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Comparative Analysis of Carbapenem-Resistant Enterobacteriaceae in Community- and Hospital-Acquired Urinary Tract Infections in a Tertiary Care Hospital of Northern India

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ABSTRACT

Background: Carbapenem-resistant Enterobacteriaceae (CRE) have emerged as a significant public health concern due to limited therapeutic options and increasing prevalence in urinary tract infections (UTIs). Comparative data on CRE-associated UTIs in community- and hospital-acquired settings in Northern India remain limited.

Methods: A cross-sectional observational study was conducted over six months (July-December) in the Department of Microbiology at Hamdard Institute of Medical Sciences and Research, New Delhi. A total of 1687 urine samples were processed, yielding 589 Enterobacteriaceae isolates. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method and the VITEK 2 Compact system according to CLSI guidelines. Carbapenem resistance was confirmed phenotypically, and carbapenemase production was detected using the Modified Hodge Test. Biofilm production was assessed using the microtiter plate method. Statistical analysis was performed using the chi-square test.

Results: Of the 589 Enterobacteriaceae isolates, 122 (20.7%) were CRE. CRE were more frequently isolated from inpatients (53.2%) than outpatients (46.7%). Escherichia coli (50.8%) was the predominant CRE isolate. High resistance was observed to nalidixic acid (82.7%) and ampicillin (76.2%), whereas colistin (96%) showed the highest sensitivity. Biofilm production was detected in 51.6% of CRE isolates and was significantly associated with hospital-acquired UTIs (p=0.03).

Conclusion: CRE prevalence remains substantial in both community and hospital settings, with higher biofilm production among hospitalized and catheterized patients. Strengthening antimicrobial stewardship and infection control measures is essential.

Key-words: Carbapenem-resistant Enterobacteriaceae, Urinary tract infection; Antimicrobial resistance, Biofilm, Hospital-acquired infection

INTRODUCTION

Enterobacteriaceae are among the most clinically significant families of Gram-negative bacilli and remain the major etiological agents of urinary tract infections (UTIs) worldwide [1].

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UTIs are responsible for a large number of outpatient visits, hospital admissions, and antibiotic prescriptions in all age groups. *Escherichia coli* remains the most common uro-pathogen, followed by Klebsiella species, Proteus species, and Citrobacter species. While most community-acquired UTIs are traditionally amenable to treatment with oral antimicrobial agents, the growing emergence of multidrug-resistant strains has radically changed therapeutic strategies [2]. Carbapenems have long been considered the cornerstone of treatment of infections caused by extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae because of their stability to most beta-lactamases and favourable

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pharmacokinetic properties [3]. However, the development and spread of carbapenem-resistant Enterobacteriaceae (CRE) worldwide are serious public health concerns. Resistance to carbapenems is mostly mediated by production of carbapenemase enzymes such as *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo- v-lactamase (VIM) and OXA-48-like enzymes. The addition of other mechanisms, such as the loss of porins associated with ESBL production and efflux pump overexpression, contributes to resistance phenotypes [4,5]. The speed with which CRE is spreading is especially concerning in developing countries, where regulation of antibiotic use may be inconsistent and over-the-counter sale of antimicrobials may remain [6]. In India, the burden of antimicrobial resistance has been worsened by high population density, widespread antibiotic exposure, inadequate infection-control infrastructure in some settings, and horizontal gene transfer via plasmids and transposons [7]. The finding of NDM-producing strains in community settings blurs the historical distinction between the acquisition of drug resistance in clinical settings and in the community [8]. Urinary tract infections can be broadly classified as community-acquired UTIs (CA-UTIs) and hospital-acquired UTIs (HA-UTIs), including catheter-associated UTIs (CAUTIs). Hospitalized patients are especially susceptible to invasive procedures, length of hospitalization, immunosuppression, and prior antibiotic exposure. Indwelling urinary catheters are a nidus for microbial colonization, biofilm formation, and persistent infection [9,10]. Biofilms represent complex microbial communities encased in extracellular polymeric substances that confer enhanced resistance to antimicrobial agents and host immune responses [11].

MATERIALS AND METHODS

Study Design and Setting- This cross-sectional observational study was conducted over six months (July-December) in the Department of Microbiology, Hamdard Institute of Medical Sciences and Research (HIMSR), New Delhi, a tertiary care hospital.

Study Population- A total of 1687 urine samples received in the microbiology laboratory during the study period were included. Samples were obtained from patients of all age groups and both genders presenting with clinical suspicion of UTI. Both midstream clean-catch urine samples and catheter-collected urine specimens were processed according to standard guidelines.

Inclusion criteria- Urine samples yielding isolates belonging to the Enterobacteriaceae family.
Samples from both community-acquired and hospital-acquired infections.

