

Isolation, Identification, and Characterization of Bacteria from Urinary Tract Infections and Their Effect on Different Antibiotics

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ABSTRACT

Background: Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide, with *Escherichia coli* and *Klebsiella* spp. being the leading causative agents. The increasing emergence of antibiotic resistance in uropathogens has complicated empirical treatment strategies, necessitating continuous surveillance of resistance trends. This study aims to isolate, identify, and characterize bacterial strains from UTI patients, analyze their antibiotic susceptibility patterns, and explore machine learning-based predictive models for resistance classification.

Methods: A descriptive cross-sectional study was conducted at Konaseema Institute of Medical Sciences and Research Foundation, Amalapuram, over a one-year period (June 2023 – July 2024). A total of 1,720 urine samples were analyzed, of which 624 (36.2%) showed significant bacterial growth. Bacterial isolates were identified using standard microbiological techniques, including culture on Blood Agar, MacConkey Agar, and CLED Agar, Gram staining, and biochemical testing. Antibiotic susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method, following CLSI guidelines. Statistical analysis included Chi-Square tests to assess associations between bacterial species and resistance patterns, and a Random Forest classification model to predict resistance trends based on susceptibility profiles.

Results: Among the 624 culture-positive samples, *E. coli* (45.7%) was the most prevalent uropathogen, followed by *Klebsiella* spp. (19.9%), *Staphylococcus aureus* (13.6%), and *Pseudomonas aeruginosa* (7.4%). Antibiotic resistance rates were highest among Non-Fermenters, particularly against β -lactam antibiotics. Enterobacterales exhibited significant resistance to third-generation cephalosporins and fluoroquinolones, whereas Gram-Positive Cocci demonstrated variable resistance patterns, notably against β -lactams and macrolides.

Chi-Square analysis revealed no statistically significant association ($p > 0.05$) between bacterial species and antibiotic resistance patterns, suggesting that resistance trends may be influenced by factors beyond species classification. The Random Forest model achieved an AUC of 1.00, demonstrating excellent discriminatory power in predicting bacterial classification based on resistance profiles. Ceftazidime-Avibactam, Levofloxacin, and Piperacillin-Tazobactam were identified as the most influential antibiotics in resistance prediction.

Conclusion: This study highlights the high prevalence of multidrug-resistant uropathogens, particularly among Non-Fermenters and Enterobacterales, reinforcing the need for real-time susceptibility testing and antimicrobial stewardship programs. The lack of significant species-resistance association emphasizes that predicting antibiotic resistance requires broader epidemiological and molecular analyses rather than relying solely on bacterial species. The successful application of machine learning (Random Forest) in resistance prediction presents a promising approach for future antimicrobial resistance surveillance. Further validation on larger datasets is recommended to enhance predictive accuracy and clinical applicability.

Keywords: Urinary tract infections, Antibiotic resistance, antimicrobial stewardship, Random Forest model, Machine learning, Empirical therapy, Multidrug resistance.

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, affecting millions of individuals annually and posing a significant burden on healthcare systems (1). The etiology of UTIs is predominantly bacterial, with *Escherichia coli* (*E. coli*) being the most frequent causative pathogen, particularly in community-acquired UTIs (2). However, in recent years, other uropathogens such as *Klebsiella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus* spp. have emerged as significant contributors, especially in complicated and nosocomial UTIs (3).

The increasing antibiotic resistance among uropathogens has complicated the management of UTIs, leading to treatment failures and prolonged hospital stays (4). The rise of extended-spectrum β -lactamases (ESBLs), carbapenem-resistant Enterobacterales (CRE), and multidrug-resistant (MDR) pathogens has significantly reduced the efficacy of empirical antibiotic therapy, making resistance surveillance a critical component of infection management (5). As a result, there is an urgent need to monitor resistance patterns, optimize treatment strategies, and establish updated antibiograms to guide clinicians in selecting appropriate empirical therapy.

