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Antibiotic Susceptibility Pattern of Escherichia Coli at Tertiary Care Hospital, Islamabad

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ABSTRACT

Objectives: The objectives of this study are; 1. To comprehensively investigate the antibiotic susceptibility pattern of Escherichia coli, focusing on its virulence factors to understand its pathogenicity and address the challenges posed by antibiotic resistance.
2. To assess the prevalence of citrate and TSI in E. coli isolated from urine to evaluate the impact of antibiotics according to Clinical and Laboratory Standards Institute (CLSI 2023) guidelines.
Study Design: Cross-sectional study.
Study Place: Tertiary Care Hospital, Al Nafees Medical Hospital, Islamabad, Pakistan.
Study Duration: March 2023 to October 2023.
Methodology: Informed consent was obtained from the microbiology lab of Al Nafees Medical College. The study utilized biochemical tests, including citrate and TSI, for identification and differentiation of E. coli based on metabolic characteristics. A total of 50 urine samples were collected for isolation and identification of E. coli. The Kirby-Bauer disc diffusion technique was employed to determine antibiotic susceptibility, identifying nitrofurantoin and Fosfomycin as the most effective choices for bacterial infections.
Results: Antibiotic efficacy assessments revealed nitrofurantoin as notably effective, surpassing other antibiotics according to CLSI guidelines. In contrast, ciprofloxacin demonstrated the lowest efficacy among the tested antibiotics. The research highlights the importance of judicious antibiotic selection for optimal therapeutic outcomes in clinical settings, emphasizing the need for continuous monitoring and optimization of antibiotic treatment strategies.
Conclusion: The findings underscore nitrofurantoin's efficacy and advocate for vigilant monitoring to optimize treatment strategies, offering valuable insights for improved clinical outcomes.

INTRODUCTION

Bacteria are single-celled microorganisms that inhabit nearly all environments on Earth, presenting a diversity of sizes and shapes, such as spheres, rods, and spirals. They are categorized based on various characteristics, including their Gram stain reaction, which indicates cell wall composition. Bacteria can be both beneficial and harmful to humans and other organisms (Zhang et al., 2023).

While some bacteria are used in food production, medicine, and other industries, others cause diseases like strep throat, tuberculosis, and Lyme disease. Researching bacteria's roles in different environments and their biotechnological potential is crucial (Viegas et al., 2020).

Gram-negative bacteria, a subgroup distinguished by their unique cell wall structure, appear pink or red when stained with Gram stain (Beveridge, 1999).

These bacteria are found in diverse settings, including soil, water, and the human body. Examples include Salmonella, Escherichia coli (E. coli), and Pseudomonas aeruginosa. Pathogenic Gram-negative bacteria can cause a range of diseases, from minor infections to severe conditions, and are often resistant to antibiotics, complicating treatment (Li et al., 2015).

Escherichia coli (E. coli) is a Gram-negative bacterium commonly present in the intestines of humans and animals. Most E. coli strains are harmless or beneficial, but some can be pathogenic, causing symptoms such as diarrhea, abdominal pain, and fever (Croxen et al., 2013). Severe cases can lead to dehydration, kidney failure, and other complications. E. coli outbreaks often occur in hospitals, nursing homes, and childcare centers and are typically spread through contaminated food or water (Gorbach et al., 1975). Preventing E. coli infections requires proper food handling, hygiene practices, and cooking food to the appropriate temperatures (Ebert, 2018).

E. coli is the leading cause of urinary tract infections (UTIs). When E. coli enters the urinary tract, it can adhere to the bladder and urethra linings, causing infection. Symptoms of an E. coli-related UTI include lower abdominal pain, frequent urination, and painful urination (Idayat, 2015). If the infection spreads to the kidneys, it can cause more severe symptoms such as fever, chills, and back pain. UTIs caused by E. coli are typically treated with antibiotics, and completing the entire course of treatment is essential to prevent recurrence (Goulart, 2021). Drinking plenty of water and urinating frequently can help flush out bacteria and prevent further infection.

E. coli biofilms, which are complex communities of bacteria protected by a matrix of extracellular polymeric substances, can cause persistent UTIs by adhering to surfaces in the urinary tract, such as catheters and kidney stones. These biofilms shield bacteria from the immune system and antibiotics (Ramírez-Larrota & Eckhard, 2022). E. coli produces virulence factors, such as fimbriae, that aid in adherence to host cells and tissues, promoting colonization and evasion of the immune system, leading to chronic infections (Totsika et al., 2012).