Exclusion criteria- Samples yielding non-Enterobacteriaceae organisms. Duplicate isolates from the same patient during the same episode. Hospital-acquired UTI was defined as an infection occurring ≥48 hours after hospital admission, in accordance with CDC guidelines.

Microbiological Processing- Urine samples were subjected to semi-quantitative culture using a calibrated loop method (0.001 mL loop). Samples were inoculated onto Blood agar and MacConkey agar plates and incubated aerobically at 37°C for 18-24 hours. Significant bacteriuria was defined as $\geq 10^5$ CFU/mL for midstream urine samples, in accordance with standard microbiological criteria.
Preliminary identification was based on colony morphology, blood agar, lactose fermentation on MacConkey agar, Gram staining, and standard biochemical reactions, including the indole test, citrate utilization test, urease test, triple sugar iron (TSI) agar reaction, methyl red test, and Voges-Proskauer test. Identification confirmation was performed using the VITEK 2 Compact automated system, as applicable.

Antimicrobial Susceptibility Testing
Antimicrobial susceptibility testing was performed using:
1. Kirby-Bauer disk diffusion method on Mueller-Hinton agar.
2. VITEK 2 Compact automated system for confirmation.

Interpretation of zone diameters and minimum inhibitory concentrations (MICs) was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Isolates resistant to one or more carbapenems were categorized as CRE.

Detection of Carbapenem Resistance- Phenotypic detection of carbapenemase production was performed using the Modified Hodge Test (MHT). A lawn culture of

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carbapenem-susceptible *E. coli* ATCC 25922 was inoculated on Mueller-Hinton agar. A carbapenem disk was placed at the center of the plate, and the test isolate was streaked from the disk to the periphery. After incubation, a cloverleaf-shaped indentation indicated carbapenemase production.

Biofilm Detection- Biofilm formation was assessed using the quantitative microtiter plate method. Briefly, overnight cultures of CRE isolates were inoculated into tryptic soy broth supplemented with 1% glucose and incubated at 37°C for 24 hours. The cultures were diluted and inoculated into sterile 96-well flat-bottom microtiter plates. After incubation, wells were gently washed with phosphate-buffered saline to remove planktonic cells.
The adherent biofilm was fixed with methanol, stained with 0.1% crystal violet, and excess stain was removed by washing. The bound dye was solubilized using an ethanol-acetone solution, and optical density (OD) was measured at 570 nm using a microplate reader. Based on OD values, isolates were categorized as non-biofilm producers, weak, moderate, or strong biofilm producers.
This method allows quantitative assessment of biofilm biomass and is widely accepted for in vitro biofilm analysis. Biofilm production was correlated with hospital-acquired infection status and catheterization history.

Statistical Analysis- Data were entered into Microsoft Excel and analysed using SPSS version 22. Categorical variables were expressed as percentages. The association between biofilm production and hospital-acquired infection was assessed using the chi-square test. A p-value <0.05 was considered statistically significant.

Ethical Approval- Ethical approval for the study was obtained from the Institutional Ethics Committee of Jamia Hamdard University (Approval No: IEC/HIMSR/2018/14/18). Patient confidentiality was strictly maintained, and all data were anonymized before analysis.

RESULTS

In our study, out of a total of 1687 bacterial isolates, 589 (34 9%) strains of Enterobacteriaceae and 1098 (65.1%) strains of other bacteria were isolated. Out of the total 589 isolates of Enterobacteriaceae, 467 (79.3%) were Carbapenem-sensitive Enterobacteriaceae and 122 (20.7%) were Carbapenem-resistant Enterobacteriaceae s depicted in Table 1.

Table 1: Prevalence of Enterobacteriaceae and CRE

Parameter	Number	Percentage (%)
Total urine samples	1687	100
Enterobacteriaceae	589	34.9
CRE	122	20.7

The present study showed that isolation of CRE strains was higher in female patients (82, 35.24%) than in male patients (40, 17.21%). Isolation of CRE strains varied across age groups. The highest number of patients was in the 16-35 years age group, 58 (47.5%), followed by >50 years, 30 (24.5%), and 27 (22.3%) in the 36-50 years age group. It was lowest in the 5-15 years age group, 7 (5.7%).
Table 2 shows the age-wise distribution of CRE strains in IPD and OPD. CRE were significantly more common among IPD patients 65 (53.2%) compared to OPD patients 57 (46.7%) (p = 0.04).

Table 2: Distribution of CRE in IPD and OPD

IPD/OPD	Number of CRE samples (n)	Percentage (%) (n/Nx100)
IPD	65	53.2
OPD	57	46.72
Total (N)	122	100

Among the 122 total CRE strains, the most commonly isolated organism was *E. coli* (58.8%), followed by Klebsiella species (19.6 %) and Proteus species (19.6%). The highest prevalence was for E. coli (57.5%), which was higher in IPD patients than in OPD patients among the 122 CRE.