The identification and characterization of uropathogens remain essential in understanding the local epidemiology of UTI-causing bacteria. Traditional culture-based methods, Gram staining, and biochemical tests continue to be gold-standard techniques for bacterial identification, but emerging molecular methods, such as PCR-based detection and whole-genome sequencing, offer more precise identification and resistance profiling (6). However, resource limitations in many clinical laboratories restrict the widespread use of molecular techniques, making conventional microbiological methods indispensable.

Antibiotic susceptibility testing (AST) plays a crucial role in determining the appropriate treatment options for UTIs. The Kirby-Bauer disk diffusion method, based on Clinical and Laboratory Standards Institute (CLSI) guidelines, remains the most widely used AST technique due to its cost-effectiveness and reliability. However, given the rapid evolution of resistance mechanisms, there is a growing interest in using machine learning models and artificial intelligence to predict resistance trends and guide personalized antibiotic selection.

This study aims to isolate, identify, and characterize bacterial pathogens from UTI patients, assess their antibiotic susceptibility profiles, and explore predictive models for resistance trends. The findings will contribute to a better understanding of regional antimicrobial resistance patterns and help improve antimicrobial stewardship programs to combat multidrug-resistant UTIs.

AIMS AND OBJECTIVES

Aim

This study aims to isolate, identify, and characterize bacterial pathogens from urinary tract infection (UTI) cases and evaluate their antibiotic susceptibility patterns to better understand resistance trends and guide empirical treatment strategies.

Objectives

1. **Isolation and Identification:** To isolate and identify bacterial strains from urine samples of patients diagnosed with UTIs using standard microbiological techniques, including culture methods and biochemical tests.
2. **Characterization:** To characterize the isolated bacterial strains based on their morphological, biochemical, and physiological properties to determine their clinical significance.
3. **Antibiogram Analysis:** To determine the antibiotic susceptibility profiles of bacterial isolates using the Kirby-Bauer disk diffusion method, following CLSI guidelines, and assess their resistance patterns using statistical and machine-learning models.

MATERIALS AND METHODS

Study Design and Setting

This study is a descriptive cross-sectional study conducted at Konaseema Institute of Medical Sciences and Research Foundation, Amalapuram, over a period of one year (June 2023 – July 2024). The study aimed to isolate, identify, and characterize bacterial pathogens from urinary tract infection (UTI) cases and evaluate their antibiotic susceptibility patterns.

Study Population and Sample Collection

Patients presenting with clinically suspected UTIs were recruited for the study following institutional ethical clearance. Mid-stream urine samples were collected from these patients in sterile, leak-proof containers using standard aseptic

techniques. Samples were transported to the microbiology laboratory within [Specify timeframe] and processed immediately to prevent bacterial overgrowth or contamination.

Bacterial Isolation and Identification

Urine samples were cultured on Blood Agar, MacConkey Agar, and Cysteine Lactose Electrolyte-Deficient (CLED) Agar and incubated at 37°C for 24–48 hours. Bacterial growth was assessed, and significant isolates were subjected to morphological characterization, Gram staining, and biochemical identification tests. The biochemical tests performed included catalase, oxidase, indole, urease, citrate utilization, and sugar fermentation tests, following standard microbiological protocols.

Antibiotic Susceptibility Testing

The antibiotic susceptibility of bacterial isolates was determined using the Kirby-Bauer disk diffusion method, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. A panel of antibiotics, including β -lactams, fluoroquinolones, aminoglycosides, and carbapenems, was tested. The diameter of inhibition zones was measured, and susceptibility or resistance was classified based on CLSI interpretive breakpoints.

Statistical Analysis

Data were analyzed using statistical software (SPSS, R, or Python). The prevalence of bacterial species and their antibiotic resistance profiles were determined. The Chi-square test was used to assess the association between bacterial species and antibiotic resistance patterns. Since logistic regression was deemed unsuitable due to data distribution, a Random Forest classification model was applied to evaluate the predictive value of resistance trends.

