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Epidemiological analysis of extended-spectrum β-lactamase-producing uropathogenic bacteria

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Abstract

Urinary tract infections (UTIs), particularly those caused by extended-spectrum β-lactamase (ESBL)-producing bacteria, pose a significant and escalating global health challenge, affecting nearly 100 million individuals annually. This study investigates the prevalence and resistance patterns of ESBL-producing uropathogenic bacteria in Chennai, focusing on pathogens such as *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas* spp.A total of 100 urine samples were collected from in-patients and out-patients at multispecialty hospitals, representing diverse demographic groups. Pathogen identification was conducted using standard microbiological methods, including Gram staining, selective media isolation, and biochemical profiling. Antibiotic susceptibility testing and Multiple Antibiotic Resistance (MAR) index calculations revealed high resistance levels, particularly to β-lactam antibiotics, necessitating the use of stronger treatments like carbapenems.Results showed *E. coli* as the predominant uropathogen, with females significantly more affected than males (84% vs. 12%). In-patients had a higher positivity rate (53%) compared to out-patients (37%), likely due to healthcare-associated factors such as catheterization and extended hospital stays. These findings emphasize the urgent need for targeted infection control measures in healthcare settings and region-specific treatment guidelines to address local resistance patterns.Routine antibiotic susceptibility testing, robust antimicrobial stewardship, and public awareness campaigns are vital to curb multidrug-resistant pathogens. Comprehensive strategies, including gender-sensitive interventions and stringent infection control policies, are critical to managing ESBL-producing UTIs and mitigating the public health burden of antimicrobial resistance.

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INTRODUCTION

The widespread prevalence of urinary tract infections (UTIs), particularly those caused by extended-spectrum β-lactamase (ESBL)-producing bacteria, presents a growing global health challenge. Each year, UTIs affect nearly 100 million people worldwide, placing a significant burden on healthcare systems. These infections, although common across all age groups, disproportionately impact women due to anatomical susceptibility. The involvement of

multidrug-resistant bacteria, especially those producing ESBL enzymes, further complicates treatment, as these infections exhibit resistance to many standard antibiotic therapies, including commonly used β-lactams. ESBL enzymes, frequently produced by Gram-negative bacteria such as Escherichia coli and Klebsiella pneumoniae, confer antibiotic resistance by degrading a broad range of β-lactam antibiotics, such as penicillins and cephalosporins. As a result, infections involving these bacteria often require alternative antibiotics, which may be less effective or carry a higher risk of side effects. The rise in ESBL-producing uropathogens has been closely linked to the overuse and misuse of antibiotics, especially in healthcare settings. Additionally, the genes responsible for ESBL production can transfer between bacterial species, fueling the spread of these resistant strains across both hospital and community settings.

Epidemiological studies indicate that UTIs due to ESBL-producing bacteria are not only harder to treat but also associated with increased morbidity, extended hospital stays, and elevated healthcare costs. In specific regions, such as Coimbatore, research has shown that ESBL-producing E. coli is the most frequently isolated uropathogen in both hospital-acquired and community-acquired UTIs. Alarmingly, resistance rates to antibiotics like cefotaxime and ceftazidime often approach 100%, underscoring an urgent need for improved infection control measures and updated antibiotic use policies. The persistent increase in multidrug-resistant pathogens underscores the importance of routine antibiotic susceptibility testing and rigorous antimicrobial stewardship. Healthcare experts advocate for developing region-specific treatment guidelines tailored to local resistance patterns. Coupled with public health efforts to promote responsible antibiotic use, these measures could help curb the spread of resistant uropathogens. Understanding the epidemiology of ESBL-producing bacteria is essential to formulating effective strategies for managing and preventing these resistant UTIs, ultimately leading to better health outcomes.

MATERIALS AND METHODS

The study employed standard microbiological procedures to isolate and identify extended-spectrum β-lactamase (ESBL)-producing bacteria associated with urinary tract infections (UTIs). A total of 100 urine samples were collected from patients across multi-specialty hospitals in Coimbatore, following ethical approval.

