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Burden of Multidrug-Resistant Gram-Negative Bacterial Infections in a Tertiary Care Hospital

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Abstract

Multidrug-resistant (MDR) Gram-negative bacterial infections have emerged as a major public health concern. The aim of the present study was to detect the rate of infections due to MDR Gram-negative bacteria (GNB) in a tertiary care hospital, the rate of Carbapenemases and AmpC-β-lactamases production and the Antimicrobial susceptibility test pattern (AST) among MDR GNB. The rate of MDR GNB during the study period was 25.70%. Urine samples showed the highest contribution to the total MDR GNB. Among the total MDR GNB isolates, 166 were randomly selected and included in the present study. A higher rate of MDR GNB was reported among male patients (61.5%) compared to the females (38.5%) and most of them were from the patients aged between 61-70 years (30.7%). The most prevalent MDR GNB was *Klebsiella pneumoniae* 80 (48.12%), followed by *Escherichia coli* 43 (25.9%). AST of MDR GNB revealed their significant resistance to β-lactamases/β-lactamases inhibitors, cephalosporins, fluoroquinolones and carbapenem drugs (98%). Of 123 MDR Enterobacterales, 83% of them were found to be Metallo β-lactamase (MBLs) producers by mCIM and eCIM methods. Of 43 MDR non-fermenters, 29 (67.4%) of them were found to be carbapenemase producers by MHT. About 29.51% of MDR GNB isolates were found to be AmpC producers by AmpC disk test. A reliable and rapid phenotypic method to detect carbapenemases and AmpC β-lactamases among MDR GNB in a routine microbiology laboratory method is clinically important to guide antibiotic therapy and implementation of effective infection control practices.

Keywords: Multidrug-resistant, Gram-negative Bacteria, Hospital Acquired Infections, Carbapenemase, AmpC β-lactamase, Modified Hodge Test, Carbapenemase Inactivation Method

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INTRODUCTION

Antimicrobial resistance (AMR) has emerged as a pivotal concern of the 21st century.[1,2] It presents a critical and escalating challenge to the world, with an increased risk of morbidity and mortality, and it is recognized by the World Health Organization (WHO) as a public health concern.[3,4] Multidrug-resistance (MDR) is defined as non-susceptibility to at least one agent in three or more antimicrobial groups. Infections caused by MDR Gram-negative bacteria (GNB) constitute a significant concern due to limited therapeutic options. MDR-GNB, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*, are responsible for major hospital-acquired infections (HAIs), including urinary tract infections (UTIs), ventilator-associated pneumonia (VAP), intra-abdominal infections (IAIs), surgical site infections (SSIs), and bacteremia.[5-7]

The Centers for Disease Control and Prevention (CDC) in the USA have classified Carbapenem-resistant(CRE) and ESBL-producing Enterobacteriaceae, Carbapenem-resistant Acinetobacter baumannii (CRAB), and MDR and ESBL-producing *Pseudomonas aeruginosa* as urgent threats.[8] The root causes of the rise in MDR infections are multifaceted, including a lack of antibiotic stewardship program, irrational use of existing antimicrobial agents in hospitalized patients, and the use of antibiotics in veterinary feeds, which contribute to the development of adaptive resistance mechanisms.[9]

Gram-negative bacteria (GNB) exhibit a high degree of adaptability to various antimicrobial agents through the utilization of diverse mechanisms. These mechanisms may either be intrinsic to a given species or acquired. Intrinsic resistance mechanisms arise from mutations in chromosomal genes and include enzymes that inactivate drugs, increased efflux of antimicrobials, altered outer membrane porins (OMPs), and modifications to target sites. On the other hand, acquired mechanisms result from the transfer of resistance genes via mobile genetic elements (MGEs), most notably the beta-lactamase genes encoded on plasmids, or mechanisms that do not involve enzymes, such as Qnr, which confers resistance to fluoroquinolones in *Enterobacterales*.[7,10,11]

