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Antimicrobial Susceptibility Profile and Phenotypic Screening of Carbapenem-Resistant Enterobacteriaceae Isolated from Pus Samples at a Tertiary Care Hospital

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ABSTRACT

Introduction: Carbapenem-resistant Enterobacteriaceae (CRE) are important multidrug-resistant pathogens causing wound infections. This study was conducted to assess the antimicrobial susceptibility profile and phenotypic screening of CRE isolated from pus samples.
Materials and Methods: This prospective cross-sectional study was conducted in the Department of Microbiology, Bidar Institute of Medical Sciences, Bidar. A total of 100 non-duplicate Enterobacteriaceae isolates from pus samples were included. Isolates were identified by standard microbiological methods. Antimicrobial susceptibility testing was done by Kirby–Bauer disc diffusion method as per CLSI guidelines. Carbapenem resistance was screened phenotypically using imipenem and meropenem discs.
Results: Wound infection was the most common diagnosis, accounting for 58.0% of cases. Females constituted 78.0%, and the most common age group was 21–40 years (52.0%). Most samples were received from the Obstetrics and Gynaecology department (47.0%). Klebsiella pneumoniae was the most common isolate (28.0%), followed by Escherichia coli (27.0%). CRE was detected in 41.0% of isolates. Piperacillin–tazobactam showed the highest sensitivity (78.0%), while highest resistance was observed against ceftriaxone (70.0%), cefepime (57.0%), and ciprofloxacin (54.0%).
Conclusion: The study showed a high prevalence of CRE among pus isolates. Routine culture, antimicrobial susceptibility testing, rational antibiotic use, and strict infection control measures are essential to reduce the spread of CRE.

***Keywords**: Carbapenem-resistant Enterobacteriaceae, pus samples, antimicrobial susceptibility, Klebsiella pneumoniae, Escherichia coli, imipenem, meropenem.*

INTRODUCTION

Enterobacteriaceae constitute an important group of Gram-negative bacilli responsible for a wide spectrum of community-acquired and hospital-acquired infections[1]. These organisms are commonly implicated in urinary tract infections, gastrointestinal infections, respiratory tract infections, bloodstream infections, wound infections and infections associated with invasive medical devices.[2] Among these, pus and wound infections represent a major clinical burden, particularly in surgical wards, orthopaedic units, intensive care units, burns units, and other hospital settings where prolonged hospitalization, invasive procedures, underlying comorbidities, and prior antibiotic exposure increase the risk of infection with resistant organisms.[3]The clinical management of wound infections depends largely on early isolation of the causative organism and timely initiation of appropriate antimicrobial therapy. However, treatment has become increasingly difficult due to the emergence and spread of multidrug-resistant Gram-negative bacteria. Enterobacteriaceae possess a remarkable ability to acquire and disseminate antimicrobial resistance determinants through plasmids, transposons, integrons, and other mobile genetic elements.[4] This facilitates rapid spread of resistance within the same bacterial species as well as across different species, thereby creating a significant challenge for clinicians, microbiologists, and infection control teams.Carbapenems are broad-spectrum β-lactam antibiotics and are often considered reserve or last-line agents for the treatment of severe infections caused by multidrug-resistant Gram-negative bacilli. They are especially useful against organisms producing extended-spectrum β-lactamases and AmpC β-lactamases.[4,5] However, the increasing emergence of carbapenem-resistant Enterobacteriaceae has become a serious global public health concern. Carbapenem-resistant Enterobacteriaceae are associated with limited therapeutic options, prolonged hospital stay, increased healthcare expenditure, higher risk of treatment failure, and increased morbidity and mortality.[1,5]