Table 3: Distribution of CRE Isolates (n=122)

Organism	Number	Percentage (%)
E. coli	62	50.8
Klebsiella spp.	24	19.6
Proteus spp.	20	16.3
Citrobacter spp.	8	6.5

Antimicrobial susceptibility testing revealed high resistance to commonly used oral antibiotics, particularly nalidixic acid (82.7%) and ampicillin (76.2%).

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Fluoroquinolone and cotrimoxazole resistance were also substantial, limiting empirical oral treatment options. Among the tested drugs, colistin demonstrated the highest susceptibility (96%). Moderate susceptibility was observed with nitrofurantoin and aminoglycosides such as amikacin. Carbapenem resistance remained significant, with resistance observed to imipenem (58.1%) and meropenem (45%). Overall, reserve antibiotics retained greater activity than conventional agents.

A total of 65 IPD CRE isolates were obtained, of which 50 (79.9%) patients were catheterised. Among 122 isolates, 63 (51.6%) isolates produced biofilm. Biofilm production was significantly higher among hospital-acquired UTI isolates compared to community-acquired isolates.

DISCUSSION

Enterobacteriaceae are major contributors to the intrinsic human gut flora. They can also colonize patients' guts and spread through the community via the faeco-oral route. Thus, the spread of CRE is deeply disconcerting in a country such as India, with a population of more than 1.4 billion. [12,13] The first step in dealing with the problem of CRE is the identification of infected patients. Antibiotic resistance in bacterial pathogens is an ever-evolving survival tactic that has enabled bacteria to outlast available antibiotics [14]. The increasing resistance to available antibiotics and the emergence of new resistance in common bacterial pathogens are now of major concern worldwide.

The present study was carried out on 122 CRE isolates obtained from urine specimens collected over 6 months. The results were analyzed for the prevalence of CRE in hospitals, antimicrobial sensitivity patterns, carbapenem detection, and biofilm production. In the present study, the overall prevalence of Enterobacteriaceae strains was 34.9%, which was similar to that reported by Deshpande *et al.* [12] (36.2%). In our study, 122/589 carbapenem-resistant enterobacteriaceae were isolated from urine samples (20.7%), which is consistent with a study by Gupta et al., which reported similar isolation rates (17 to 22%) of CRE from urine samples [1]. In contrast, another study by Wattal *et al.* [15] reported a higher prevalence (13-51%).

Our study showed that the maximum number of isolates was from females (53.2%) compared with male patients (46.7%). A similar result was reported in a study by Bardoloi and Yogeesha Babu *et al*. [9], which showed a greater number of isolates from females (55%) than from male patients (45%). The isolation rate of CRE varies by age group. The high prevalence of UTI in this study was observed in the age group of 16-35 years (53.3%), which is in accordance with other studies by Niveditha S. et al. (2012) and 15-90 years (62%). [16] In our study, *E. coli* (50.8%) was the most commonly isolated organism, followed by *K. pneumoniae* (6.5%), with the remaining members of the Enterobacteriaceae family. Similar results were reported in the study by Nair *et al.* [17], with low prevalence rates of *E. coli* (23%) and *K. pneumoniae* (4%). In comparison, another study conducted by Bardoloi and Yogeesha Babu [9] showed higher prevalence of *E. coli* (46.05%) and Klebsiella species (61.5%).

In our study, among all CRE samples, samples from patients were more from IPD (53.2%) than from OPD (46.72%), which is consistent with another study by Nair *et al*. [17], which reported IPD (68%) and OPD (19%). The reason may be more catheterizations and a longer hospital stay for the patient. 79.9% of our IPD patients were catheterized, and the rest had UTIs due to other reasons. In our study, the overall biofilm production among the bacterial isolates from urine samples was 51.63%. The study demonstrated that the CA-UTI showed a higher BPP rate (40.1%) than the Com-UTI (11.4%). A similar study by Abdallah *et al.* reported a lower prevalence of BPP in Com-UTI than in CA-UTI using different genotypic and phenotypic methods. [18] In contrast, a study by Bardoloi and Yogeesha Babu [9] showed a higher BPP prevalence in Com-UTI (76.19%) than in CA-UTI (60.78%).

In our study, the total Enterobacteriaceae samples received in the laboratory (IPD & OPD) were 589; of them, 122 were Carbapenem-Resistant Enterobacteriaceae (20.7%), which include several members of the Enterobacteriaceae family such as *E. coli* (50.8%), *K. pneumoniae* (6.5%), Klebsiella species (19.6%), Citrobacter species (6.5%), and Proteus species (16.3%). These bacteria show inherent resistance to the most commonly used antibiotics, which is of concern because they are the commonest agents of hospital- and community-acquired infections.

