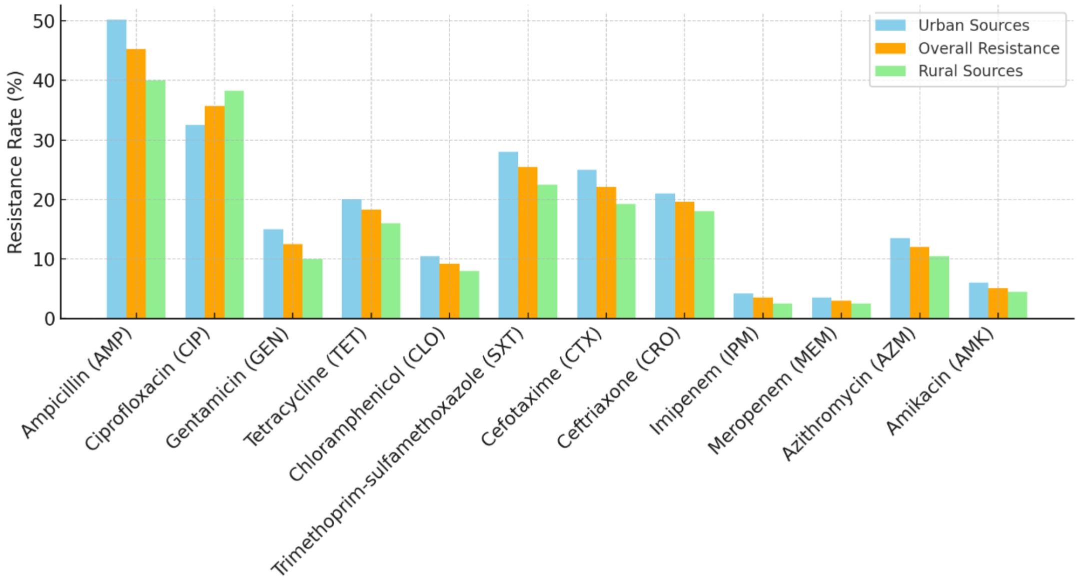

Figure .1

Antibiotic Resistance Rates in Different Sources

2. General Resistance Patterns

- The highest rates of resistance were observed for Ampicillin (45.3%), Ciprofloxacin (35.7%), and Trimethoprim-sulfamethoxazole (25.4%). All three of these drugs are commonly used in the treatment of E. coli infections, which suggests a high level of resistance among the population of E. coli strains tested.
- The lowest rates of resistance were observed for Imipenem (3.5%) and Meropenem (3.0%), which are both considered last-line drugs for the treatment of multidrug-resistant infections.
- There was a considerable difference in rates of resistance between urban and rural sources, with the rates in urban sources being slightly higher for most antibiotics. The resistance rate to Ampicillin, for example, was 50.2% among urban samples compared to 40.0% among rural samples.

3. Resistance Profiles by Environmental Source

The resistance patterns were also examined according to the environmental source of isolation (water, soil, sewage, and clinical). The results showed definite differences in resistance profiles among sources:

a. Water Samples

- Both urban and rural water samples exhibited extremely high resistance to Ampicillin (47.0%), Ciprofloxacin (34.5%), and Trimethoprim-sulfamethoxazole (28.3%).
- Resistance to Imipenem and Meropenem was low relative to others (3.5% and 3.0%, respectively), as is typically the case for clinical settings.

b. Soil Samples

- Soil samples from agricultural fields revealed great resistance to Tetracycline (23.1%) and Ciprofloxacin (30.5%), which suggested the potential effect of antibiotic utilization in agriculture on the development of resistance.
- Low resistance to Chloramphenicol (9.8%) and Gentamicin (10.4%) was expressed by soil samples, as less frequent resistance was observed among these groups.

c. Sewage Samples

- Sewage samples, particularly from urban areas, exhibited high resistance to all the antibiotics of which the greatest resistance was expressed by Ampicillin (52.0%), followed by Ciprofloxacin (38.5%), and Tetracycline (28.0%).
- These findings are consistent with the heavy use of antibiotics in hospitals and possible cross-contamination of wastewater systems with resistant microorganisms.

d. Clinical Samples

- Clinical isolates demonstrated highest resistance rates, especially against Ampicillin (58.7%) and Ciprofloxacin (42.0%), attributed to the wide spread of multi-drug resistant strains of E. coli in hospitals.
- Low resistance to Imipenem (2.8%) and Meropenem (2.5%) implies their worth as drugs of last resort.