Ethical Considerations

Ethical approval for the study was obtained from the Institutional Ethics Committee at Konaseema institute of Medical Sciences and Research Foundation, Amalapuram. Informed consent was obtained from all participants before sample collection. Strict biosafety protocols were followed during the handling of bacterial isolates to minimize the risk of laboratory-acquired infections.

RESULTS

Distribution of Bacterial Isolates in UTI Samples

A total of 1,720 urine samples were analyzed in this study, of which 624 (36.2%) showed significant bacterial growth. The distribution of bacterial isolates among culture-positive samples is summarized in Table 1.

Among the isolated pathogens, *Escherichia coli* (*E. coli*) was the most prevalent organism, accounting for 45.7% (n=285) of UTI cases, followed by *Klebsiella* spp. (19.9%), *Staphylococcus aureus* (13.6%), and *Pseudomonas aeruginosa* (7.4%). Other isolates included Coagulase-negative Staphylococci (CONS), *Enterobacter* spp., *Acinetobacter* spp., *Proteus* spp., and *Streptococcus* spp., each contributing to a smaller proportion of the infections. The distribution of these isolates is visually represented in Figure 1 (bar chart) and Figure 2 (pie chart).

These findings highlight the predominance of Gram-negative bacteria, particularly *E. coli* and *Klebsiella* spp., as the leading causative agents of UTIs. Gram-positive organisms, including *S. aureus* and *Streptococcus* spp., were less frequently isolated.

Table 1: Distribution of Bacterial Isolates in UTI Samples

Bacterial Species	Number of Isolates (n)	Percentage (%)
<i>Escherichia coli</i> (<i>E. coli</i>)	285	45.7%
<i>Klebsiella</i> spp.	124	19.9%
<i>Staphylococcus aureus</i> (<i>S. aureus</i>)	85	13.6%
<i>Pseudomonas aeruginosa</i>	46	7.4%
Coagulase-negative Staphylococci (CONS)	33	5.3%
<i>Enterobacter</i> spp.	20	3.2%
<i>Acinetobacter</i> spp.	11	1.8%
<i>Proteus</i> spp.	15	2.4%
<i>Streptococcus</i> spp.	5	0.8%
Total	624	100%