Each sample was initially inoculated in Luria Bertani (LB) broth and incubated overnight at 37°C to facilitate bacterial growth. After incubation, cultures were streaked onto selective agar media to enable isolation of specific bacterial genera. The media used included Eosine Methylene Blue (EMB) agar for Escherichia coli, MacConkey Agar for Klebsiella spp., Nutrient Agar for Proteus spp., and King's B medium for Pseudomonas spp. To confirm bacterial identity, Gram staining and a series of biochemical tests were performed. The isolated bacteria were then subjected to antibiotic susceptibility testing to determine resistance patterns. This testing was conducted using the disc diffusion method on Mueller-Hinton agar plates. Pure bacterial cultures, cultivated in nutrient broth for 6-8 hours, were swabbed onto the agar plates, and antibiotic discs were applied with care to prevent overlapping inhibition zones. Following an incubation period of 18-24 hours at 37°C, inhibition zones were measured, and the isolates were classified as sensitive, intermediate, or resistant according to standard interpretative guidelines. Additionally, a Multiple Antibiotic Resistance (MAR) index was calculated for each isolate by dividing the number of antibiotics to which the isolate showed resistance by the total number of antibiotics tested. A MAR index above 0.2 suggested that the isolate likely originated from a high-risk source of contamination. This comprehensive methodology provided valuable insights into the prevalence and antibiotic resistance profiles of ESBL-producing uropathogens, aiding in the development of targeted antimicrobial strategies for effective treatment and control of resistant UTIs.

Table 1 Selective medium for isolation of pathogens

Selective media	Bacterial genera
Eosine Methylene Blue agar (EMB)	*E. coli*
Mac Conkey Agar	*Klebsiella* spp.
Nutrient Agar (NA)	*Proteus* spp.
King's B medium	*Pseudomonas* spp.

The selective media used for isolating various uropathogenic bacteria in **Table 1** allowed for targeted identification of specific bacterial genera. Escherichia coli was isolated on Eosine Methylene Blue (EMB) agar, which is selective for Gram-negative bacteria and enables E. coli to be easily identified based on its characteristic growth and color changes. Klebsiella spp. was cultured on MacConkey Agar, which differentiates lactose-fermenting bacteria like Klebsiella by producing distinct colonies. Proteus spp. was grown on Nutrient Agar (NA), providing a general-purpose medium suitable for this genus. Finally, Pseudomonas spp. was isolated using King's B medium, specifically formulated to promote the growth of Pseudomonas species and aid in their identification.

Result

Urine samples were gathered from both in-patients and out-patients at multispecialty hospitals around Coimbatore, covering both genders across various age groups. Of the 100 samples collected, 81 were from in-patients and 19 from out-patients. The isolates underwent Gram staining and were cultured on selective media. Since none of the isolates tested positive for Gram-positive bacteria, further screening for Gram-positive organisms on selective media was discontinued. The epidemiological analysis of extended-spectrum β-lactamase (ESBL)-producing uropathogenic bacteria reveals concerning trends in antimicrobial resistance within urinary tract infections (UTIs). ESBL-producing bacteria, notably among uropathogens like Escherichia coli and Klebsiella species, demonstrate resistance to a broad spectrum of β-lactam antibiotics, including penicillins and cephalosporins. This resistance complicates treatment protocols, often requiring the use of more potent and expensive antibiotics, such as carbapenems, which should ideally be reserved to mitigate the rise of multidrug-resistant strains.

The analysis of uropathogenic bacteria in **Table 2**shows that Escherichia coli is the predominant organism isolated from positive samples, accounting for 41 isolates, which represents 29% of the total samples. This indicates that E. coli is the most common causative agent of UTIs in the sampled population, aligning with its well-known association with these infections. Klebsiella spp. and Pseudomonas spp., however, were much less frequently isolated, with only 7 isolates (3%) and 2 isolates (0.7%), respectively. These lower percentages suggest that while Klebsiella and Pseudomonas species are less common, they still contribute to the overall burden of uropathogenic infections, especially in certain patient subsets or in cases of complicated UTIs. The gender distribution within positive samples further emphasizes that these infections are more prevalent in females across all isolated organisms, particularly for E. coli, underscoring the need for targeted infection control and preventive measures tailored to this higher-risk group.