ESBLs are plasmid-encoded β-lactamases that confer resistance to extended-spectrum β-lactam antibiotics in GNB.[12] AmpC β-lactamases, like cephalosporinases can be both plasmid and chromosomally encoded, and they confer resistance to most β-lactam drugs in GNB.[13] Carbapenemase genes provide a persistent and transferable form of resistance, enabling their dissemination among naive bacteria.[14,15] It is important to consider both carbapenemase-producing bacteria and those employing alternative carbapenem resistance mechanisms.[16]

The effective management of infections caused by MDR GNB necessitates a comprehensive approach. Reliable and rapid phenotypic methods for detecting carbapenemases, AmpC β-lactamases, and extended-spectrum β-lactamases in MDR GNB in routine microbiology laboratory is of clinical significance. Such methods guide antibiotic therapy and facilitate the implementation of effective infection control practices, ultimately improving the care of critically ill patients at high risk of harboring MDR GNB.[6,14] The present study aims to provide insights into the incidence of infections caused by MDR GNB, their resistance mechanisms, and the prevalence of AmpC β-lactamases and carbapenemase-producing MDR GNB among patients at a tertiary care hospital in Mysuru.

MATERIALS AND METHODS

This was a laboratory based prospective study conducted in the Department of Microbiology, for a period of one year (May 2022 – April 2023). All MDR GNB isolated from clinical samples were included in this study and processed according to the standard protocol. The sociodemographic details of the patients and microbiological records were obtained from the digital data stored online at the Hospital.

Species identification, AST & storage

Identification of GNB and AST was done by Automated VITEK 2 system (bioMerieux VITEK). The isolates which were found to be resistant for at least one agent in three or more antimicrobial

classes were considered as MDR GNB. The pure cultured growth of these MDR GNB isolates on MacConkey agar were sub-cultured onto nutrient agar vials and stored at 4°C.

A randomly selected 166 MDR GNB isolates were screened for Carbapenemase and AmpC β-lactamase production.

Phenotypic detection of carbapenemase **production**

All the MDR GNB isolates were brought to room temperature and sub-cultured onto MacConkey agar before conducting the phenotypic testing.

The MDR *Enterobacterales* isolates were screened for Carbapenemase production by Modified Carbapenemase Inactivation method (mCIM), Ethylene diamine tetra acetic acid (EDTA) Carbapenemase Inactivation method (eCIM). Non-fermenters were screened for Carbapenemase production by Modified Hodge test (MHT).

Screening for Carbapenemase production by MHT[17]
Test procedure
- Lawn culture of ATCC 25922 *E. coli* was done on Muller Hinton Agar (MHA) plates
- Meropenem disk (10 µg) was placed in the center of each plate, 3-5 colonies of each test organism inoculated in the form of a straight line from the meropenem disk edge to the end of the plate and incubated at 37°C for 16-18 hours.

Interpretation

MHA plate were checked for the growth of ATCC *E. coli* around each test organism at the intersection of the inoculated line and inhibition zone. Positive growth indicates Carbapenemase production and no growth indicates no Carbapenemase production.

mCIM and eCIM test for suspected carbapenamse production in *Enterobacterales* according to CLSI guidelines[17]
Test procedure for mCIM
- For each isolate to be tested, 1 µL loopful of bacteria from an overnight pure culture plate was inoculated into 2 mL of Tryptic soy broth (TSB) and vortexed for 10-15 seconds.

- 10 µg meropenem disk was added to the tube using sterile forceps so that entire disk was immersed and incubated at 37°C for 4 hours.
- MHA plate was lawn cultured with *E. coli* ATCC 25922.
- Meropenem disk from each TSB-meropenem disk suspension was removed using the flat side of a sterile 10 µL loop and excess broth was drained by pressing the loop along the inside edge of the tube. The disk was removed from the tube and placed on the MHA plate inoculated with the *E. coli* ATCC 25922. The MHA plates were inverted and incubated at 37°C for 18-24 hours.
- Zones of inhibition was measured as routine disk diffusion method.