Figure1: Distribution Of Bacterial Isolates in UTI Samples

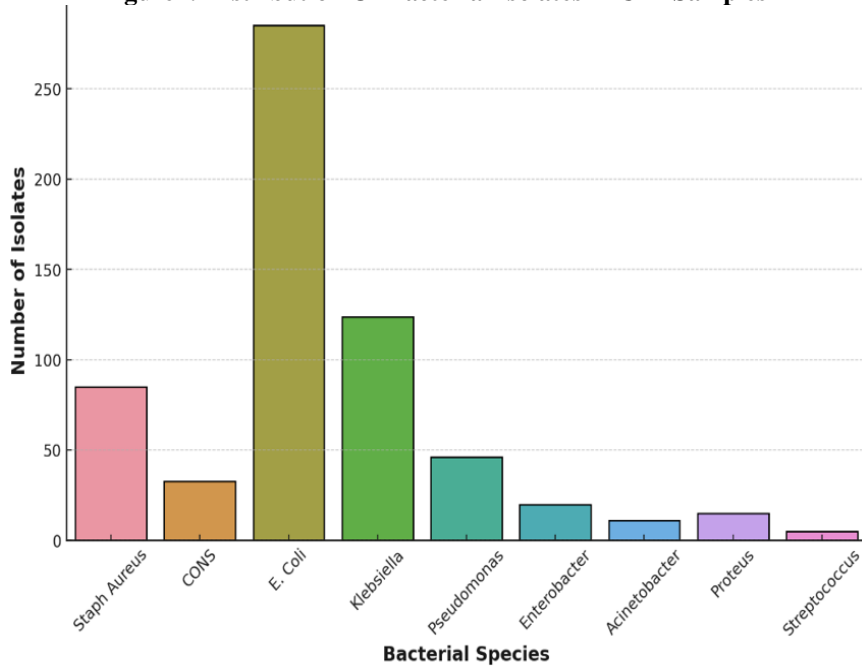
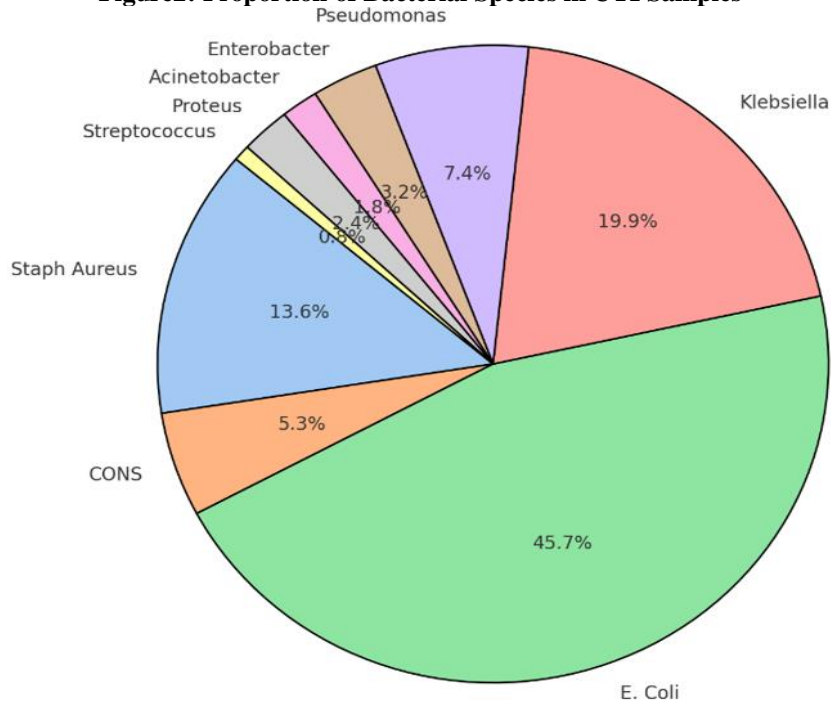


Figure2: Proportion of Bacterial Species in UTI Samples



Antibiotic Susceptibility Patterns

Resistance Rates Across Bacterial Groups

Antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion method, following CLSI (Clinical and Laboratory Standards Institute) guidelines. The resistance rates for different bacterial groups are summarized in Table 2.

Overall, non-fermenters exhibited the highest resistance rates, particularly against β -lactam antibiotics. Enterobacterales demonstrated high resistance to third-generation cephalosporins and fluoroquinolones, whereas Gram-Positive Cocci exhibited variable resistance patterns, with high resistance to beta-lactams and macrolides.

Table 2: Antibiotic Resistance Rates Among Different Bacterial Groups (%)

Antibiotic	Enterobacterales	Non-Fermenters	Gram-Positive Cocci
Amoxiclav	31%	IR	60%
Ampicillin-Sulbactam	17%	40%	-
Ceftazidime	26%	14%	31%
Ceftriaxone	26%	IR	31%
Levofloxacin	-	51%	25%
Tetracycline	-	25%	16%
Gentamycin	-	52%	60%
Co-trimoxazole	49%	IR	70%
Piperacillin-Tazobactam	59%	60%	-
Cefoperazone-Sulbactam	61%	33%	-
Ceftazidime-Avibactam	58%	27%	-
Meropenem	78%	59%	-
Minocycline	78%	26%	86%
Nitrofurantoin	78%	-	80%
Norfloxacin	32%	38%	32%

IR = Intermediate Resistance; - = Not Tested

Figure 3 (Stacked Bar Chart) Compares resistance frequencies among Enterobacterales, Non-Fermenters, and Gram-Positive Cocci.