The biochemical profiling in **Table 3** outlines the distinct characteristics of Escherichia coli, Klebsiella spp., and Pseudomonas spp., facilitating their identification in clinical microbiology settings. All three organisms are Gram-negative rods, yet they exhibit unique biochemical reactions that differentiate them from each other. E. coli is characterized by a positive methyl red (MR) test and catalase activity, along with the ability to ferment lactose but not sucrose, maltose, or glucose, and a lack of urease production. It is motile, which aids in its identification among uropathogenic organisms. Klebsiella spp. shows a broader biochemical profile with positive results in the Voges-Proskauer (VP), urease, and triple sugar iron (TSI) tests, as well as fermentation of glucose, lactose, maltose, and sucrose. This organism is notably non-motile, which is a distinctive feature compared to E. coli and Pseudomonas. Pseudomonas spp. also displays a

Table 2 Percentage of uropathogenic bacteria

S. No.	Positive samples		Organism isolated	Total no. of isolates	Percentage %
	Male	Female			
1.	15	24	*E. coli*	41	29%
2.	2	4	*Klebsiella* spp.	7	3%
3.	5	-	*Pseudomonas* spp.	2	0.7%

Table 3 Typical biochemical profile of E. coli, Klebsiella spp. And Pseudomonas spp

Gram's staining	Indole	MR	VP	Urease	TSI	Catalase	Glucose	Lactose
G-ve Rod	-	-	+	-	+	+	-	+
G-ve Rod	-	+	-	+	+	+	+	+
G-ve Rod	-	-	-	+	+	+	+	-

Table 3 Typical biochemical profile of E. coli, Klebsiella spp. And Pseudomonas spp (continued)

Gram's staining	Maltose	Sucrose	motility	Oxidase	Suspected organism
G-ve Rod	-	-	+	-	*E. coli*
G-ve Rod	+	+	-	-	*Klebsiella* sp.
G-ve Rod	+	+	+	+	*Pseudomonas* sp.

Table 4 Demograhic data of UTI among patients

Category	No. of samples	Male	Female	No. of Positive samples	Percentage %
IP	58	60	30	38	53%
OP	42	4	6	12	37%

unique profile, particularly with positive results for oxidase and motility, distinguishing it from E. coli and Klebsiella spp. It can utilize sucrose and maltose but does not ferment lactose or glucose. Additionally, it shows positive reactions for urease and catalase but lacks the ability to produce indole or methyl red. These biochemical markers are essential for the accurate identification and differentiation of these organisms, aiding clinicians in selecting appropriate treatments, especially given the varying resistance patterns associated with each pathogen in urinary tract infections.

The demographic data in **Table 4** indicate a higher prevalence of positive urinary tract infection (UTI) cases among in-patient (IP) samples compared to out-patient (OP) samples. Specifically, 53% of the in-patient samples tested positive for UTIs, with a significant portion of these cases identified in female patients. In contrast, out-patient samples showed a lower positivity rate at 37%. This discrepancy in infection rates suggests that patients in in-patient settings are more susceptible to UTIs, potentially due to prolonged exposure to healthcare environments and increased use of invasive procedures, such as catheterization, which are common in hospital settings. The higher rate in females across both IP and OP categories is consistent with known demographic trends, where anatomical factors predispose females to higher UTI incidence. This data underscores the importance of targeted infection control practices within healthcare facilities to mitigate UTI prevalence among in-patients.

Table 5 Gender wise distribution of uropathogenic bacteria in UTI samples

Gender	Total no. of samples	No. of Positive samples	Percentage %
Male	53	14	12%
Female	47	36	84%

The gender-wise distribution of uropathogenic bacteria in UTI samples, as shown in **Table 5**, reveals a pronounced difference in infection rates between males and females. Among the total samples, 84% of females tested positive for uropathogenic bacteria, a stark contrast to the 12% positivity rate observed in male samples.

This significant disparity highlights that females are considerably more prone to urinary tract infections compared to males, a trend that is well-supported by anatomical and physiological differences that increase UTI susceptibility in females. This finding suggests the need for heightened awareness, prevention, and early intervention strategies targeting the female population to address the high prevalence of UTIs. It also emphasizes the importance of gender-specific research and healthcare approaches in managing and preventing UTIs effectively.

Discussion

The epidemiological analysis of urinary tract infections (UTIs) caused by extended-spectrum β-lactamase (ESBL)-producing bacteria reveals critical insights into the prevalence, resistance patterns, and demographic distribution of these infections which complicate treatment options due to their resistance to commonly used β-lactam antibiotics. This challenge aligns with global trends in antimicrobial resistance and highlights the importance of monitoring regional prevalence, as observed in Chennai, where a notable frequency of ESBL-producing strains has emerged. The data on uropathogenic bacteria isolated in this study, especially the predominance of E. coli (29%) among positive UTI cases, align with existing literature on UTIs, where E. coli is commonly cited as the primary uropathogen. However, the presence of other organisms such as Klebsiella spp. and Pseudomonas spp., albeit at lower frequencies (3% and 0.7%, respectively), underscores the diversity of pathogens involved in UTIs. These pathogens, while less common than E. coli, play a significant role, particularly in patients with complicated UTIs or those exposed to prolonged antibiotic treatments, further exacerbating resistance patterns.