Test procedure for eCIM
- 20 µL 0.5 M EDTA was added to a 2 mL TSB tube to obtain a final concentration of 5 mM EDTA.
- For each isolate to be tested, 1 µL loopful of bacteria from an overnight pure culture plate was inoculated into EDTA-TSB broth and vortexed for 10-15 seconds.
- 10 µg meropenem disk was added to each tube using sterile forceps so that entire disk was immersed and incubated at 37°C for 4 hours.
- Meropenem disk from the EDTA-TSB-meropenem disk suspension was removed as in mCIM test and placed on the MHA plate which was inoculated with ATCC *E. coli* 25922.

Test interpretation: mCIM
- mCIM positive: Zone diameter of 6-15 mm or presence of pinpoint colonies within a 16-18 mm zone
- mCIM negative: Zone diameter of ≥19 mm (clear zone)
- mCIM indeterminate: Zone diameter of 16-18 mm or zone diameter of ≥19 mm with the presence of pinpoint colonies within the zone.

eCIM – Interpret only when mCIM test is positive
- eCIM positive: A ≥5 mm increase in zone diameter for eCIM vs zone diameter for mCIM. For eCIM test, ignore pinpoint colonies within any zone of inhibition.

Table 1. Reporting of mCIM and eCIM test

mCIM	eCIM	Report
Negative	Do not interpret	Carbapenemase not detected
Positive	Negative	Serine carbapenemase detected
Positive	Positive	Metallo-β –lactamase detected
Indeterminate	Do not interpret	Testing inconclusive for the presence of carbapenemase

mCIM- modified carbapenemase inactivation method, eCIM- EDTA Carbapenemase inactivation

- eCIM negative: A ≤4 mm increase in zone diameter for eCIM vs zone diameter of mCIM
- The test was reported as depicted in the Table 1.

Phenotypic detection of AmpC production
Screening for AmpC production by AmpC disk test (Disk Diffusion method)

All the MDR GNB isolates (166) were tested for AmpC production.

Test procedure
- A lawn culture of *Escherichia coli* ATCC 25922 indicator strain was made on the MHA plate.
- Sterile disk (6 mm) was moistened with sterile saline (10 µl) and inoculated with a suspension of the test organism.
- The cefoxitin disk (30 µg) and sterile disk inoculated with test organism were placed adjacent to each other on the MHA plate and incubated overnight at 37°C.

Interpretation
- Positive result: flattening or distortion of the cefoxitin inhibition zone

Table 2. Contribution of each sample to the total MDR GNB during the study period

	No. of MDR GNB	Percentage of MDR
Pus	461	30.07%
Blood	116	7.56%
ET	220	14.35%
Sputum	113	7.37%
Urine	476	31.05%
BAL	91	5.9%
Bile	23	1.5%
Synovial fluid	1	0.06%
Tissue	19	1.23%
Ear swab	8	0.52%
Vaginal Swab	5	0.32%
Total	1533	

ET-endotracheal aspirate, BAL- bronchoalveolar lavage

- Negative result: undistorted cefoxitin inhibition zone.[18]

RESULTS

Of the 26775 samples received in the microbiology laboratory for culture and sensitivity,
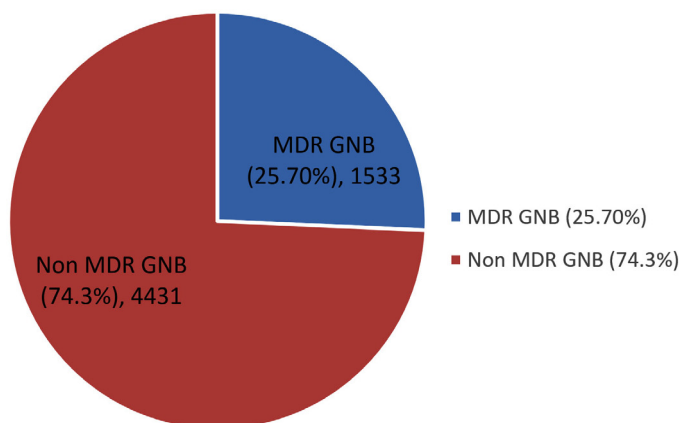