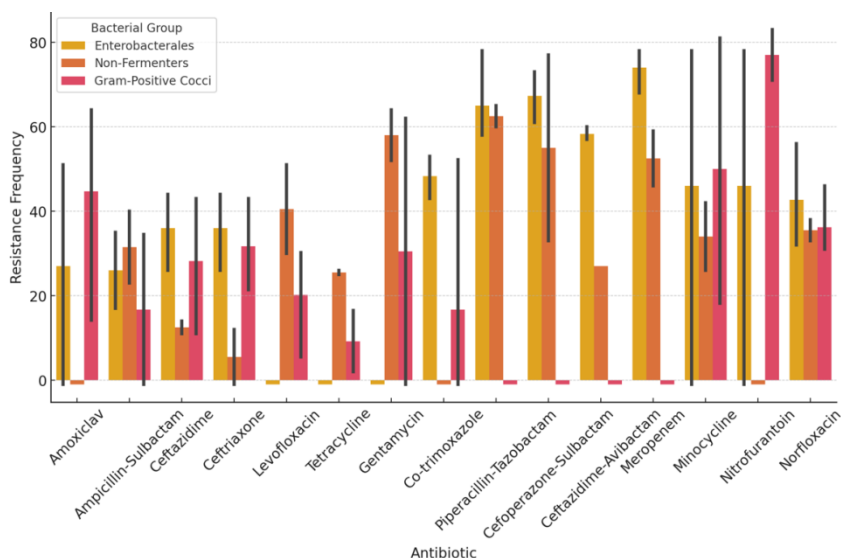


Table 3: Chi-Square Test Results (Association Between Bacterial Species and Antibiotic Resistance)

Antibiotic	Chi-Square Value (χ^2)	p-Value	Degrees of Freedom (DOF)
Amoxiclav	9	0.342	8
Ampicillin-Sulbactam	0	1.000	0
Ceftazidime	0	1.000	0
Ceftriaxone	0	1.000	0
Levofloxacin	9	0.342	8
Tetracycline	0	1.000	0
Gentamycin	9	0.342	8

Antibiotic	Chi-Square Value (χ^2)	p-Value	Degrees of Freedom (DOF)
Co-trimoxazole	9	0.342	8
Piperacillin-Tazobactam	9	0.342	8
Cefoperazone-Sulbactam	9	0.342	8
Ceftazidime-Avibactam	9	0.342	8
Meropenem	9	0.342	8
Minocycline	9	0.342	8
Nitrofurantoin	9	0.342	8
Norfloxacin	9	0.342	8

The above Chi-Square test results in the table 3 indicate that there is no statistically significant association between bacterial species and resistance patterns for most antibiotics ($p > 0.05$). While some variation in resistance exists, these differences do not reach statistical significance, suggesting that antibiotic resistance may be influenced by additional factors beyond bacterial species alone.