Carbapenem resistance in Enterobacteriaceae may occur through multiple mechanisms. The most important mechanism is production of carbapenemase enzymes, which hydrolyse carbapenems and other β-lactam antibiotics. Other mechanisms include loss or alteration of outer membrane porins, increased efflux pump activity, production of β-lactamases combined with reduced membrane permeability, and changes in penicillin-binding proteins.[4,5] The genes encoding carbapenemases are often located on plasmids, enabling rapid horizontal transfer and dissemination within healthcare settings. Therefore, early laboratory detection of carbapenem resistance is essential not only for appropriate patient management but also for implementation of infection prevention and antimicrobial stewardship measures.[1,6]In India, carbapenem-resistant Enterobacteriaceae have been increasingly reported from various clinical samples, including urine, pus, wound swabs, blood, respiratory secretions, and endotracheal aspirates. Previous Indian studies have shown variable prevalence of carbapenem resistance among Enterobacteriaceae, reflecting differences in geographical region, hospital setting, sample type, patient population, antibiotic usage patterns, and infection control practices.[7-10] Pawar et al. reported a high prevalence of carbapenem-resistant Enterobacteriaceae in a tertiary teaching hospital from rural western India, with pus/wound swabs being one of the important sources of isolates.[7] Thomas and Sarwat also observed carbapenem-resistant Enterobacteriaceae in a tertiary care hospital in North India, highlighting the growing burden of resistance in hospital settings.[8] Similarly, studies from South India and Karnataka have reported carbapenem resistance among Enterobacteriaceae isolated from various clinical samples, including pus samples.[9,10]Pus samples are particularly important because wound infections are commonly encountered in both outpatient and inpatient settings. Resistant organisms in such infections may contribute to delayed wound healing, chronic infection, surgical site complications, prolonged hospitalization, and nosocomial transmission.[3,7,10] Hence, routine monitoring of bacterial isolates from pus samples and their antimicrobial susceptibility pattern is essential for guiding empirical therapy and strengthening hospital infection control practices.Phenotypic screening using carbapenem discs such as imipenem and meropenem by the Kirby–Bauer disc diffusion method remains a practical and widely used approach in routine diagnostic microbiology laboratories, especially in resource-limited settings. Although molecular methods provide definitive identification of resistance genes, they may not be available in many tertiary care hospitals due to cost and infrastructural limitations. Hence, standardized phenotypic screening as per Clinical and Laboratory Standards Institute guidelines plays an important role in detecting suspected carbapenem-resistant isolates and guiding further clinical and infection control decisions.[6]

Knowledge of the local antimicrobial susceptibility profile is essential because resistance patterns vary from one region to another and even between different units within the same hospital. Periodic surveillance of Enterobacteriaceae isolated from pus samples can help identify the burden of carbapenem resistance, guide empirical antibiotic policies, reduce irrational antibiotic use, and support hospital infection control programmes.[8-10] With this background, the present study was undertaken to isolate and identify Enterobacteriaceae from pus samples, determine their antimicrobial susceptibility pattern, phenotypically screen them for carbapenem resistance.

MATERIALS AND METHODS

This prospective cross-sectional study was conducted in the Department of Microbiology, Bidar Institute of Medical Sciences, Bidar, Karnataka. The study included pus samples received from patients with clinically suspected wound infections attending outpatient departments or admitted to various hospital wards.The study was conducted over a period of six months, from January 2026 to June 2026. A total of 100 non-duplicate Enterobacteriaceae isolates obtained from pus samples were included. IEC Approval Letter No.: 348/BRIMS/IEC 2026-1

Inclusion and exclusion criteria

Pus samples showing significant aerobic growth of Enterobacteriaceae and collected before initiation of antibiotics or within 48 hours of antibiotic therapy were included. Duplicate isolates, mixed bacterial growth, isolates other than Enterobacteriaceae, contaminated or improperly labelled samples, and samples collected after prolonged antibiotic therapy were excluded.

Sample collection and processing

Pus samples and wound swabs were collected aseptically from the active edge of the wound infected site using sterile swabs and transported immediately to the microbiology laboratory. Samples were processed within two hours of collection. Direct Gram staining was performed, and samples were inoculated on blood agar and MacConkey agar. Plates were incubated aerobically at 37°C for 18–24 hours.

Identification of isolates
Enterobacteriaceae isolates were identified based on colony morphology, Gram staining, oxidase test, and standard biochemical tests including indole, methyl red, Voges–Proskauer, citrate utilization, triple sugar iron agar, urease, and motility tests.

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing was performed by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar as per CLSI guidelines. The antibiotics tested were cefepime, ciprofloxacin, piperacillin–tazobactam, ceftriaxone, meropenem, imipenem, and amikacin. Zones of inhibition were measured and interpreted according to CLSI breakpoints.[17]

Phenotypic screening for carbapenem resistance
All confirmed Enterobacteriaceae isolates were screened for carbapenem resistance using imipenem 10 µg and meropenem 10 µg discs by the Kirby–Bauer disc diffusion method. Isolates showing reduced susceptibility or resistance to imipenem and/or meropenem were labelled as carbapenem-resistant Enterobacteriaceae.

Statistical analysis
Data were entered in Microsoft Excel and analysed using SPSS.29statistical software. Categorical variables were expressed as frequency and percentage. Results were presented in the form of tables and graphs.

