

Study of Prevalence of Multidrug Resistant Gram-Negative Bacteria Isolated From Various Clinical Specimens in a Tertiary Care Hospital of Jaipur, Rajasthan

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Abstract

Introduction: Infection due to Gram negative bacilli (GNB) is the leading cause of mortality in hospitalized patients including ICU and ward. These GNB are capable of production of various enzymes like ESBL, carbapenemase. Anti-microbial Resistance (AMR) is raising serious threat to public health. Increased emergence of ESBL and CRE pathogens causing further makes the treatment difficult. This study aims to investigate the prevalence of MDR Gram-negative pathogens and proportion of ESBL producer and Carbapenem-resistant Enterobacterales, Acinetobacter baumannii, Pseudomonas aeruginosa, and to determine susceptibility pattern to various antibiotics.

Materials and Methods: This study was conducted over a period of 6 month from March to August 2025. The specimens were processed at the Microbiology Laboratory of JNUIMSRC Medical College, Jaipur, Rajasthan. Standard procedures were used to process the clinical specimens. ESBL producer and Carbapenem-resistant isolates were screened according to CLSI 2025 guideline.

Results: This study includes 200 isolates of Multidrug Resistant (MDR) Gram-negative Bacterial isolates of Enterobacterales, Acinetobacter baumannii, Pseudomonas aeruginosa. Of these in the Enterobacterales (108/148) 72.5% were ESBL-producing isolates and 79.50% (n = 159/200) were carbapenem resistant. The breakup is as follows: 108 (67.92%) were Enterobacterales, 42 (26.41%) were A. baumannii, and 10 (6.28%) were P. aeruginosa.

Conclusion: A high prevalence of ESBL production and Carbapenem resistant isolates was observed among Gram negative isolates. Early detection, isolation and contact precaution to these MDR organism and judicious usage of antibiotic will help to prevent dissemination of these isolates.

Keywords: Gram-negative bacteria, carbapenem-resistant, Enterobacterales, ESBL.

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Introduction

According to the World Health Organisation (WHO), resistance to commonly used antimicrobial agents is currently the most urgent problem to public health. [1]

Multidrug-resistant (MDR) bacteria carry much type of virulence factors and have acquired resistance mechanisms to counteract the most effective antibiotics. [2] Among MDR bacteria, the following pathogens like: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Enterobacter species, Acinetobacter baumannii and Pseudomonas aeruginosa, have been designated as the acronym “ESKAPE” because of their ability to escape the bactericidal activity of conventional antibiotics. [3] In February 2017, the WHO

developed a priority pathogen list where ESKAPE. Vancomycin-resistant Enterococcus (VRE) and methicillin-resistant Staphylococcus aureus (MRSA) were classified as high priority pathogens. Carbapenem-resistant Acinetobacter baumannii (CRAB), carbapenem-resistant Pseudomonas aeruginosa (CRPA), and carbapenem-producing or extended-spectrum beta-lactamase (ESBL)-producing strains of Enterobacterales were stratified as critical priority pathogens because they cause life-threatening infections such as pneumonia and bloodstream infections. [4,5]

Anti-microbial Resistance (AMR) is on the rise and is a serious threat because of overuse and misuse and incomplete course of antibiotics as well as in

empirical course of antibiotics is most common cause of increasing resistance to common antibiotic in the community and in the hospital setting and therapy becomes difficult with this rising trends of resistance pattern to antimicrobial drugs. [6,7]

As per the World Health Organization (WHO) fact sheet, these antimicrobial-resistant organisms infections can result in failure of treatment, increased cost of medical treatment, increased hospitalization stay and increased socioeconomic burden. [8,9] Multi-drug resistance (MDR) is defined as resistance to at least one agent in three or more antimicrobial categories. [10]

The rise of MDR pathogens has been linked to various factors, including by phenotypic method like production of enzyme like ESBL, carbapenemase, change the binding protein for antibiotic agents, overuse of antibiotics, poor infection control practices.

This study aims to investigate the prevalence of MDR Gram-negative pathogens e.g. Carbapenem-resistant *Acinetobacter baumannii* (CRAB), *Pseudomonas aeruginosa* (CRPA), and carbapenem-resistant or extended-spectrum beta-lactamase (ESBL)-producing strains of Enterobacterales and their antimicrobial susceptibility pattern to better inform to clinician for choosing most effective treatment strategies and containment measures.