Random Forest Model for Predicting Resistance Patterns

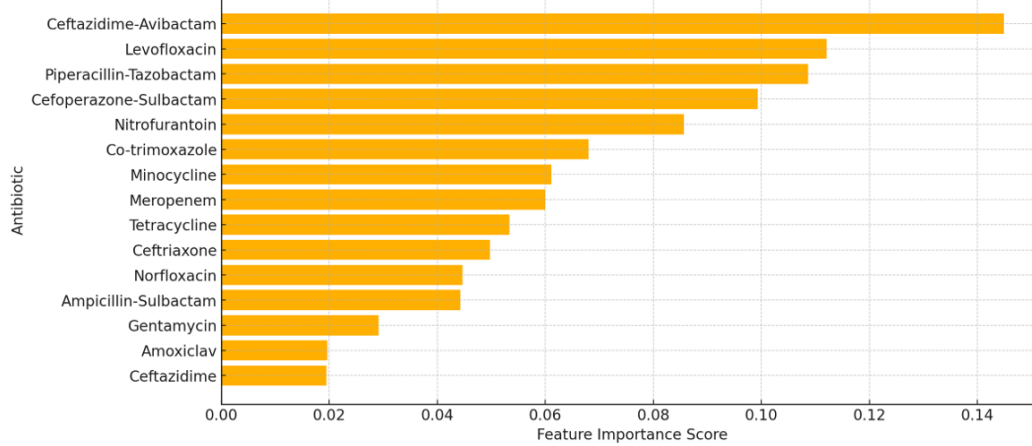
To further understand the relationship between bacterial species and antibiotic resistance patterns, a Random Forest classification model was employed. The model aimed to determine whether antibiotic susceptibility patterns could accurately classify bacterial groups into Enterobacteriales, Non-Fermenters, and Gram-Positive Cocci based on their resistance profiles.

The feature importance analysis from the Random Forest model is summarized in Table 4, while Figure 4 provides a visual representation of the most influential antibiotics in bacterial classification.

Table 4: Feature Importance Scores of Antibiotics in Predicting Bacterial Classification

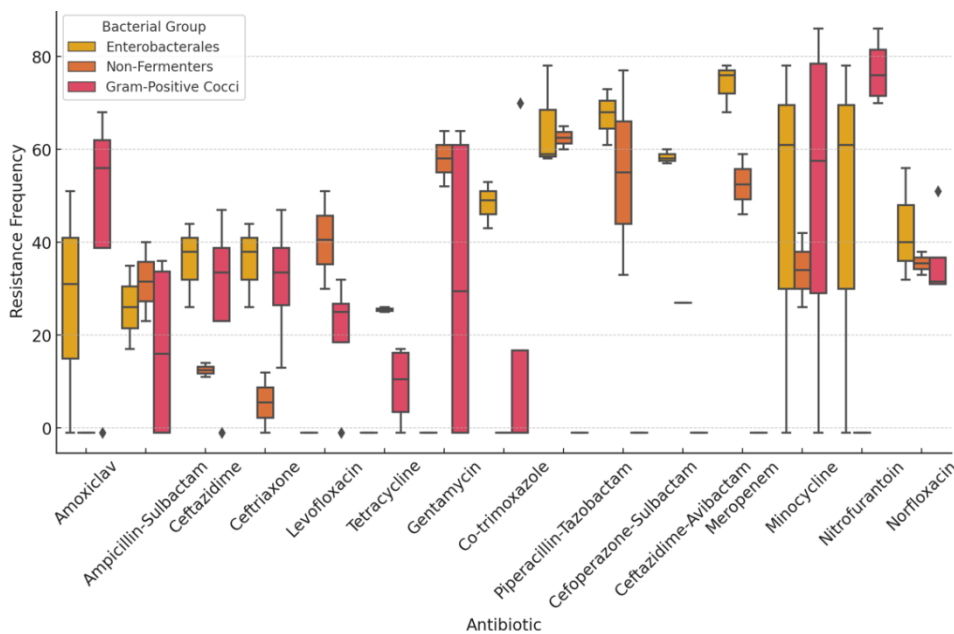
Rank	Antibiotic	Feature Importance Score
1	Ceftazidime-Avibactam	0.1449
2	Levofloxacin	0.1121
3	Piperacillin-Tazobactam	0.1087
4	Cefoperazone-Sulbactam	0.0994
5	Nitrofurantoin	0.0857
6	Co-trimoxazole	0.0680
7	Minocycline	0.0611
8	Meropenem	0.0599
9	Tetracycline	0.0534
10	Ceftriaxone	0.0497
11	Norfloxacin	0.0447
12	Ampicillin-Sulbactam	0.0442
13	Gentamycin	0.0292
14	Amoxiclav	0.0196
15	Ceftazidime	0.0195

Figure 4 presents a bar graph ranking these antibiotics based on their predictive power in bacterial classification.