RESULTS
A total of 100 Enterobacteriaceae isolates obtained from pus samples were included in the present study. The most common clinical diagnosis was wound infection, observed in 58 cases (58.0%), followed by purulent ear discharge and diabetic foot ulcer in 11 cases (11.0%) each. Abscess was present in 10 cases (10.0%), cellulitis in 7 cases (7.0%), and necrotizing fasciitis in 3 cases (3.0%).

In the present study, females constituted the majority of cases, accounting for 78 cases (78.0%), while males constituted 22 cases (22.0%). Age-wise distribution showed that the maximum number of cases belonged to the 21–40 years age group, comprising 52 cases (52.0%). This was followed by 41–60 years with 27 cases (27.0%), 0–20 years with 12 cases (12.0%), and >60 years with 9 cases (9.0%).

Department-wise distribution showed that the highest number of samples was received from the Department of Obstetrics and Gynaecology, accounting for 47 cases (47.0%), followed by Surgery with 21 cases (21.0%), Medicine with 13 cases (13.0%), ENT with 11 cases (11.0%), and Orthopedics with 8 cases (8.0%).

Among the Enterobacteriaceae isolates, *Klebsiella pneumoniae* was the most commonly isolated organism, accounting for 28 isolates (28.0%), followed closely by *Escherichia coli* with 27 isolates (27.0%). *Proteus mirabilis* was isolated in 15 cases (15.0%), Citrobacter koseri in 13 cases (13.0%), *Klebsiella* oxytoca in 8 cases (8.0%), Citrobacter freundii in 7 cases (7.0%), and *Proteus vulgaris* in 2 cases (2.0%).

Phenotypic screening for carbapenem resistance showed that 41 isolates (41.0%) were carbapenem-resistant Enterobacteriaceae, while 59 isolates (59.0%) were carbapenem-sensitive. Thus, the prevalence of carbapenem-resistant Enterobacteriaceae among pus isolates in the present study was 41.0%.

Antimicrobial susceptibility testing showed that the highest sensitivity was observed to piperacillin–tazobactam, with 78 isolates (78.0%) being sensitive, followed by meropenem and imipenem with 59 isolates (59.0%) sensitive to each. Amikacin sensitivity was observed in 51 isolates (51.0%), ciprofloxacin in 46 isolates (46.0%), cefepime in 43 isolates (43.0%), and ceftriaxone in 30 isolates (30.0%).

The highest resistance was observed against ceftriaxone, with 70 isolates (70.0%) resistant, followed by cefepime in 57 isolates (57.0%), ciprofloxacin in 54 isolates (54.0%), and amikacin in 49 isolates (49.0%). Resistance to both meropenem and imipenem was observed in 41 isolates (41.0%) each, while piperacillin–tazobactam showed the lowest resistance, with only 22 isolates (22.0%) resistant.

Overall, the study demonstrated that wound infection was the most common clinical presentation, females and the 21–40 years age group were predominantly affected, and the maximum number of isolates were received from the Obstetrics and Gynaecology department. *Klebsiella pneumoniae* and *Escherichia coli* were the predominant organisms isolated. A high prevalence of carbapenem resistance was observed, with 41.0% of isolates being CRE-positive, indicating a significant burden of carbapenem-resistant Enterobacteriaceae among pus samples.

Table 1. Distribution of Cases According to Clinical Diagnosis (n=100)

Diagnosis	Number of Cases (n)	Percentage (%)
Wound infection	58	58.0
Purulent ear discharge	11	11.0
Diabetic foot ulcer	11	11.0
Abscess	10	10.0
Cellulitis	7	7.0
Necrotizing fasciitis	3	3.0
Total	100	100.0

Table 2. Demographic Distribution of Study Participants (n=100)

Variable	Category	Number of Cases (n)	Percentage (%)
Age group	0–20 years	12	12.0
	21–40 years	52	52.0
	41–60 years	27	27.0
	>60 years	9	9.0
Gender	Female	78	78.0
	Male	22	22.0

Table 3. Distribution of Enterobacteriaceae Isolates (n=100)

Organism Isolated	Number of Isolates (n)	Percentage (%)
Klebsiella pneumoniae	28	28.0
Escherichia coli	27	27.0
Proteus mirabilis	15	15.0
Citrobacter koseri	13	13.0
Klebsiella oxytoca	8	8.0
Citrobacter freundii	7	7.0
Proteus vulgaris	2	2.0
Total	100	100.0