Methods

The study was conducted in JNUIMSRC Medical College, Jaipur, Rajasthan, India, at a tertiary care teaching hospital's Department of Microbiology from March 2025 to August 2025. Throughout this time, the hospital received and processed 5327 different clinical specimens from patients who had been hospitalised to different clinical departments and in the different OPDs. Samples were transferred and processed in to laboratory as early as possible. Each specimen was inoculated on blood agar and Mac-Conkey agar and incubated aerobically at 37°C for 24–48 h. After incubation, the colony morphology of bacterial growth was observed and further processing, viz. Gram staining and standard biochemical reactions were performed.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing (AST) was performed for all the GNB isolates by the Kirby-Bauer disc diffusion method, and the results were interpreted as per the Clinical Laboratory Standard Institute guidelines 2024.^[11] The following antibiotic discs from various groups, commercially procured from HiMedia, Mumbai, India, were used: amikacin (AK, 30 µg), gentamicin (GEN, 10 µg), ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), ciprofloxacin (CIP, 5 µg), co-trimoxazole

(CM, 25 µg), imipenem (IMP, 10 µg), meropenem (MRP, 10 µg), piperacillin-tazobactam (PIT, 100/10/µg). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as control strains for Gram-negative fermenters and non-fermenters, respectively.

Identification of multidrug-resistant isolates:

The isolate showing non-susceptibility to at least one antibiotic from three different antibiotic groups was considered MDR. [10] In present study, it includes resistance to any three of the following groups – cephalosporins, fluoroquinolones, aminoglycosides, and carbapenem.

For detection of ESBL producing Enterobacterales, screening was done using disc diffusion method with ceftazidime (30 µg), and confirmation was done by simultaneous testing with and combination of ceftazidime-clavulanate (30/10 µg) by phenotypic confirmatory disc diffusion test (PCDDT). For detection of CRE, screening was done using disc diffusion method with imipenem. [11]

Similarly for detection of Carbapenem Resistance *Pseudomonas* spp and *Acinetobacter* spp, screening will be done by disc diffusion method with carbapenem agents (e.g. Imipenem 10 µg) [11]

Statistical analysis: Statistical analysis was expressed in frequency distribution (percentage), whereas MDR was calculated as described earlier. The comparison of categorical variables was analysed using the Chi-square test, and a P value < 0.05 was considered statistically significant for a confidence interval of 95%.

Results

Of 5327 clinical samples received in the Laboratory, 1695 samples (31.81%) were found to be culture positive for microorganisms. Out of these 200 (11.79%) multidrug resistant Gram negative organism included in this study.

The majority of the MDR GNB isolates were from male patients (57%, 114/200), whereas the remaining 43% (86/200) belonged to female patients. Culture-positive specimens were received from patients ranging from 1 month to 90 years, most commonly from the 41 to 60 year (35%) followed by > 60 year age group (33.5%), 21- 40 (21.5%), and least were from age group less than 20 year (10%). (Figure no.1)

Maximum isolates were from urine sample (28.5%) followed by pus and endotracheal aspirate (20.5% each), sputum (17.5%), blood (10.5%) and least from body fluid (2.5%). (Table 1).

Among the GNB isolates obtained from various samples (n = 200), 74% (n =148) of the isolates were Gram-negative fermenters

(Enterobacteriaceae isolates), while the remaining (26%, 52/200) were Gram-negative non-fermenters. The predominant isolate was *Escherichia coli* 85 (42.5%), followed by *Klebsiella* spp 56 (28%), *Acinetobacter baumannii* spp 42 (21%), *Pseudomonas aeruginosa* 10 (5%) and *Proteus* spp 5 (2.5%) and *Citrobacter* spp 2 (1%). (Figure no.2)

Most of them were isolated in specimens from patients who attended IPD (57%, n = 114) and from ICU (32%, n=64), whereas the remaining isolates, i.e., 11% (n = 22), were obtained from those attending OPD. From ward maximum patients from medicine (58/114, 50.8%) and surgery (21/114, 18.4%) department. From ICU (64) maximum patient from MICU (45/64, 70.3%) and RICU (11/64, 17.18%)

Among *E.coli* (85) maximum resistance was observed with ceftazidime, cefepime (100% and 98% respectively) and for ciprofloxacin (97.6%) followed by piperacillin-tazobactem (89.4%), carbapenems (63.5%) ceftazidium-avivectem (60%). Least resistance was seen against aminoglycoside (52.9% to 37.6%).

Among *Klebsiella pneumoniae* (57) isolates show more resistance than *E.coli*. Resistance pattern was ceftazidime (100%), piperacillin-tazobactem

(98%), ceftazidium-avivectem (73.68%), cotrimoxazole (82.4%) and carbapenems (87.7%).

