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Characterization of ESBL-Producing *Klebsiella spp.* *Escherichia coli* and *Staphylococcus aureus* in Clinical Samples

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ABSTRACT

Background: *The increasing prevalence of Extended-Spectrum Beta-Lactamase (ESBL)-producing pathogens is a significant concern in clinical microbiology, as these organisms resist a wide range of beta-lactam antibiotics, complicating treatment strategies. This study aimed to characterize ESBL-producing Klebsiella spp. Escherichia coli and Staphylococcus aureus isolated from clinical samples, including urine, wound swabs, sputum, and vaginal swabs.*

Materials and methods: *A total of 74 clinical samples were analyzed, comprising 51 urine, 11 wound swab, 4 sputum and 8 vaginal swab samples collected from patients presenting with symptoms indicative of infections between June 2024 and September 2024. Bacterial isolates were identified using standard microbiological techniques and antimicrobial susceptibility testing was performed using the disk diffusion method. ESBL production was detected using the phenotypic confirmatory test.*

Results: *Among the isolated pathogens, E. coli was the most prevalent, accounting for 49.0% of all samples, followed by Klebsiella spp. (36.4%) Staphylococcus aureus (9.1%) Pseudomonas aeruginosa (4.5%) and Proteus mirabilis (1.0%). The prevalence of ESBL production was high amongKlebsiella spp. (75%) and E. coli (72%) particularly in urine and wound isolates. Resistance rates to commonly used antibiotics were notably high, with E. coli showing 88% resistance to ampicillin, 76% to ciprofloxacin, and 75% to ceftriaxone. However, carbapenems remained effectivewith a low resistance rate of approximately 4%.*

Conclusions: *The study highlighted the alarming prevalence of ESBL-producing E. coli and Klebsiella spp. with significant resistance to beta-lactams and fluoroquinolones. These findings underscore the need for stringent antibiotic stewardship, infection control measures and the use of alternative antibiotics like carbapenems for treating infections caused by multidrug-resistant organisms. Continued surveillance and molecular analysis are essential to track the spread of resistant pathogens and guide clinical treatment.*

Key words : Antimicrobial resistance; Drug-resistant pathogens; *E. coli;* ESBL; *Klebsiella spp.*

Introduction

The rising threat of Antimicrobial Resistance (AMR) has become one of the most critical global public health challenges of the 21st century.[1] Among the many drivers of AMR, the inappropriate or excessive use of antimicrobial agents-often due to misdiagnosis, improper prescription practices and poor treatment compliance-plays a leading role.[2] Numerous studies have confirmed a strong association between antibiotic consumption patterns and the emergence of resistant bacterial strains.[3] One of the key resistance mechanisms in Gram-negative bacteria, particularly among members of the Enterobacteriaceae family, is the production of Extended-Spectrum Beta-Lactamases (ESBLs).[4] ESBLs are enzymes capable of hydrolyzing the β-lactam ring of penicillins and third-generation cephalosporins, rendering these antibiotics ineffective, although carbapenems and cephamycins often retain activity.[5] The production of beta-lactamases, especially ESBLs, is among the most widespread strategies by which Gram-negative bacteria evade beta-lactam antibiotics, contributing significantly to the current resistance crisis worldwide.[4,5] Typically, ESBLs are plasmid-mediated, enabling horizontal gene transfer and the hydrolysis of a wide range of beta-lactam antibiotics, including

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penicillins, cephalosporins, and aztreonam. Importantly, their enzymatic activity can be inhibited by beta-lactamase inhibitors like clavulanic acid[2,3]. Phenotypic detection methods such as the double-disk diffusion test and E-test are commonly used to screen for ESBL production, but these methods are not always sufficient. As a result, molecular techniques like Polymerase Chain Reaction (PCR) and gene sequencing are increasingly employed for accurate identification[6].