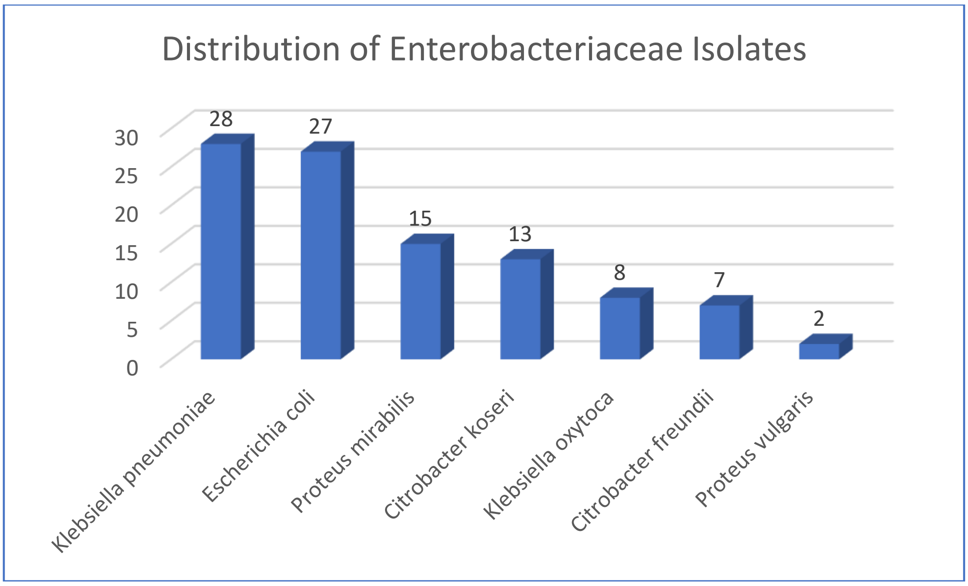

Figure 1: Distribution of Enterobacteriaceae Isolates (n=100)

Table 4. Antimicrobial Susceptibility Profile of Enterobacteriaceae Isolates (n=100)

Antibiotic	Sensitive n (%)	Resistant n (%)
Cefepime	43 (43.0)	57 (57.0)
Ciprofloxacin	46 (46.0)	54 (54.0)
Piperacillin–Tazobactam	78 (78.0)	22 (22.0)
Ceftriaxone	30 (30.0)	70 (70.0)
Meropenem	59 (59.0)	41 (41.0)
Imipenem	59 (59.0)	41 (41.0)
Amikacin	51 (51.0)	49 (49.0)

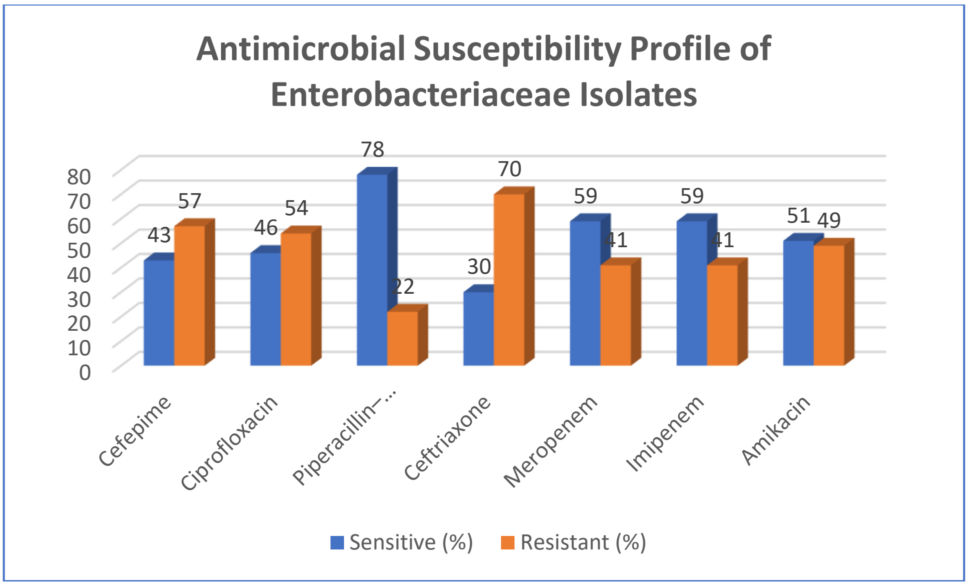

Figure 2: Antimicrobial Susceptibility Profile of Enterobacteriaceae Isolates

DISCUSSION

In the present study, wound infection was the most common clinical diagnosis (58.0%), followed by purulent ear discharge (11.0%), diabetic foot ulcer (11.0%), abscess (10.0%), cellulitis (7.0%), and necrotizing fasciitis (3.0%). The predominance of wound infections reflects the high burden of surgical site and soft tissue infections encountered in tertiary care hospitals. These findings are comparable to those of Rajput K et al.[11], who reported wound and pus infections as the major source of bacteriological specimens with a bacterial isolation rate of 58.3%. Similarly, Bhujugade SR et al.[12] highlighted wound infections as one of the most frequent causes of specimen submission in microbiology laboratories, emphasizing their contribution to healthcare-associated infections.