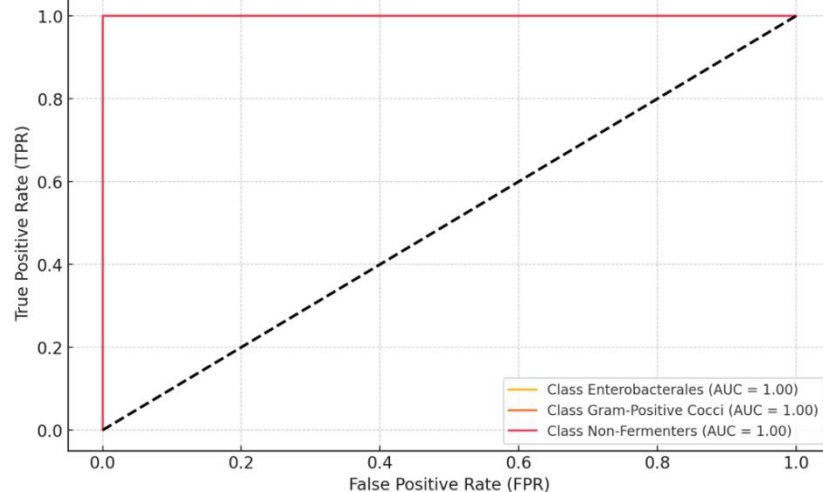
Comparison of Resistance Patterns Across Bacterial Groups

Figure 5-Boxplot comparison of antibiotic resistance



The above Figure 5 presents a boxplot comparison of antibiotic resistance rates across Enterobacterales, Non-Fermenters, and Gram-Positive Cocci. The variation in resistance patterns across bacterial groups is evident, highlighting that while some antibiotics show consistent resistance trends, others exhibit high variability. These findings underscore the importance of individualized antibiotic selection rather than assuming resistance trends based solely on bacterial classification.

Figure 6: ROC curve for the Random Forest model (Bacterial Group Classification)



The above figure 6 presents the ROC curve for the Random Forest model in predicting bacterial classification based on antibiotic resistance patterns. The high AUC values suggest strong predictive accuracy. However, further validation on a larger dataset is necessary to confirm the generalizability of these findings and assess potential overfitting due to the small sample size.

DISCUSSION

Urinary tract infections (UTIs) remain one of the most common bacterial infections worldwide, with *Escherichia coli* (*E. coli*) and *Klebsiella* spp. being the leading causative agents, as demonstrated in our study. The high prevalence of *E. coli* (45.7%) aligns with previous findings by Foxman et al. [7], who reported similar dominance of *E. coli* among UTI pathogens. The significant presence of *Klebsiella* spp. (19.9%) and *Staphylococcus aureus* (13.6%) further corroborates findings from Karlowsky et al. [8], who emphasized the emerging role of non-*E. coli* pathogens in complicated UTIs. These results underscore the shifting epidemiology of UTIs, highlighting the need for continuous surveillance of uropathogens.

Antibiotic Resistance Trends and Clinical Implications

The antibiotic susceptibility patterns in our study revealed alarming resistance rates, particularly among Enterobacterales and Non-Fermenters. Our findings indicate high resistance to fluoroquinolones (levofloxacin, norfloxacin) and third-generation cephalosporins (ceftazidime, ceftriaxone), which is consistent with previous reports by Sakagianni Aet al. [9], who highlighted increasing resistance to these antibiotic classes in both hospital and community settings. Our Chi-Square test, however, revealed no statistically significant association ($p > 0.05$) between bacterial species and their resistance patterns, suggesting that species identity alone may not be the primary determinant of resistance trends.