Figure 1. Burden of MDR Gram-negative organisms

Table 3. Overall susceptibility of MDR Enterobacterales

Organism	Total isolates	Antibiotics resistant to	Number and Percentage of Resistance	Organism	Total isolates	Number and Percentage of Resistance
Klebsiella pneumoniae	80	Amoxicillin clavulanate	78 (97.5%)	*E. coli*	43	42 (97.67%)
		Piperacillin tazobactam	80 (100%)			43 (100%)
		Cefuroxime	80 (100%)			42 (97.67%)
		Cefuroxime axetil	78 (97.5%)			42 (97.67%)
		Ceftriaxone	78 (97.5%)			43 (100%)
		Cefaperazone sulbactum	77 (96.25%)			41 (91.34%)
		Cefipime	78 (97.5%)			43 (100%)
		Ertapenem	80 (100%)			42 (97.67%)
		Imepenem	75 (93.75%)			43 (100%)
		Meropenem	79 (98.75%)			41 (91.34%)
		Amikacin	19 (23.75%)			23 (53.5%)
		Gentamicin	69 (86.25%)			28 (65.11%)
		Ciprofloxacin	80 (100%)			42 (97.67%)
		Tigecycline	5 (6.25%)			0
		Fosfomycin	4 (5%)			0
		Colistin	2 (2.5%)			1 (2.32%)
		Trimethoprim sulfamethoxazole	61 (76.25%)			37 (86.04%)
		Nitrofurantoin	14 (17.5%)			6 (27.27%)

8997 (33.60%) samples were culture positive, of which 5964 (22.27%) samples yielded growth of GNB and among them, 1533 (25.70%) isolates were found to be MDR. The rate of MDR GNB during the study period was 25.70% (Figure 1).

Urine samples contributed highest 476 (31.05%) to the total MDR GNB, followed by endotracheal aspirate 220 (14.35%), blood 116 (7.56%), sputum 113 (7.37%), bronchoalveolar lavage 91 (5.93%), bile 23 (1.5%), tissue 19 (1.23%),
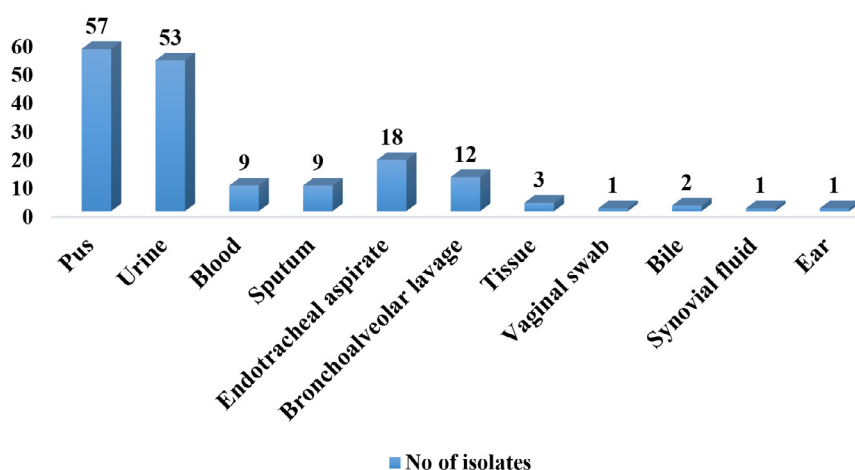