Our study demonstrated that the highest Ampicillin resistance was found in E. coli (85.4%), which is similar to the study by Perry *et al.* [19] (94%), and the highest

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ampicillin resistance rate was observed in the study by Ejaz *et al.* [20] (100%). Similarly, the present study is similar to a study done by Von Baum and Marre (2007) (76%) [21].

Among Enterobacteriaceae, *E. coli* and *K. pneumoniae* are common causes of UTI in humans [22,23]. The sites of infection are usually important for treatment considerations, because the kinetics and fate of a drug in the body depend on several factors, such as its release from the dosage form. Absorption from the site of administration into the bloodstream, distribution to various parts of the body, including the site of action, and the rate of elimination from the body via metabolism or excretion of the unchanged drug.

Among the non-beta-lactam antibiotics, the highest resistance rates were observed for nalidixic acid, ciprofloxacin, and co-trimoxazole. The observation may be due to the widespread use of these drugs empirically because they are relatively cheap and, as oral antibiotics, easy to administer. In *E. coli* isolates, overall resistance to nalidixic acid, ciprofloxacin, and co-trimoxazole was 88.7%, 80.6%, and 79%, respectively. In *K. pneumoniae* isolates, the overall resistance to nalidixic acid, ciprofloxacin, and co-trimoxazole was 75%, 62.5%, and 50%, respectively.

A similar resistance pattern was observed in a study by Kothari and Sagar [23], which showed Nalidixic acid, Ciprofloxacin, and Co-trimoxazole at 67.8%, 63.51%, and 60.52%, respectively, in *E. coli* isolates. Nalidixic acid, Ciprofloxacin, and Co-trimoxazole were 60.73%, 62.16%, and 58.18%, respectively, in *Klebsiella pneumoniae*. Higher resistance rates for these antibiotics were also observed in other studies (Moyo *et al.* [24]). Therefore, resistance to Co-trimoxazole may be due to its widespread use for prophylaxis against opportunistic infections.

All CRE isolates were tested for resistance to two aminoglycosides, amikacin and gentamicin. Overall, 43.5% of *E. coli* and 37.5% of *K. pneumoniae* isolates were resistant to amikacin. [24,25]. Another study reported by Khatri *et al.* showed resistance rates of 60% for amikacin and 55% for gentamicin among *K. pneumoniae* isolates [26]. The high degree of Cefuroxime resistance (68.8%) reflects extensive use of broad-spectrum antibiotics, especially third-generation cephalosporins, which are recommended as first-line drugs for the treatment of nosocomial and community-acquired infections, similar to the study by Solomkin *et al*. [27] that reported a similar resistant rate.

Carbapenems are used as a last resort for treating infections caused by ESBL-producing, multidrug-resistant bacteria. Carbapenems such as imipenem and meropenem have broad-spectrum activity and are resistant to β-lactamases, including ESBLs [28]. The resistance patterns observed in our study for the different isolates from urine samples were as follows: E. coli (53.2% carbapenem resistance), *K. pneumoniae* (7.3%), K. species (20.4%), Proteus species (26.2%), and Citrobacter species (7.3%). A similar study by Gupta *et al*. [29] demonstrated meropenem (50.78%) and imipenem (13.02%) among Enterobacteriaceae isolates. A similar study reported a lower incidence of imipenem resistance than of meropenem resistance among nosocomial pathogens. It is now well documented that the indiscriminate use of antibiotics in healthcare facilities has led to resistance among isolates [30].

CONCLUSIONS

Carbapenem-resistant Enterobacteriaceae pose a growing threat to the management of UTIs in the community and hospitals. The finding of CRE in almost one-fifth of Enterobacteriaceae isolates in the current study underscores the need to strengthen antimicrobial use programs and infection prevention measures. The increased prevalence in patient groups who are hospitalized and on catheters underlines the need to optimize the unnecessary device use and antimicrobial prescribing practices. The high proportion of biofilm-forming CRE isolates further complicates therapy, contributing to persistent infection, reinfection, and increased healthcare costs. This twin challenge of resistance and virulence requires a multi-pronged strategy that incorporates microbiological surveillance and good antimicrobial stewardship and addresses infection control vigilance and clinician awareness. Early recognition of CRE, rational selection of antibiotics based on susceptibility testing, and periodic review of institutional antibiogram databases are key to maintaining the effectiveness of therapeutic options. Future studies should include molecular characterization of resistance determinants to better resolve transmission dynamics and make effective targeted containment strategies. Multicentric studies across different geographical regions would strengthen epidemiological

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understanding and policy formulation. In conclusion, containment of CRE requires sustained institutional commitment, interdisciplinary collaboration, integration with microbiological surveillance, and rational implementation of antimicrobial policies.

CONTRIBUTION OF AUTHORS

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