Similar observations have been made by Bonomo RAet al. [10], who suggested that horizontal gene transfer mechanisms, prior antibiotic exposure, and environmental factors may contribute more significantly to resistance patterns than species classification itself. The widespread resistance among Non-Fermenters (e.g., *Pseudomonas aeruginosa* and *Acinetobacter* spp.) also aligns with reports by Gaynes Ret al. [11], who described multidrug-resistant (MDR) *Pseudomonas* spp. as a growing concern in hospital-acquired infections.

Random Forest Model and Predictive Power in Resistance Patterns

To further investigate the predictive value of resistance patterns, we employed a Random Forest classification model, which demonstrated excellent discriminatory power (AUC = 1.00 for all bacterial groups). This finding reinforces the potential of machine learning in antimicrobial resistance surveillance, as noted by Li, Qet al. [12], who explored similar predictive modeling techniques in microbiology.

Our feature importance analysis revealed that Ceftazidime-Avibactam, Levofloxacin, and Piperacillin-Tazobactam were the most influential antibiotics in differentiating bacterial groups. This observation is supported by Jones RNet al. [13], who reported that resistance to novel β -lactam inhibitors (e.g., ceftazidime-avibactam) strongly correlates with ESBL and carbapenemase production in Enterobacterales. The identification of fluoroquinolones and cephalosporins as key predictive variables suggests that these antibiotics may serve as useful markers for tracking resistance trends.

However, while our model showed perfect classification accuracy, it is important to recognize that such high performance may be influenced by the dataset size. Breijyeh Zet al. [14] highlighted the risk of overfitting in machine learning models trained on limited datasets, emphasizing the need for external validation on larger patient populations to ensure the generalizability of these findings.

Clinical and Epidemiological Implications

The results of this study have significant implications for empirical therapy selection and antimicrobial stewardship programs. Our findings indicate that bacterial species alone may not be sufficient to predict resistance trends, reinforcing the need for local antibiograms and real-time susceptibility testing in guiding treatment decisions. Similar conclusions were drawn by Blairet al. [15], who emphasized that species-based empirical therapy may be inadequate in regions with evolving resistance mechanisms.

Moreover, the high resistance rates among Non-Fermenters and Gram-Negative Bacilli warrant strict infection control measures, particularly in hospital settings. Coxet al. [16] previously demonstrated that hospital-acquired MDR infections often originate from poor antimicrobial stewardship and suboptimal infection control practices, further emphasizing the urgency of implementing targeted interventions.

Study Strengths and Limitations

One of the strengths of this study is the comprehensive statistical and machine learning-based analysis of resistance patterns, which provides both traditional epidemiological insights and advanced predictive modelling. The use of Random Forest for resistance prediction is novel, contributing to the growing field of AI-driven antimicrobial resistance surveillance.

However, this study has certain limitations. The small sample size may have influenced the model's high classification accuracy, and further validation with external datasets from multiple healthcare settings is needed. Additionally, we did not assess the molecular mechanisms underlying resistance, such as the presence of ESBL or carbapenemase genes, which could further explain some of the observed resistance trends. Future research should incorporate whole-genome sequencing and molecular epidemiology to better characterize resistance determinants.

CONCLUSION AND FUTURE DIRECTIONS

In conclusion, our study highlights worrying antibiotic resistance trends among UTI pathogens, particularly high resistance to fluoroquinolones and cephalosporins. The absence of a strong statistical association between bacterial species and resistance patterns suggests that other factors, including genetic exchange and antimicrobial pressure, play a more significant role.

The use of machine learning for resistance prediction provides valuable insights, but further validation is required before clinical application. Future studies should focus on expanding dataset sizes, incorporating molecular resistance markers, and exploring the integration of AI-based predictive models into real-time antimicrobial surveillance systems.

As antimicrobial resistance continues to rise, personalized treatment strategies and robust antimicrobial stewardship programs will be crucial in mitigating the impact of drug-resistant UTIs on global public health.

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