The antibiotic susceptibility pattern of E. coli varies based on the strain and infection site. Generally, antibiotics such as fluoroquinolones (e.g., ciprofloxacin, levofloxacin), trimethoprim-sulfamethoxazole, and nitrofurantoin are effective against E. coli strains causing UTIs (Johnson et al., 2008). However, the rise in antibiotic resistance complicates treatment, requiring alternative or combination therapies (Zalewska-Piątek & Piątek, 2020). Adhering to prescribed treatment protocols is vital to ensure the complete eradication of the infection.

E. coli's susceptibility to antibiotics can be categorized as susceptible, intermediate, or resistant. Antibiotics such as amoxicillin, ampicillin, and cephalosporins are often less effective due to high resistance levels. In contrast, trimethoprim-sulfamethoxazole, nitrofurantoin, and fluoroquinolones like levofloxacin and ciprofloxacin are generally effective, though resistance is increasing (Ortega et al., 2009). Consulting a healthcare professional is essential to select the most appropriate medication for treating E. coli-related UTIs.

Antibiotics targeting E. coli work by disrupting the bacterial cell's DNA, cell wall, or protein synthesis machinery. For example, trimethoprim-sulfamethoxazole inhibits folic acid synthesis, crucial for bacterial growth and survival. Nitrofurantoin damages bacterial DNA, preventing reproduction and infection spread. Fluoroquinolones, such as ciprofloxacin and levofloxacin, inhibit bacterial enzymes involved in DNA replication and repair, killing the bacteria (Abdu et al., 2018). By targeting these critical bacterial functions, antibiotics effectively eliminate E. coli and treat infections caused by this bacterium (Allison & Lambert, 2024).

E. coli can cause infections in various body parts, including the bloodstream, lungs, and abdominal cavity, beyond the urinary tract. The symptoms of an E. coli infection vary depending on the infection site and severity. For example, bacteremia (E. coli in the bloodstream) can cause fever, chills, and low blood pressure, while pneumonia (E. coli in the lungs) can cause coughing, shortness of breath, and chest pain. Severe cases can lead to life-threatening complications such as sepsis and organ failure (Breijyeh et al., 2020).

Due to its frequent occurrence in the gastrointestinal tracts of humans and animals, E. coli is involved in various metabolic processes, including vitamin production and the breakdown of complex compounds (Bader et al., 2016). E. coli contributes to maintaining a balanced gut microbiome by competing with pathogenic bacteria for nutrients and producing antibiotic substances that inhibit harmful bacteria growth (Holmes et al., 2021). Additionally, E. coli assists in degrading complex carbohydrates (Debnath et al., 2022).

While most E. coli strains are harmless or beneficial, some can cause various diseases. Pathogenic E. coli strains produce virulence factors like adhesins, toxins, and invasins, enabling them to invade and damage host tissues. For instance, some E. coli strains produce Shiga toxin, which can severely damage the intestinal lining and cause complications such as hemolytic uremic syndrome (HUS), potentially fatal (Bányai et al., 2023).

Rationale of Study

Urinary tract infections (UTIs) caused by Escherichia coli (E. coli) present a significant global health challenge, demanding a nuanced and comprehensive approach to address both its pathogenicity and the escalating concern of antibiotic resistance. This study aims to delve into the intricate interplay between E. coli's virulence factors and antibiotic susceptibility patterns, shedding light on essential aspects of its behavior and the challenges posed by antimicrobial resistance. The scientific rationale underlying this investigation is grounded in the following key aspects:

The study employs a robust methodology aligned with the Clinical and Laboratory Standards Institute (CLSI) guidelines for the year 2023. This ensures not only the scientific rigor of the research but also its direct applicability to clinical settings, where adherence to standardized procedures is paramount.

The identification of Nitrofurantoin as notably effective, coupled with the revelation of lower efficacy with Ciprofloxacin, underscores the importance of judicious antibiotic selection. The study calls for vigilance in monitoring and optimizing antibiotic treatment strategies, contributing essential knowledge for achieving optimal therapeutic outcomes in clinical settings.

