

Prevalence and Antimicrobial Resistance Patterns of ESKAPE Pathogens Isolated from Urinary Tract Infections in a Tertiary Care Hospital in Tripura

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ABSTRACT

Introduction: Being the 'escapers' from the action of antibiotics, the ESKAPE pathogens - *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.* - are presently considered greatly by the health sector because of their highly infective nature and influence on mortality and morbidity.

Objective: This study was conducted to determine the prevalence of ESKAPE pathogens in UTI patients, with a primary focus on phenotypic characterisation of antibiotic resistance.

Materials and Methods: A study was conducted using urine samples from patients attending a Tertiary Care Hospital in Tripura over 1 year (May 2023 - April 2024). Following the universal standard method of urine collection, 7641 samples were collected and processed for isolation of ESKAPE pathogens. Bacterial identification was performed using standard microbiological techniques, including Gram's staining, morphological & cultural characteristics, and biochemical properties. Determination of isolates exhibiting VRE (Vancomycin Resistant Enterococci), ESBL (Extended Spectrum β -lactamases), Carbapenemases & MRSA (Methicillin-resistant *Staphylococcus aureus*)

characteristics was done following standard procedures after processing for AST on the isolates by using MHA by Kirby-Bauer disc diffusion method.

Results: Out of the total 7641 samples, 488(6.38%) samples were found to be ESKAPE pathogen isolates. Among them, more than 49% were *Enterococcus faecium*, followed by 30.12% of *Klebsiella pneumoniae*, *Staphylococcus aureus* (11.06%), *Pseudomonas aeruginosa* (5.32%), *Acinetobacter baumannii* (2.45%) & *Enterobacter spp.* (2.43%). MRSA was 37.03% & VRE was 16.52%. ESBL & MBL producing *Klebsiella pneumoniae* was 16.32% & 6.8%; *Acinetobacter baumannii* 75% each; *Pseudomonas aeruginosa* 11.53% & 7.69% respectively.

Conclusion: This study estimates the burden of ESKAPE pathogens in urine samples and their antimicrobial resistance pattern in a hospital setting. A high percentage of drug resistance was noted. Thus, the results of this study support implementing targeted antimicrobial strategies to specifically target these pathogens.

Keywords: ESKAPE pathogens, Urinary Tract Infections, Antimicrobial Resistance, MRSA, VRE, ESBL

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections encountered in healthcare settings and continue to pose a significant global health burden. These infections affect individuals of all age groups and contribute substantially to patient morbidity, prolonged hospitalisation, recurrent illness, and increased healthcare costs. UTIs are responsible for a large proportion of both community-associated and hospital-associated infections, especially among elderly individuals, catheterised patients, immunocompromised hosts, and critically ill patients.¹ The treatment of UTIs has become increasingly challenging because of the growing prevalence of multidrug-resistant (MDR) bacteria. Antimicrobial resistance (AMR) is now recognised as a major threat to public health worldwide, reducing the effectiveness of routinely used antibiotics and complicating infection management.^{2,3}

Among the organisms frequently associated with healthcare-associated infections, the ESKAPE group of pathogens has emerged as a major clinical concern. The term ESKAPE includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species.^{4,5} These pathogens are well known for their remarkable ability to survive exposure to multiple antimicrobial agents and persist in hospital environments.⁴ They are commonly implicated in nosocomial infections, including UTIs, bloodstream infections, ventilator-associated pneumonia, surgical site infections, and device-related infections.^{5,6} Their importance in clinical medicine is largely attributed to their capacity to acquire and disseminate resistance determinants through genetic mutation, horizontal gene transfer, and adaptive mechanisms.^{6,7}

The increasing prevalence of MDR ESKAPE pathogens has created serious challenges for empirical antimicrobial therapy.⁸ These organisms utilise several resistance strategies, including production of extended-

spectrum β -lactamases (ESBLs), carbapenemases, altered penicillin-binding proteins, reduced outer membrane permeability, overactive efflux pumps, and biofilm formation. As a result, they exhibit resistance to multiple classes of antibiotics, including β -lactams, aminoglycosides, carbapenems, glycopeptides, and fluoroquinolones.⁹ Resistant phenotypes, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), ESBL-producing *Klebsiella pneumoniae*, and metallo- β -lactamase (MBL)-producing Gram-negative bacilli, have become major therapeutic concerns in hospitals worldwide.^{6,8} In addition, the emergence of carbapenem-resistant *Enterobacterales* and resistant non-fermenting Gram-negative organisms has significantly limited available treatment choices and increased reliance on reserve antibiotics.^{8,3}