Figure 2. Sample wise distribution of the isolates

Table 4. Overall susceptibility of MDR Non-fermenters

| Organism	Total isolates	Antibiotics resistant to	Number and Percentage of Resistance	Organism	Total isolates	Number and Percentage of Resistance
Acinetobacter baumannii	27	Piperacillin tazobactam	27 (100%)	*Pseudomonas aeruginosa*	16	16 (100%)
		Ceftazidime	27 (100%)			14 (87.25%)
		Cefaperazone sulbactum	22 (81.5%)			13 (81.25%)
		Cefipime	23 (85.18%)			13 (81.25%)
		Imepenem	27 (100%)			15 (93.75%)
		Meropenem	27(100%)			16 (100%)
		Amikacin	22 (81.5%)			13 (81.25%)
		Gentamicin	25 (92.6%)			16 (100%)
		Ciprofloxacin	27 (100%)			16 (100%)
		Levofloxacin	4 (14.81%)			16 (100%)
		Minocycline	7 (25.92%)			-
		Tigecycline	0			-
		Colistin	0			0
		Trimethoprim sulfamethoxazole	24 (88.9%)			-
		Nitrofurantoin	4 (14.8%)			-
		Aztreonam	-			16 (100%)

ear swab 8 (0.52%), vaginal swab 5 (0.32%) and synovial fluid 1 (0.06%) (Table 2).

Among the 1533 MDR GNB isolates reported during the study period, 166 isolates were randomly selected and included in the present study to detect the various resistant mechanisms. Of the 166 randomly selected isolates, 65 (39.15%) of them were reported among the patients in the age group 61-80 years. Higher rate of MDR GNB were isolated from male patients (61.5%) as compared to the female patients (38.5%). The maximum number 106 (63.8%) of MDR GNB were isolated from the wards, followed by ICUs 36 (21.68%) and outpatient department 24 (14.45%).

Maximum number of isolates were selected from pus samples 57 (34.33%), followed by urine 53 (31.92%), endotracheal aspirate 18 (10.84%), bronchoalveolar lavage 12(7.22%), blood 9 (5.42%), sputum 9 (5.42%), tissue 3 (1.80%), 2 (1.02%) from bile samples and 1 (0.60%) each from vaginal swab, synovial fluid & ear (Figure 2).

Out of 166 MDR GNB isolates, 123 (74.09%) were *Enterobacterales* and 43 (25.90%)
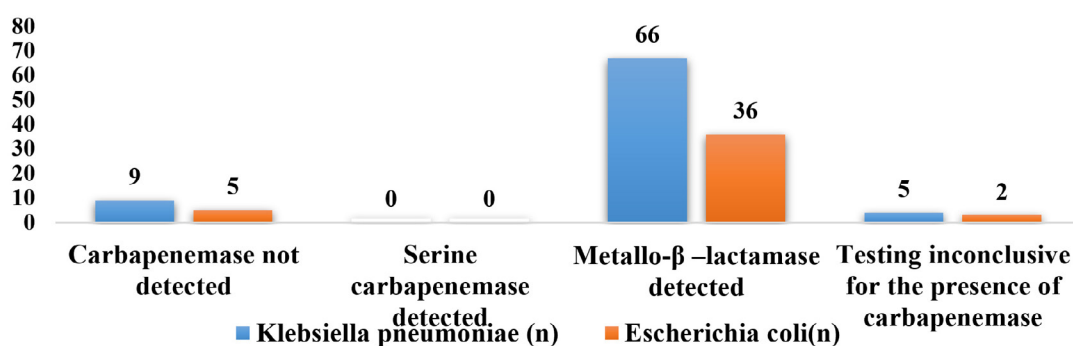
Figure 3. Report of carbapenemase detection in *K. pneumoniae* and *E. coli* by mCIM and eCIM

were non-fermenters. Most prevalent MDR GNB was *Klebsiella pneumoniae* 80 (48.12%), followed by *Escherichia coli* 43 (25.9%), *Acinetobacter baumannii* 27 (16.2%) and *Pseudomonas aeruginosa* 16 (9.6%). The antimicrobial susceptibility pattern of MDR GNB showed that the most of the isolates were resistant to β-lactamases/β-lactamases inhibitors, cephalosporins, fluoroquinolones and carbapenem drugs (98%). AST results of MDR *Enterobacterales* and Non-fermenters are shown in Table 3 and Table 4, respectively.