The gender-wise distribution data highlight a stark contrast between male and female susceptibility to UTIs, with females representing 84% of positive cases compared to just 12% in males. This pronounced gender disparity is well-documented, with females being more prone to UTIs due to anatomical factors, such as a shorter urethra and its proximity to the anus, which facilitates bacterial colonization. This finding calls for targeted interventions in females, such as improved hygiene practices and more effective, gender-specific educational programs, to help mitigate this disparity. Another important aspect revealed by the study is the difference in UTI positivity rates between in-patient (IP) and out-patient (OP) samples. In-patients exhibited a higher positivity rate of 53%, compared to 37% among out-patients. This observation may be attributed to factors prevalent in healthcare settings, such as the increased use of invasive devices (e.g., catheters), frequent antibiotic administration, and prolonged hospital stays, which create an environment conducive to the development and spread of antibiotic-resistant pathogens. This highlights the importance of stringent infection control measures, particularly in hospitals, to reduce the risk of healthcare-associated UTIs. The biochemical profile provided in this study offers valuable insights into the diagnostic markers essential for identifying and differentiating uropathogenic bacteria. For instance, the presence of urease in Klebsiella spp. and Pseudomonas spp., combined with their unique patterns of sugar utilization and motility, assists in accurate identification. Such differentiation is crucial in clinical settings, as each pathogen presents distinct resistance profiles that necessitate tailored treatments.

The growing prevalence of ESBL-producing bacteria in UTIs, demonstrated by the resistance to common antibiotics like cefotaxime and ceftazidime, underscores the need for antimicrobial stewardship and routine susceptibility testing. High rates of resistance to these antibiotics suggest that alternative treatment regimens, including carbapenems, are increasingly required, though these should be reserved to avoid inducing resistance to last-resort drugs. Furthermore, the data suggests that a Multiple Antibiotic Resistance (MAR) index exceeding 0.2 in certain isolates indicates potential contamination from high-risk sources, emphasizing the need for vigilant infection control and environmental hygiene. This study provides essential epidemiological data that can inform local healthcare policies and intervention strategies to manage and control the spread of ESBL-producing uropathogens. Implementing regional guidelines based on resistance patterns, promoting public awareness on responsible antibiotic use, and enhancing infection control practices in healthcare facilities are crucial steps toward mitigating the impact of these resistant pathogens and improving patient outcomes in UTI management.

Conclusion

The epidemiological analysis of extended-spectrum β-lactamase (ESBL)-producing uropathogenic bacteria underscores a significant public health challenge due to the increased prevalence of multidrug-resistant urinary tract infections (UTIs). This study highlights that Escherichia coli remains the dominant causative

agent in UTIs, followed by Klebsiella spp. and Pseudomonas spp., albeit at lower frequencies. The predominance of ESBL-producing strains among these bacteria poses considerable obstacles in treatment, given their resistance to a wide range of β-lactam antibiotics, including commonly used penicillins and cephalosporins. This resistance often necessitates the use of more potent, last-resort antibiotics like carbapenems, underscoring the critical need for antimicrobial stewardship to prevent further resistance development. Gender-based analysis shows that females are disproportionately affected by UTIs, with a positivity rate far exceeding that of males. This trend, supported by biological susceptibility, highlights the need for focused interventions in female populations to manage and prevent UTIs effectively. Additionally, the higher prevalence of positive UTI cases among in-patients, as compared to out-patients, suggests a strong association with hospital environments. Factors like catheterization, prolonged stays, and frequent antibiotic exposure contribute to higher infection and resistance rates in healthcare settings, indicating an urgent need for enhanced infection control measures in hospitals. The findings of this study emphasize the importance of routine antibiotic susceptibility testing and the establishment of region-specific guidelines to address local resistance patterns. Adopting rigorous infection control practices, promoting responsible antibiotic use, and implementing educational initiatives on UTI prevention could collectively help curb the spread of ESBL-producing bacteria. This comprehensive approach is essential to mitigate the impact of resistant uropathogens, reduce the burden on healthcare systems, and improve patient outcomes in managing UTIs effectively.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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