Research has focused on identifying and characterizing the genes responsible for ESBL production, including **TEM, SHV, OXA** and **CTX-M** types.[6,7] Among these, CTX-M-type enzymes have become the most dominant ESBLs globally, with more than 130 variants described and classified into at least five subgroups according to the Lahey beta-lactamase database.[7-9] Given the plasmid-mediated nature of these genes, ESBLs can rapidly disseminate across bacterial populations, leading to both sporadic cases and widespread outbreaks.[10] Furthermore, ESBL-producing strains often carry additional resistance to other antibiotic classes such as aminoglycosides, sulfonamides and fluoroquinolones, posing significant challenges for patient management.[11] *Klebsiella pneumoniae* and *Escherichia coli* are recognized as the predominant ESBL-producing organisms worldwide, frequently causing infections such as urinary tract infections and surgical site infections.[6,12,13,14] Studies from Bangladesh have also highlighted the significant burden of ESBL-producing bacteria, with reports showing that 43.2% of *E. coli* and 39.5% of *K. pneumoniae* isolates from a hospital setting were ESBL-positive.[15] However, there remains a notable lack of comprehensive data regarding the molecular characterization of ESBL-producing organisms in Bangladesh. In addition, while most studies on ESBLs have primarily focused on Gram-negative bacteria, Gram-positive organisms like Staphylococcus aureus also pose major clinical concerns, particularly due to their multidrug-resistant nature and virulence factors. Given this background, the present study aims to characterize ESBL-producing *Klebsiella spp. Escherichia coli* and *Staphylococcus aureus* isolated from clinical samples. It will focus on both phenotypic detection methods and the molecular identification of key resistance genes, thereby providing valuable insights into the local epidemiology of ESBL producers and guiding effective infection control strategies.

Materials and methods

This cross-sectional microbiological study was conducted at Ibn Sina Hospital and Nurul Islam Diabetic Hospital,Jasorein South-West Bangladesh, aiming to characterize Extended-Spectrum Beta-Lactamase (ESBL)-producing *Klebsiella spp. Escherichia coli* and *Staphylococcus aureus in clinical samples. The study included a total of 74 clinical samples,* consisting of 51 urine samples, 11 wound swabs, 4 sputum samples, and 8 vaginal swabs, collected from patients presenting with symptoms indicative of infections between June 2024 and September 2024. The samples were collected following strict aseptic techniques to ensure accurate microbial isolation. Urine samples were obtained from patients with suspected Urinary Tract Infections (UTIs) using the midstream clean-catch method, ensuring minimal contamination. Wound swabs were collected from patients with suspected soft tissue or surgical site infections using sterile cotton-tipped applicators to sample the base of the wound. Vaginal swabs were collected from female patients presenting with symptoms of vaginal or pelvic infections, while sputum samples were obtained from patients showing signs of lower respiratory tract infections, such as pneumonia or bronchitis, after they were instructed to produce sputum by deep coughing. All samples were transported to the laboratory within two hours of collection to prevent specimen degradation. The samples were inoculated onto appropriate culture media: Cystine-Lactose-Electrolyte-Deficient (CLED) agar and MacConkey agar for urine samples, Blood agar and MacConkey agar for wound and vaginal swabs, and Blood agar and MacConkey agar for sputum samples. These media were selected to encourage the growth of both Gram-negative and Gram-positive pathogens commonly associated with infections in these anatomical sites. The inoculated plates were incubated at 37°C for 18-24 hours, after which bacterial colonies were examined for growth and morphology.

Bacterial identification was performed through Gram staining and a series of standard biochemical tests, including catalase, coagulase, oxidase, indole and motility testing. Commercial systems like API 20E (bioMérieux, France) were used to confirm the identification of Escherichia coli and Klebsiella spp. Isolates suspected to be Staphylococcus aureus were confirmed using coagulase tests. The presence of ESBL-producing isolates was determined using the Double-Disk Synergy Test (DDST). Isolates resistant to third-generation cephalosporins, such as ceftriaxone or ceftazidime, were tested for ESBL production by placing a disk containing clavulanic acid near the third-generation cephalosporin disk on the agar plate. A significant increase in the zone of inhibition around the cephalosporin disk in the presence of clavulanic acid confirmed the ESBL production.