Conducted in a tertiary care hospital in Islamabad, Pakistan, the study reflects the local context, acknowledging the specific challenges and characteristics of bacterial infections in this region. This geographical specificity enhances the study's relevance and potential impact on regional healthcare practice.

Material AND Methodology

This study utilized a prospective, cross-sectional design to assess antibiotic susceptibility patterns. The research was conducted in the Microbiology Department of Al-Nafees

Medical College, Isra University Islamabad campus. The study was carried out from September 26, 2023, to January 31, 2024. A total of 350 urine samples were included in the study. A non-probability convenient sampling technique was employed.

Inclusion Criteria: Only urine samples were included in the study.

Exclusion Criteria: All other types of samples were excluded.

Operational Definitions

Antibiotic Susceptibility Testing: A procedure to measure the susceptibility of bacteria to antibiotics.

Resistance: The natural process by which bacteria become resistant to antibiotics over time, often due to genetic changes.

Sensitivity: The degree to which bacteria respond to the effects of antibiotics.

Materials

CLED Agar Plates: Prepared in-house, these plates facilitated the growth of urinary tract pathogens like E. coli while inhibiting contaminating bacteria. The composition typically included peptones, lactose, agar, and various electrolytes.

Antibiotics: The study followed CLSI 2022 guidelines, using a range of antibiotics, including penicillin, cephalosporin, nitrofurantoin, ciprofloxacin, and tetracycline.

Antibiotic Disks: Disks impregnated with known concentrations of antibiotics were placed on the agar surface to allow diffusion.

Incubator: The inoculated agar plates were incubated at a specified temperature range.

Zone Diameter Measurement Device: Post-incubation, the diameter of the inhibition zones around each antibiotic disk was measured using a caliper or specialized measuring tools.

Methodology

Ethical Considerations: The study was approved by the ethical committee of Al-Nafees Medical College, Isra University Islamabad campus.

Data Protection: Collected data was stored on a password-protected personal computer to ensure confidentiality.

Collection of Specimen: The study was conducted in the Microbiology Department of Al-Nafees Medical College and Hospital, Islamabad, from September 2023 to December 2023.

Sample Collection: Samples were collected from both male and female patients using a non-probability convenient sampling technique.

CLED Agar Preparation: Ingredients included CLED agar powder, distilled water, and specific equipment like autoclaves and pH meters. The agar was prepared, sterilized, cooled, and poured into sterile petri dishes under aseptic conditions. Urine samples were inoculated on CLED agar and incubated at 37°C for 24 hours. Biochemical tests were conducted for bacterial confirmation.

Biochemical Tests: Citrate Test was utilized to identify species of *E. coli* and these bacterial colonies were streaked onto a citrate agar slant and incubated at 35–37°C for 18–24 hours. A color change from green to blue indicated a positive result. After that, TSI (Triple Sugar Iron) Test was used to identify enteric bacteria by their ability to ferment specific sugars, produce gas, and generate hydrogen sulfide. Bacteria were inoculated on TSI agar slants and incubated at 35–37°C for 18–24 hours. Post-incubation, reactions such as color changes, gas production, and H2S production were observed.

Then antibiotic susceptibility testing was applied using, Kirby-Bauer Disc Diffusion Technique. Pure bacterial cultures were grown on appropriate agar media. Bacteria were transferred to a saline solution or broth, adjusted to match the turbidity of a 0.5 McFarland standard. Mueller-Hinton agar was poured into sterile petri dishes. Bacterial suspensions were spread uniformly across the agar surface. Discs with specific antibiotic concentrations were placed on the agar surface, ensuring proper spacing, and plates were incubated at 35–37°C for 16–18 hours. Post-incubation, inhibition zones were measured, and their diameters compared to CLSI interpretive standards to determine bacterial sensitivity or resistance.

RESULTS

Our study demonstrated that Nitrofurantoin, belonging to the Nitrofuran class, exhibited the highest efficacy among the tested antibiotics according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for 2023. Nitrofurantoin showed the most significant effectiveness, with inhibition zones present in 268 out of 350 samples. This highlights its potential as a preferred treatment option for bacterial infections, as depicted in Graph 1. Nitrofurantoin achieved an impressive 84% efficacy, indicating its superior ability to treat the targeted conditions compared to other antibiotics.