Among *Acinetobacter baumannii* (42) maximum resistance was observed with Cephalosporins, Carbapenems, Aminoglycoside, Ciprofloxacin (100% each), cefepime, ampicillin-sulbactem, cotrimoxazole (88% to 95%). Least resistance was seen against Doxycycline and Minocycline (26% each).

Among *Pseudomonas aeruginosa* (10) maximum resistance was observed with Carbapenems (100%) followed by cephalosporins (90%), piperacillin-tazobactem (80%) and Tobramycin (70%). Least resistance was seen against flouroquinolones, ceftazidium- avivectem (60% each) and Aztreonam (40%). (Table -2)

Out of 148 Enterobacterales isolate, 56% (83/148) were ESBL-producing Enterobacterales. Of these 83/148 (56%) included 72.2% (60/83) of all *E.coli* and 24% (20/83) of *Klebsiella pneumoniae*, 3.6% (3/83) of *Proteus mirabilis*. Out of 200 isolates, 79.50% (n = 159) were found to be carbapenem resistant. The breakup is as follows: 108/159 (67.92%) were Enterobacterales, 41/159 (25.78%) were *A. baumannii*, and 10/159 (6.28%) were *P. aeruginosa*. Out of 67.92% (108) of CRE, *E.coli* were (56/108, 51.8%) and *Klebsiella pneumoniae* were (52/108, 48%).