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Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method, testing a range of antibiotics depending on the sample type. Antibiotics tested for urine isolates included ampicillin, amoxicillin-clavulanic acid, nitrofurantoin, ciprofloxacin, levofloxacin, gentamicin, ceftriaxone and imipenem. For wound and vaginal swab isolates, antibiotics tested included ampicillin, amoxicillin-clavulanic acid, ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, piperacillin-tazobactam and meropenem. Sputum isolates were tested against ampicillin, ceftriaxone, ciprofloxacin, gentamicin, doxycycline and vancomycin. The results were interpreted based on the Clinical and Laboratory Standards Institute (CLSI) guidelines.

The data collected from the microbiological analysis were analyzed using descriptive statistics, including frequencies and percentages, to determine the prevalence of ESBL-producing strains and their antimicrobial resistance patterns across the different clinical sample types. Ethical approval was obtained from IRB of two hospitals, and informed consent was collected from all participating patients, ensuring adherence to ethical standards and patient confidentiality. This methodology ensured a thorough investigation of ESBL-producing pathogens from various clinical samples, with a focus on common pathogens such as *Klebsiella spp. Escherichia coli* and *Staphylococcus aureus,* offering valuable insights into the antimicrobial resistance landscape in the region.

Results

A total of 74 clinical samples, comprising 51 urine specimens, 11 wound swabs, 4 sputum samples and 8 vaginal swabs, were collected and analyzed for the presence of ESBL-producing *Klebsiella spp. Escherichia coli* and *Staphylococcus aureus*. The distribution of bacterial isolates and the presence of ESBL-producing strains in each sample type has been presented below.

Table I Distribution of bacterialisolates by sample type

Pathogen	Urine Isolates (n=51)	Percentage (%)	Wound Isolates (n=11)	Percentage (%)	Sputum Isolates (n=4)	Percentage (%)	Vaginal Swab Isolates (n=8)	Percentage (%)
Escherichia coli	25	49.0%	1	9.1%	2	50.0%	3	37.5%
Klebsiella spp.	12	23.5%	4	36.4%	1	25.0%	1	12.5%
Staphylococcus aureus	8	15.7%	2	18.2%	0	0.0%	2	25.0%
Pseudomonas aeruginosa	4	7.8%	3	27.3%	1	25.0%	0	0.0%
Proteus mirabilis	2	3.9%	0	0.0%	0	0.0%	0	0.0%
Enterococcus faecalis	0	0.0%	0	0.0%	0	0.0%	1	12.5%
Total	51	100%	11	100%	4	100%	8	100%

This Table revealed a summary of the distribution of bacterial isolates across different clinical sample types, which include urine, wound swabs, sputum and vaginal swabs. The pathogens identified were *Escherichia coli, Klebsiella spp., Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis* and *Enterococcus faecalis*. Our study shows the number and percentage of each pathogen isolated from the different sample types. For example, E. coli was predominantly isolated from urine (49.0%) while Klebsiella spp. was most frequently found in wound samples (36.4%). This distribution highlights the variation in bacterial pathogens across different infection sites and underscores the clinical relevance of each pathogen.

Table II Prevalence of ESBL-producingisolates

Pathogen	ESBL-Positive in Urine Isolates (n=51)	Percentage (%)	ESBL-Positive in Wound Isolates (n=11)	Percentage (%)	ESBL-Positive in Sputum Isolates (n=4)	Percentage (%)	ESBL-Positive in Vaginal Swab Isolates (n=8)	Percentage (%)
Escherichia coli	18	72.0%	1	9.1%	1	50.0%	2	66.7%
Klebsiella spp.	8	66.7%	3	75.0%	0	0.0%	0	0.0%
Staphylococcus aureus	3	37.5%	1	50.0%	0	0.0%	1	50.0%
Pseudomonas aeruginosa	0	0.0%	2	66.7%	1	100%	0	0.0%
Proteus mirabilis	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Enterococcus faecalis	0	0.0%	0	0.0%	0	0.0%	1	100%
Total ESBL-Positive	29	56.9%	7	63.6%	2	50.0%	4	50.0%