In our current research, Fosfomycin, a member of the Phosphonic antibiotics class, demonstrated the second highest efficacy among all tested antibiotics, based on the CLSI 2023 guidelines. Fosfomycin showed inhibition zones in 260 out of 350 samples, further supporting its effectiveness, as shown in Graph 1. This significant efficacy positions Fosfomycin as a strong alternative to Nitrofurantoin for treating bacterial infections.

Conversely, Ciprofloxacin, chosen based on the CLSI 2023 guidelines, exhibited the lowest efficacy in our study. Ciprofloxacin showed inhibition zones in only 52 out of 350 samples, marking an efficacy rate of 30%. This finding underscores Ciprofloxacin's limited effectiveness in treating the tested samples, emphasizing the necessity to monitor antibiotic efficacy continuously. The observed low performance of Ciprofloxacin suggests potential challenges in using it as a primary antibiotic choice, necessitating further research to understand the reasons behind its comparatively lower effectiveness. This insight is crucial for optimizing antibiotic treatment strategies to ensure better therapeutic outcomes.

Additionally, our study found that Ceftriaxone, belonging to the Cephems class, showed the second least efficacy among all tested antibiotics. Ceftriaxone exhibited inhibition zones in 298 out of 50 samples, which translates to an efficacy rate of 76%, as depicted in Graph 2. This relatively lower efficacy suggests that Ceftriaxone may be less preferable for treating bacterial infections compared to other antibiotics tested. The findings highlight the importance of selecting antibiotics based on their efficacy profiles to achieve optimal treatment outcomes in clinical settings.

The results underscore the necessity of considering alternative antibiotics with higher success rates, especially when dealing with infections where Ciprofloxacin and Ceftriaxone demonstrate limited effectiveness. The study highlights the importance of judicious antibiotic selection to enhance therapeutic outcomes, reduce the risk of antibiotic resistance, and ensure effective infection management in clinical practice.

DISCUSSION

The sensitivity testing results demonstrate that E. coli is highly susceptible to nitrofurantoin, suggesting its potential effectiveness in treating E. coli infections. Nitrofurantoin's mechanism likely targets specific bacterial cell components, inhibiting or destroying the pathogen, which is crucial given the rise in antibiotic resistance. Careful use is imperative to maintain its efficacy. Our findings are consistent with previous research showing high sensitivity rates of E. coli to nitrofurantoin (90%) among 350 isolates, with significant resistance observed for ampicillin (2%), ciprofloxacin (15%), and others(Swetha et al., 2022).

Fosfomycin also displayed high sensitivity to E. coli, positioning it as a strong candidate for treating E. coli-related infections. Its effectiveness can reduce the reliance on other antibiotics, thereby mitigating resistance issues. Studies involving 3044 E. coli isolates revealed fosfomycin susceptibility at 97.6%, underscoring its reliability in clinical use(Saeed et al., 2021).

Amikacin was effective against E. coli, supporting its use for treating infections caused by this bacterium. The study indicated that amikacin, ciprofloxacin, and other antibiotics could effectively treat E. coli and K. pneumoniae infections (Ezechukwu, 2019).

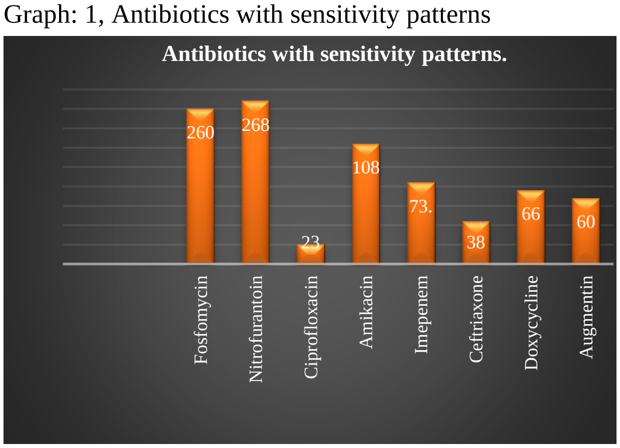
However, the findings highlight concerning resistance patterns. Ciprofloxacin resistance in E. coli, noted in this study, raises significant public health concerns due to its widespread use. Previous research supports this, showing high resistance levels for ampicillin (76.6%), tetracycline (68.4%), and others (Afema et al., 2019). Similarly, ceftriaxone resistance suggests the need for alternative treatments and highlights the genetic adaptability of bacteria (Gelaw et al., 2022). Amoxicillin resistance further underscores the pressing issue of antibiotic misuse and the necessity for judicious antibiotic stewardship (Musa et al., 2020).