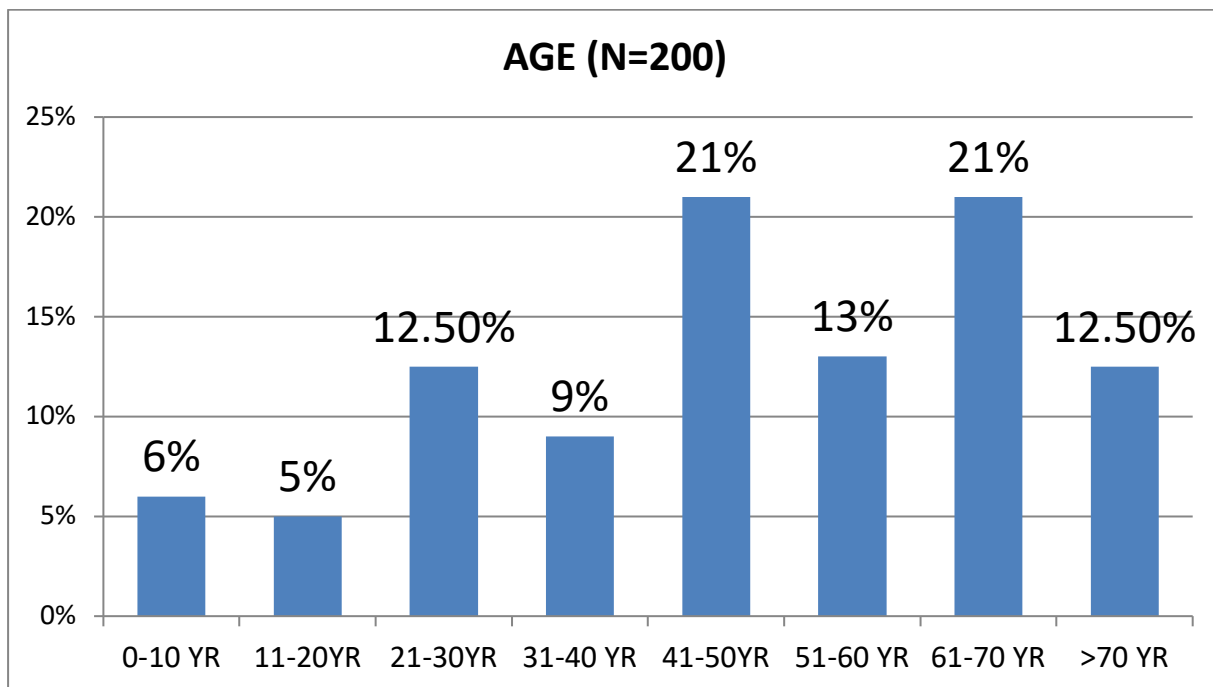


Figure 1: Age Wise Distribution

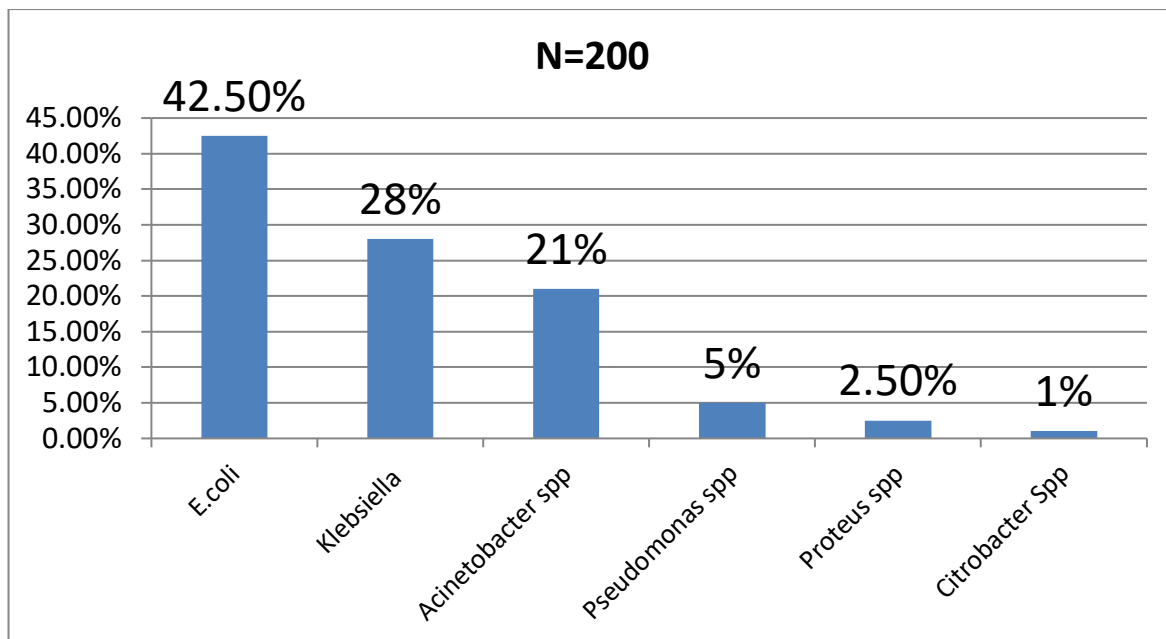


Figure 2: Distribution of Isolated Organisms

Table 1: Profile of Different Clinical Specimens

Samples	No	%
Urine	57	28.5%
Pus	41	20.5%
Et Secretion	41	20.5%
Sputum	35	17.5%
Blood	21	10.5%
Body Fluid	5	2.5%

Table 2: Anti-microbial Resistance pattern of isolates

Antibiotic	E.coli (N=85)	Klebsiella pneumoniae (N=57)	Acinetobacter baumannii (N=42)	Pseudomonas aeruginosa (N=10)
Ampicillin	91.7%	-	-	-
Amoxyclav	91.7%	100%	-	-
Ampicili-sulbactem	-	-	92.8%	-
Piperacillin-tazobactem	89.4%	98.2%	100%	80%
Ceftazidime	100%	100%	100%	90%
Cefepime	98%	94.7%	95.2%	90%
Imipenem	63.5%	87.7%	100%	100%
Meropenem	63.5%	87.7%	100%	100%
Amikacin	52.9%	87.7%	100%	-
Gentamicin	64%	84.2%	100%	-
Tobramycin	-	-	100%	70%
Ciprofloxacin	97.6%	100%	90.4%	60%
Levofloxacin	94.1%	98.2%	90.4%	60%
Cotrimoxazole	82.35%	-	88.1%	-
Ceftazidime-avibactem	60%	73.68%	-	60%
Minocycline	-	-	45.2%	-
Aztreonam	-	-	-	40%
Doxycycline	-	-	26.2%	-

Discussion

Gram negative bacteria are leading cause of infection due to antibiotic resistance which can be due to a great number of factors like usage of broad spectrum antibiotics, frequent invasive procedures, prolonged hospital stay and other co-morbid

conditions. Among 200 GNB isolates, 57%, were from male patients, 43% belonged to female with a Male to Female ratio of 1.32:1. Other studies also had a male preponderance with Joseph JN and Boloor R et al[12] & Garland A et al. [13] reporting Male to Female ratio of 1.9:1 & 1.75

respectively. This may be due to the fact that men are more likely to suffer from chronic condition needing hospitalization because of lifestyle choices like occupation.

The majority of patient were from 41 to 60 year age group (35%) followed by > 60 year age group (33.5%). A study by Joseph JN and Bolor R et al [12] agree with our finding and they report maximum isolation of MDR GNB from 51 to 70 age group (23.2%) and Devi KD et al. [14] reported a median age of GNB isolation 18 to 40 age group 44.9% in Tripura which is in contrast to our finding.