This Table shows the prevalence of ESBL (Extended-Spectrum Beta-Lactamase)-producing isolates of the major pathogens identified in the clinical samples. The table lists the number and percentage of ESBL-positive isolates for each pathogen across the different sample types: urine, wound swabs, sputum and vaginal swabs. For example, E. coli had a high percentage of ESBL-positive isolates in urine (72.0%) and Klebsiella spp. showed a notable rate of ESBL positivity in wound swabs (75.0%). This data is crucial in understanding the antimicrobial resistance patterns and the prevalence of ESBL-producing strains in different types of infections.

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Table III Antibiotic Resistance profiles of Key Pathogens

Antibiotic	*E. coli* (n=25)	*Klebsiella spp.* (n=12)	*Staphylococcus aureus* (n=8)	*Pseudomonas aeruginosa* (n=4)	*Proteus mirabilis* (n=2)
Ampicillin	22 (88%)	10 (83.3%)	6 (75%)	2 (50%)	2 (100%)
Amoxicillin-Clavulanic Acid	18 (72%)	9 (75%)	4 (50%)	2 (50%)	1 (50%)
Ceftriaxone	20 (80%)	10 (83.3%)	3 (37.5%)	3 (75%)	2 (100%)
Ciprofloxacin	19 (76%)	8 (66.7%)	4 (50%)	2 (50%)	2 (100%)
Levofloxacin	17 (68%)	7 (58.3%)	3 (37.5%)	1 (25%)	2 (100%)
Gentamicin	13 (52%)	5 (41.7%)	2 (25%)	1 (25%)	2 (100%)
Nitrofurantoin (Urine Only)	10 (40%)	5 (41.7%)	N/A	N/A	N/A
Imipenem	2 (8%)	3 (25%)	N/A	2 (50%)	N/A
Meropenem	1 (4%)	2 (16.7%)	N/A	2 (50%)	N/A

This Table provides a detailed account of the antibiotic resistance profiles of the major pathogens isolated from the clinical samples and the percentage of resistance for each antibiotic tested across the key pathogens (*E. coli, Klebsiella spp., Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus mirabilis*). Antibiotics tested include ampicillin, amoxicillin-clavulanic acid, ceftriaxone, ciprofloxacin, levofloxacin, gentamicin, nitrofurantoin (For urine isolates only) and the carbapenems (Imipenem and meropenem). For example, *E. coli* exhibited high resistance to ampicillin (88%) and ciprofloxacin (76%), while resistance to carbapenems was low (4%).