The majority of patients in the present study were aged 21–40 years (52.0%), followed by 41–60 years (27.0%), and females constituted 78.0% of the study population. This predominance of younger adults may be related to greater occupational exposure and healthcare utilization. In contrast, Rajput K et al.[11] observed that most patients belonged to the 40–60-year age group with a male predominance, while Mund K et al.[13] also reported that males constituted 64.0% of wound infection cases. These differences may reflect regional and demographic variations in study populations.

Among the Enterobacteriaceae isolates, *Klebsiella pneumoniae* (28.0%) was the predominant organism, closely followed by *Escherichia coli* (27.0%), while *Proteus mirabilis* (15.0%), *Citrobacter koseri* (13.0%), *Klebsiella oxytoca* (8.0%), *Citrobacter freundii* (7.0%), and *Proteus vulgaris* (2.0%) were isolated less frequently. These findings are in agreement with Mund K et al.[13], who identified *Klebsiella pneumoniae* and *Escherichia coli* among the major Gram-negative wound pathogens. Likewise, Bhujugade SR et al.[12] reported *Klebsiella* spp. as the second most common isolate after *Staphylococcus aureus*. A five-year surveillance study by Katoch O et al.[14] similarly demonstrated that *Escherichia coli* (52.8%) and *Klebsiella* spp. (18.4%) were the predominant Enterobacteriaceae isolated from clinical specimens.

The antimicrobial susceptibility pattern showed that Piperacillin–Tazobactam exhibited the highest sensitivity (78.0%), followed by Meropenem (59.0%), Imipenem (59.0%), Amikacin (51.0%), Ciprofloxacin (46.0%), Cefepime (43.0%), and Ceftriaxone (30.0%). Resistance was highest to Ceftriaxone (70.0%), followed by Cefepime (57.0%) and Ciprofloxacin (54.0%), indicating a considerable burden of multidrug resistance. Comparable findings were reported by Katoch O et al.[14], who observed high resistance to cephalosporins and quinolones, with carbapenems remaining the most effective agents. Sirisha M et al.[15] also demonstrated higher susceptibility to Piperacillin–Tazobactam (65.0%), Meropenem (87.4%), and Amikacin (99.2%), with relatively poor susceptibility to cephalosporins. Similarly, Madhusudhan K et al.[16] reported that *Klebsiella pneumoniae* isolates were most susceptible to Meropenem, Imipenem, and Piperacillin–Tazobactam.

Overall, the findings of the present study are consistent with previous reports, demonstrating the predominance of *Klebsiella pneumoniae* and *Escherichia coli* among Enterobacteriaceae isolates and the increasing resistance to cephalosporins and fluoroquinolones. The relatively preserved activity of Piperacillin–Tazobactam and carbapenems supports their continued role in the empirical management of serious Enterobacteriaceae infections. These observations emphasize the need for continuous antimicrobial surveillance, periodic institution-specific antibiograms, and robust antimicrobial stewardship programs to optimize antibiotic use and limit the emergence of multidrug-resistant organisms.

Limitation of the study

The present study had certain limitations. It was conducted at a single tertiary care hospital with a limited sample size, so the findings may not be generalizable to other settings. Only pus isolates were included, and molecular testing for carbapenem-resistance genes was not performed. Clinical outcomes, prior antibiotic exposure, treatment response, and long-term follow-up were also not assessed.

CONCLUSION

The study showed a considerable burden of carbapenem-resistant Enterobacteriaceae among pus isolates in a tertiary care hospital. A high level of resistance to commonly used antibiotics was observed, emphasizing the growing challenge of multidrug-resistant organisms in wound infections. Phenotypic screening remains useful for early detection of carbapenem resistance in routine microbiology laboratories. Strict antimicrobial stewardship, regular surveillance, and effective infection-control practices are essential to limit the spread of resistant Enterobacteriaceae.

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