Urine was the most commonly collected sample with 28.5% of all the specimens followed by endotracheal aspirates and pus at 20.5%. This is in contrast to a study done by Moolchandani K et al [7] reported tracheal aspirate 29.9% followed by urine 19.5% and another study Balakrishnan et al [15] reported blood and urine as the most commonly collected sample.

Among the GNB isolates, 148 (74%) isolated were identified as Enterobacterales, while remainder were categorised as Gram-negative non-fermenters as 42 (21%) were *Acinetobacter baumannii* and 10 (5%) were *Pseudomonas aeruginosa*. Devi KD et al [14] also reported Gram-negative fermenters (52.9%) as the most commonly isolates.

The most commonly GNB isolated was *E.coli* (42.5%) followed by *Klebsiella* spp at 28%, *Acinetobacter baumannii* at 21% and *Pseudomonas aeruginosa* at 5%. Nagvekar V et al [16] also reported *E.coli* 22% as the most common isolate. In contrast to our study, Jha D.K. et al [17] reported *Acinetobacter baumannii* 56%, followed by *Pseudomonas aeruginosa* at 24% and *Klebsiella pneumoniae* at 16%.

In our study, the most common GNB isolated from urine and blood was *E.coli* at 46/57 and 9/21 respectively. *A. baumannii* (19/40) was common isolate in endotracheal secretion, *Klebsiella* spp (16/38) in pus sample. Saha A et al [18] reported *Klebsiella pneumoniae* (60%) in respiratory samples and Pandya et al [19] reported *Pseudomonas* spp 31% from ICU patient.

All MDR organism showing resistance to almost all the antibiotics like ceftazidime, cefepime, piperacillin-tazobactam, imipenem, carbapenem and ceftazidime avivectem ranging from 63.5 to 100%. amikacin is most effective antibiotic for enterobacteriaceae organism. *Acinetobacter baumannii* show good sensitivity to doxycycline and minocycline (26% each). Aztreonam (40%) is the most effective antibiotic to *Pseudomonas* spp. In contrast to present study Joseph JN et al [12] study showing *E. coli* was most sensitive to Amikacin (75%) and Imipenem (73%). *Klebsiella*

pneumoniae also sensitivity to Amikacin and Imipenem at 53.3% and 48.9% respectively, *Acinetobacter baumannii* too had high amount of MDR strains and Cotrimoxazole & Amikacin/Gentamicin were effective only in 34.3% and 31.2% cases respectively. Amikacin was highly efficacious for *Pseudomonas aeruginosa* (80%), followed by Imipenem/Meropenem in 72% cases.

Out of the 148 isolates of Enterobacteriaceae family, a total of 83 were ESBL producers (56%) which included 72.2% of *E.coli* and 24% of *Klebsiella pneumoniae*, 3.6% of *Proteus mirabilis*. A study by Khkhakhar VM et al. [20] showed much higher rate of ESBL Production for *E.coli* at 84.05%.

Out of 200 isolates GNB 79.50% (n = 159) were found to be carbapenem resistant. The breakup was as follows: 108 (67.92%) were Enterobacterales, 41 (25.78%) were *A. baumannii*, and 10 (6.28%) were *P. aeruginosa*. Out of 67.92% of CRE were *E.coli* (51.8%) and *Klebsiella pneumoniae* (48%). A study done by Pawar SK et al [21] showed prevalence of CRE 31.77%, in which *Klebsiella pneumoniae* (63%) and *E.coli* (19%).

Conclusion

GNB are highly resistant to many previously effective first line antibiotics like penicillin's, cephalosporins. It shows that MDR GNB infections are on the rise in ICU and physicians must be use antibiotics judiciously. ESBL production is generally accompanied by multi-drug resistance.

This study focuses on the prevalence of carbapenem resistance and ESBL production among commonly isolated gram negative bacteria, which is a major concern not only for treating physicians but also for infection control professionals.

There is urgent need for proper surveillance and appropriate infection control measures, as well as the need for the limited use of antibiotics to stop the spread of resistance. The number of drug-resistant microorganisms can be reduced by adherence to antimicrobial stewardship programs.

Limitations: Genotypic study for genes responsible for carbapenem resistance is further required to know the accurate results.

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Ethics Statement: This study was approved by the Institutional Ethics Committee, JNUIMSRC Institute of Medical Sciences, Jaipur, India, with reference no. JNUIMSRC/IEC/2025/18.

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