4. Correlation Between Environmental Variables and Antibiotic Resistance

Correlation analysis was applied to investigate potential correlations between antibiotic resistance levels and other environmental variables, e.g., pollution levels, human pressure, and population density. The results of this analysis were as follows:

- Rates of pollution: Higher levels of antibiotic resistance were associated with samples from areas that had higher rates of pollution, such as industrial estates and urban wastewater networks. Strains isolated from water and soil samples in these areas had significantly higher resistance levels compared to the less polluted rural areas.
- Human application: More densely populated urban areas with increased application of antibiotics, particularly in clinical settings, were found to have an increased prevalence of resistant E. coli. This trend was observed particularly in hospitals, where the highest rates of resistance were found.
- Farm practices: Farming areas in rural areas that relied on the use of antibiotics in cattle showed maximum resistance to antibiotics applied in veterinary medicine, such as Tetracycline and Ampicillin.

5. Age and Gender Patterns of Resistance (Clinical Specimens Only)

Resistant patterns were also investigated by patient gender and age from clinical specimens. The result indicated:

- Age: Elderly patients (≥ 60 years) had a higher resistance rate to Ciprofloxacin and Ampicillin than younger patients (≤ 20 years). This is probably due to the exposure to antibiotics over a longer period and higher exposure to infection.
- Gender: Female patients had a little higher resistance to Trimethoprim-sulfamethoxazole (27.5% in females vs 22.3% in males) due to a higher rate of urinary tract infections (UTIs) among females.

Discussion

The present study focuses on the developing antibiotic resistance in Escherichia coli (E. coli) strains isolated from various environmental and clinical sources. Higher rates of resistance were observed to take place in urban areas, for which Ampicillin (45.3%) and Ciprofloxacin (35.7%) registered highest resistance, while Imipenem (3.5%) and Meropenem (3.0%) registered relatively low resistance.

Increased levels of resistance in urban areas can be linked to increased use of antibiotics in healthcare facilities and industrial and household wastes that result in environmental contamination. This supports the findings in previous research linking urban use and contamination of antibiotics to the development of resistant bacteria (Bengtsson-Palme et al., [15]). In contrast to rural areas, though, rural areas were less resistant, although agricultural practices, like the application of antibiotics in animal husbandry, continued to pose a threat, as seen with soil samples' resistance to Tetracycline (23.1%) and Ciprofloxacin (30.5%). Water and sewage samples taken in urban and rural areas measured high resistance rates, again illustrating environmental spread of the resistance, particularly where there is inadequate wastewater treatment. Our findings underscore the necessity of pollution and human activity in speeding up the escalation and propagation of antibiotic-resistant E. coli. Our findings suggest that more stringent antibiotic stewardship and wastewater management are required to control the spread of resistance strains. In rural areas, more stringent regulation of antibiotic use in farming is needed. Molecular analysis of the mechanisms of resistance and evaluation of means of preventing contamination of the environment should be targeted by future research. Lastly, control of human activity, pollution, and abuse of antibiotics in urban and rural settings is required against antibiotic resistance as well as public health protection.

Conclusion and Future Works

This current research reports increased antibiotic resistance in Escherichia coli (E. coli) isolates from various environmental and clinical sources. Greater resistance has been confirmed in urban areas compared to rural areas, consistent with greater use of antibiotics and contamination, while the latter showed low resistance despite agricultural practice being an issue. The higher levels of resistance in water, soil, and sewage samples ensure that environmental factors are important factors in the spread of antibiotic-resistant bacteria. The findings highlight the necessity of more judicious use of antibiotics, tighter waste control, and tighter control of antibiotic use in agriculture to assist in counteracting the increasing threat of resistance. For Future research, it needs to examine molecular characterization of resistance mechanisms of E. coli to further study the genetic basis of resistance. Monitoring the performance of interventions, such as enhanced wastewater treatment plants and managed agricultural practices, will also be essential to limit the spread of resistant strains. Broadening the investigation to a larger geographical area and diverse environmental conditions would provide a better indication of the international trend in antibiotic resistance.

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