UTIs caused by ESKAPE pathogens are particularly problematic because they are often associated with recurrent infection, prolonged catheter use, invasive urological interventions, intensive care admission, and increased mortality. *Klebsiella pneumoniae* and *Enterococcus faecium* are increasingly recognised as important causes of complicated UTIs, whereas *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are frequently associated with catheter-associated urinary infections and infections among critically ill patients.⁶ The ability of these organisms to produce biofilms on urinary catheters and other medical devices enhances their persistence and resistance to antimicrobial agents, thereby contributing to chronic infection and therapeutic failure.⁷

India is considered one of the countries facing a rapidly increasing burden of antimicrobial resistance due to factors such as inappropriate antibiotic use, over-the-counter availability of antimicrobial drugs, inadequate infection control measures, and insufficient antimicrobial stewardship practices. Recent reports from different parts of India have demonstrated increasing resistance among ESKAPE pathogens,

particularly against cephalosporins, fluoroquinolones, carbapenems, and glycopeptides.⁷ The rising prevalence of MRSA, VRE, ESBL-producing organisms, and MBL-producing isolates has been documented across tertiary care hospitals nationwide, underscoring the urgent need for routine surveillance and rational antibiotic policies.^{7,3}

Prompt identification of resistant pathogens remains essential for effective treatment and infection control.^{10,6} In many resource-constrained healthcare settings, phenotypic methods continue to play a crucial role in routine microbiological diagnosis. Techniques such as cefoxitin disc diffusion for MRSA detection, vancomycin screening for VRE, combined disc tests for ESBL identification, and imipenem-EDTA double-disc synergy testing for MBL detection are widely used because of their practicality and affordability. Early recognition of these resistance mechanisms assists clinicians in selecting suitable antimicrobial therapy and helps minimise the spread of MDR pathogens within hospitals.¹⁰

Although several Indian studies have investigated antimicrobial resistance among uropathogens, there is limited information specifically addressing ESKAPE pathogens causing UTIs in northeastern India.⁸ Antimicrobial susceptibility patterns vary across geographical regions due to differences in prescribing practices, infection control measures, and local epidemiological factors.³ Furthermore, published data regarding the prevalence and resistance characteristics of ESKAPE pathogens in urinary isolates from Tripura remain scarce. Understanding regional resistance patterns is important for guiding empirical therapy, framing institutional antibiotic policies, and strengthening antimicrobial stewardship programs.^{8,3}

Therefore, the present study was designed to determine the prevalence of ESKAPE pathogens isolated from urine samples of patients attending a tertiary care hospital in Tripura and to evaluate their antimicrobial resistance profiles, including MRSA, VRE,

ESBL, and MBL production. The findings of this study may contribute to regional surveillance data and aid in the development of effective infection control measures and therapeutic strategies for the management of multidrug-resistant UTIs.⁸

MATERIALS AND METHODS

Study Design and Setting

This prospective observational study was conducted in the Department of Microbiology at a tertiary care teaching hospital in Tripura over a period of one year, from May 2023 to April 2024.

Study Population

Patients clinically suspected of urinary tract infection and attending outpatient departments, inpatient wards, and intensive care units were included in the study. Patients receiving prolonged antibiotic therapy prior to sample collection were excluded whenever an appropriate clinical history was available.

Sample Collection

A total of 7,641 urine samples were collected under aseptic precautions. Midstream clean-catch urine samples were collected from ambulatory patients, whereas catheterised urine samples were obtained using sterile techniques. Samples were transported promptly to the microbiology laboratory and processed without delay.

Isolation and Identification of Bacterial Isolates

Urine samples were cultured on Cysteine Lactose Electrolyte Deficient (CLED) agar and MacConkey agar using standard loop techniques. Plates were incubated aerobically at 37°C for 18–24 hours. Significant bacteriuria was defined by colony counts.

Bacterial isolates were identified using colony morphology, Gram staining characteristics, motility testing, and standard biochemical reactions, including the catalase, coagulase, oxidase, indole, citrate

utilisation, urease, triple sugar iron agar, and carbohydrate fermentation tests.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar, following CLSI 2023 guidelines. The antibiotics tested included β -lactams, aminoglycosides, fluoroquinolones, glycopeptides, carbapenems, and other routinely used antimicrobial agents. Zones of inhibition were interpreted as susceptible, intermediate, or resistant according to CLSI criteria.