Discussion

The findings of this study highlighted significant insights into the prevalence, distribution and antimicrobial resistance patterns of bacterial pathogens isolated from clinical samples in a healthcare setting. The study focused on common pathogens such as *Escherichia coli, Klebsiella spp. Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus mirabilis,* which are well-known contributors to urinary, wound, sputum, and vaginal infections. The findings underscore the growing threat of Antimicrobial Resistance (AMR) particularly in the context of ESBL-producing organisms, which are increasingly being reported globally. The distribution of pathogens among various clinical samples revealed that *E. coli* was the predominant pathogen, isolated from 49.0% of urine samples. This is consistent with numerous studies, as E. coli remains the most common cause of Urinary Tract Infections (UTIs) worldwide. In contrast, wound swabs exhibited a higher prevalence of *Klebsiella spp.* accounting for 36.4% of isolates. This finding aligns with reports from other regions where *Klebsiella spp.* is recognized as a leading pathogen in wound infections, particularly in healthcare-associated settings.[16-19] Pseudomonas aeruginosa, a well-known opportunistic pathogen, was isolated from 18.0% of urine samples and 36.4% of wound swabs, reflecting its role in both chronic and hospital-acquired infections. *P. aeruginosa* is notoriously difficult to treat due to its ability to produce biofilms and acquire resistance to multiple classes of antibiotics, contributing to its high isolation rate in wound and sputum samples.[17] The presence of *Staphylococcus aureus* in both wound swabs and urine samples also highlights its role as a significant pathogen in both community-acquired and hospital-acquired infections. The prevalence of ESBL-producing strains is a growing concern in clinical microbiology, as these enzymes confer resistance to beta-lactam antibiotics, including penicillins and cephalosporins.[18] In our study, *E. coli* was the most common ESBL-producer among urinary isolates, with 72.0% of *E. coli* strains being ESBL-positive. This finding is consistent with global trends, where the prevalence of ESBL-producing *E. coli* has been rising rapidly, particularly in developing countries. *Klebsiella spp.* another major producer of ESBL, showed a high rate of ESBL positivity, especially in wound isolates (75.0%).[19,20] The increasing prevalence of ESBL-producing organisms is concerning, as it limits the effectiveness of commonly used antibiotics, complicating treatment regimens and contributing to longer hospital stays and increased mortality rates.[21] The antibiotic resistance profiles demonstrated in this study highlight the alarming resistance rates among the isolated pathogens, particularly against first-line antibiotics. *E. coli* exhibited high resistance rates to ampicillin (88%), ciprofloxacin (76%), and ceftriaxone (75%). These high resistance rates to commonly used antibiotics are consistent with the growing concern of resistance to fluoroquinolones and cephalosporins, which are frequently prescribed for urinary and systemic infections. Similarly, *Klebsiella spp.* also demonstrated high resistance to these antibiotics, with 80.0% of isolates showing resistance to ceftriaxone.[22] The emergence of such resistant strains calls for more stringent antibiotic stewardship and the use of more advanced diagnostic methods to guide appropriate antibiotic therapy.[23] Interestingly, the study revealed that carbapenems, including imipenem and meropenem, retained their efficacy with low resistance rates (Around 4% for *E. coli*).[23] This suggests that carbapenems could still be a viable option for treating infections caused by Multidrug-Resistant (MDR) pathogens, although their use should be carefully controlled to prevent further resistance development.

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The low resistance to aminoglycosides such as amikacin and gentamicin is also noteworthy, suggesting that these drugs may still offer a treatment option for certain resistant infections, though their use should be based on susceptibility testing.[24,25]

Clinical implications

The high prevalence of ESBL-producing strains in clinical isolates of *E. coli* and *Klebsiella spp.,* coupled with significant resistance to commonly used antibiotics, underscores the urgent need for effective surveillance, infection control measures, and the rational use of antibiotics in healthcare settings.[26] The findings also emphasize the importance of timely and accurate microbiological diagnostics to guide appropriate antimicrobial therapy. Given the high rates of resistance to fluoroquinolones and beta-lactams, clinicians should consider alternative treatments such as carbapenems or aminoglycosides, but only after susceptibility testing.[27,28] Furthermore, the results point to the importance of infection prevention strategies, particularly in hospital settings, where *Klebsiella spp.* and *Pseudomonas aeruginosa* are frequently encountered. Improved hygiene practices, proper wound care and appropriate antimicrobial prophylaxis can help reduce the incidence of these infections and mitigate the spread of resistant strains.[29]

Limitations

While this study provides valuable data on the prevalence and resistance patterns of ESBL-producing and multidrug-resistant pathogens, it is limited by its single-center design and relatively small sample size.

Recommendation

Future studies with larger, multi-center cohorts are recommended to validate these findings and assess regional differences in resistance patterns. Additionally, molecular techniques such as PCR and whole-genome sequencing could be employed to identify specific ESBL genes and map the genetic mechanisms underlying resistance.

Conclusions

The study highlights the growing threat of ESBL-producing and multidrug-resistant pathogens in clinical infections, particularly urinary and wound infections. The high rates of resistance observed underscore the need for continued surveillance, prudent use of antibiotics, and targeted infection control measures to combat this global health challenge.

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Disclosure

Theauthors declare no conflict of interest.

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