CONCLUSION

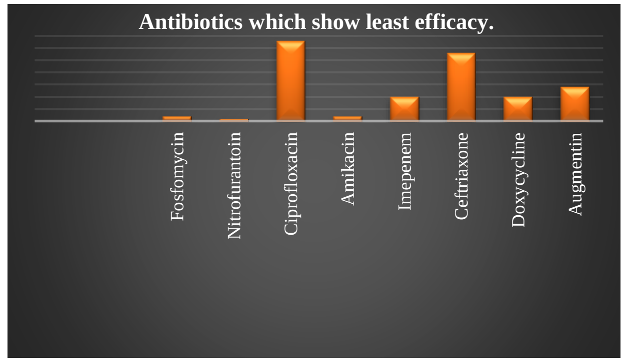
According to the investigations, nitrofurantoin is sensitive to E. coli (90% sensitivity), making it a possible treatment choice. E. coli is also susceptible to fosfomycin, opening up additional therapeutic possibilities. Amikacin demonstrates E. coli sensitivity, whereas ciprofloxacin and ceftriaxone demonstrate resistance, raising questions about potential future therapies. Additionally, the fact that amoxicillin is now ineffective against E. coli emphasizes the importance of using antibiotics responsibly in order to effectively treat bacterial infections and stop the spread of resistance. Maintaining public health still depends on combating antibiotic resistance.

Graphs

Graph: 1, Antibiotics with sensitivity patterns



Graph: 2, Antibiotics which show least efficacy



Figures

Figure III-1 Antibiotics on Antibiotic Disc

Figure III-2 Growth of E.coli on CLED Agar

Figure III-3 *E.coli* growth on CLED agar

Figure III-4 Results of Citrate Test

Figure III-5 Interpretation of TSI Test

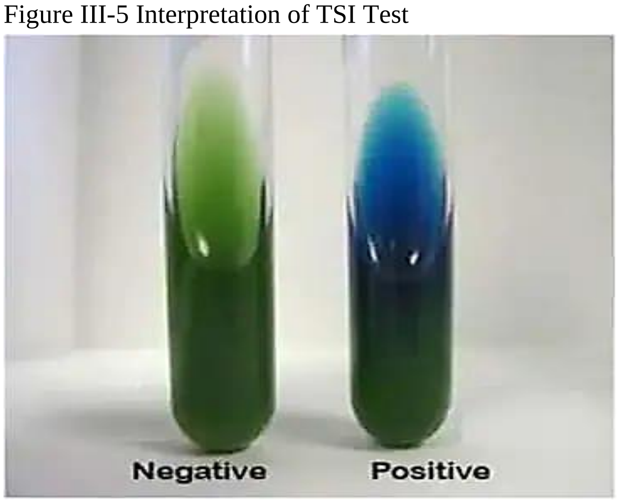
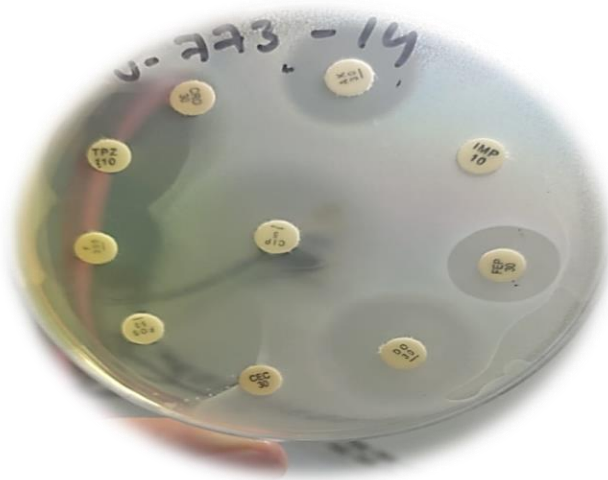


Figure III-6 Placing of Antibiotics

Figure III-7 Zones of inhibition of E.coli



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