Phenotypic Detection of Resistance Mechanisms

Detection of MRSA

Methicillin resistance among *Staphylococcus aureus* isolates was detected using cefoxitin (30 μ g) disc diffusion testing. Isolates showing resistance were considered MRSA.

Detection of VRE

Vancomycin resistance among *Enterococcus faecium* isolates was screened using vancomycin-containing media and interpreted according to CLSI recommendations.

Detection of ESBL Production

ESBL production among Gram-negative isolates was detected using the combined disc diffusion method employing ceftazidime and ceftazidime-clavulanic acid discs. An increase in zone diameter of ≥ 5 mm in the presence of clavulanic acid was considered positive for ESBL production.

Detection of MBL Production

MBL production was detected by imipenem-EDTA double-disc synergy testing. Enhancement of the inhibition zone around imipenem in the presence of EDTA indicated MBL production.

Statistical Analysis

Data were entered into Microsoft Excel and analysed using descriptive statistical methods. Results were expressed as frequencies and percentages.

RESULTS

Prevalence of ESKAPE Pathogens

Out of 7,641 urine samples processed during the study period, 488 (6.38%) yielded ESKAPE pathogens. *Enterococcus faecium* was the most frequently isolated ESKAPE pathogen, accounting for nearly half of all isolates, followed by *Klebsiella pneumoniae* (Table 1).

Table 1: Distribution of ESKAPE Pathogens Isolated from Urine Samples

Pathogen	Number of Isolates	Percentage (%)
<i>Enterococcus faecium</i>	240	49.00
<i>Klebsiella pneumoniae</i>	147	30.12
<i>Staphylococcus aureus</i>	54	11.06
<i>Pseudomonas aeruginosa</i>	26	5.32
<i>Acinetobacter baumannii</i>	12	2.45
<i>Enterobacter</i> spp.	12	2.43
Total	488	100

Antimicrobial Resistance Patterns

Table 2 shows that among the 54 *Staphylococcus aureus* isolates, 20 (37.03%) were MRSA. Among 240 *Enterococcus faecium* isolates, 16.52% exhibited vancomycin resistance. ESBL production was observed predominantly among *Acinetobacter*

baumannii isolates (75%), followed by *Klebsiella pneumoniae* (16.32%) and *Pseudomonas aeruginosa* (11.53%). Similarly, MBL production was highest among *Acinetobacter baumannii* isolates (75%), followed by *Klebsiella pneumoniae* (6.8%) and *Pseudomonas aeruginosa* (7.69%).

Table 2: Phenotypic Resistance Patterns Among ESKAPE Pathogens

Resistance Pattern	Organism	Percentage (%)
MRSA	<i>S. aureus</i>	37.03
VRE	<i>E. faecium</i>	16.52
ESBL	<i>K. pneumoniae</i>	16.32
ESBL	<i>A. baumannii</i>	75.00
ESBL	<i>P. aeruginosa</i>	11.53
MBL	<i>K. pneumoniae</i>	6.80
MBL	<i>A. baumannii</i>	75.00
MBL	<i>P. aeruginosa</i>	7.69

DISCUSSION

The present study highlights the increasing burden of multidrug-resistant (MDR) ESKAPE pathogens causing urinary tract infections in a tertiary care hospital in Tripura. Among the isolated organisms, *Enterococcus faecium* was the predominant pathogen, followed by *Klebsiella pneumoniae*. Similar observations have been reported in studies from tertiary healthcare settings, where *Enterococci* and Gram-negative bacilli have been identified as important causes of complicated and nosocomial UTIs.¹¹ The predominance of these organisms may be associated with prolonged hospitalisation, catheterisation, previous antibiotic exposure, and invasive procedures, all of which facilitate colonisation and infection by resistant pathogens. Recent global reports have also emphasised the growing contribution of ESKAPE pathogens to healthcare-associated UTIs, leading to increased morbidity and mortality.^{11,12}

The high isolation rate of *Enterococcus faecium* observed in the present study is clinically significant because *Enterococci* possess intrinsic resistance to several commonly used antibiotics and can rapidly acquire additional resistance determinants.^{11,12} The detection of vancomycin-resistant *Enterococcus* (VRE) among 16.52% of isolates is particularly concerning. Comparable VRE rates have been documented in hospital-based studies from India and other Asian countries, reflecting increasing glycopeptide use and selective antimicrobial pressure in healthcare settings. VRE infections are associated with limited therapeutic options, prolonged

hospital stays, and increased risk of treatment failure, particularly among critically ill and immunocompromised patients.¹³

Methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for 37.03% of *S. aureus* isolates in the present study. Similar MRSA prevalence has been reported from several tertiary care centres in India, indicating persistent circulation of resistant *S. aureus* strains in hospital environments. MRSA remains an important cause of hospital-acquired infections because of its resistance to β -lactam antibiotics and its ability to spread rapidly among hospitalised patients. The emergence of MRSA substantially limits empirical treatment choices and often necessitates the use of reserve antibiotics such as vancomycin and linezolid.¹⁴

Among Gram-negative pathogens, *Klebsiella pneumoniae* demonstrated considerable ESBL and MBL production. ESBL-producing organisms hydrolyse extended-spectrum cephalosporins and are commonly associated with prolonged hospitalisation, increased healthcare costs, and treatment failure.¹¹ The prevalence of ESBL-producing *K. pneumoniae* observed in this study is consistent with recent reports from India demonstrating increasing resistance among *Enterobacterales* causing UTIs. Carbapenem resistance mediated by metallo- β -lactamase (MBL) production is particularly alarming because carbapenems are considered last-line agents for severe Gram-negative infections. The World Health Organization has identified carbapenem-resistant *Enterobacterales* as critical priority pathogens requiring urgent surveillance and development of new therapeutic strategies.¹⁵

The exceptionally high rate of ESBL and MBL production among *Acinetobacter baumannii* isolates observed in this study is alarming. *A. baumannii* has emerged as one of the most problematic nosocomial pathogens due to its remarkable ability to persist in hospital environments and acquire multiple antimicrobial resistance genes.^{11,16} Similar findings have been reported in surveillance studies where *Acinetobacter* species demonstrated high resistance to carbapenems, aminoglycosides, and fluoroquinolones. The organism's capacity for biofilm formation and environmental persistence facilitates its spread in intensive care units and other high-risk healthcare settings.¹⁶

Pseudomonas aeruginosa isolates in the present study also produced ESBLs and MBLs, reflecting the complex adaptive resistance mechanisms of this pathogen. *P. aeruginosa* is intrinsically resistant to many antimicrobial agents because of low outer membrane permeability, active efflux pumps, and production of β -lactamases. Increasing carbapenem resistance among *P. aeruginosa* has been reported globally and is associated with poor clinical outcomes, prolonged hospitalisation, and increased mortality.¹⁷

The findings of the present study are consistent with both national and international surveillance reports showing a steady rise in antimicrobial resistance among ESKAPE pathogens. The rapid emergence of MDR organisms is largely driven by indiscriminate antibiotic use, self-medication, inappropriate empirical therapy, prolonged hospitalisation, and inadequate infection prevention practices. In developing countries such as India, over-the-counter availability of antibiotics and insufficient implementation of antimicrobial stewardship programs further accelerate the spread of resistant strains.¹⁸

Continuous surveillance of antimicrobial resistance patterns is essential for early detection of emerging resistant pathogens and formulation of evidence-based antibiotic policies.¹¹ Strengthening antimicrobial stewardship programs, implementing strict

infection prevention and control measures, promoting rational antibiotic prescribing, and improving hospital hygiene practices are crucial for limiting the spread of MDR ESKAPE pathogens. In addition, regular monitoring of local resistance trends can assist clinicians in selecting appropriate empirical therapy and reducing treatment failure among patients with urinary tract infections.¹⁵

CONCLUSION

The present study demonstrates the significant prevalence of multidrug-resistant ESKAPE pathogens among urinary isolates in a tertiary care hospital in Tripura. *Enterococcus faecium* and *Klebsiella pneumoniae* were the predominant pathogens isolated. The high prevalence of MRSA, VRE, ESBL, and MBL-producing organisms indicates an alarming rise in antimicrobial resistance and emphasises the urgent need for continuous microbiological surveillance, implementation of robust infection control measures, and judicious antibiotic use.

Regional antimicrobial resistance data generated through such studies are essential for guiding empirical therapy and developing effective antimicrobial stewardship policies. Early detection of resistant pathogens and rational antibiotic practices are crucial for reducing morbidity, mortality, and healthcare burden associated with multidrug-resistant urinary tract infections.

Declaration by Authors

Ethical Approval: Ethical clearance for the study was obtained from the Institutional Ethics Committee of Agartala Government Medical College and GBP Hospital.

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Conflict of Interest: The authors declare no conflict of interest.

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