The 123 *Enterobacterales* were subjected to Modified carbapenemase Inactivation method (mCIM) & EDTA carbapenemase Inactivation

method (eCIM) to detect carbapenemase production according to the standard protocol recommended by CLSI guidelines (M100) 2022, of which 83% (102) of them were found to be Metallo β-lactamase (MBLs) producers.

Of 80 *Klebsiella pneumoniae*, 66 (82.5%) were found positive for mCIM and eCIM and were considered positive for Metallo-β-lactamase production, 5 (6.25%) were found to be indeterminate and were considered inconclusive and remaining 9(11.25%) isolates were found negative for mCIM and were considered as negative for carbapenemase production. Of 43 *Escherichia coli*, 36 (83.72%) were found positive for mCIM and eCIM and were considered positive for Metallo-β-lactamase production, 2(4.65%) was found to be indeterminate and was considered inconclusive and remaining 5 (11.62%) isolates were found negative for mCIM and were considered as negative for carbapenemase production as shown in Figure 3. Figures 4, 5, and 6 shows the positive, negative, and indeterminate results of mCIM and eCIM test, respectively

Non-fermenters (n=43) were not screened by mCIM and eCIM since both the tests are not recommended by CLSI for testing carbapenemase production in *A. baumannii* and eCIM test is not recommended CLSI for testing carbapenemase production in *P. aeruginosa*.

The Non-fermenters (43) were screened for carbapenemase detection by Modified Hodge Test, of which 29 (67.4%) of them were found to be carbapenemase producers. Of 27 *A. baumannii*
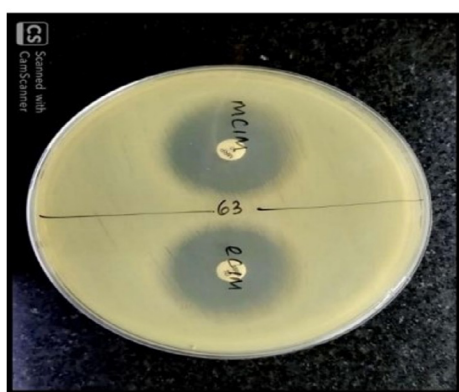

Figure 4. mCIM and eCIM test positive

Figure 5. mCIM and eCIM test negative

Figure 6. mCIM and eCIM test indeterminate

70.37% (19) of them were MHT positive, of 16 *P. aeruginosa*, 62.5% (10) of them were MHT positive (Figure 7 and 8)

All the isolates were subjected to AmpC detection by AmpC disk test, of which 29.51% (49) of them were found to be AmpC producers. AmpC β-lactamases were more frequently found among *A. baumannii* (33.33%) followed by *P. aeruginosa* (31.25%), *K. pneumoniae* (28.75%) and *E. coli* (27.9%) as depicted in the Figure 9 and 10.

DISCUSSION

Antimicrobial resistance has been a vital issue of 21st century. The emerging Antimicrobial resistance (AMR) among GNB poses an alarming threat to world healthcare and presents a substantial challenge in choosing empiric antibiotic therapy in seriously ill patients.[5]

The rate of MDR GNB in the present study was 25.7%, however, Silpi Basak *et al.*, at Jawaharlal Nehru Medical College, Wardha, India have reported higher prevalence 37.1% of MDR GNB.[19] Aisha *et al.*, from Eastern Saudi Arabia,[13] Meng *et al.*, from People's Hospital, Jining, Shandong Province, China.[20] and Vasant *et al* from Mumbai[21] have also reported higher prevalence of MDR GNB i.e., 60.4%, 42.5% and 30.8%, respectively. Ours being a teaching hospital, adherence to infection control practices and
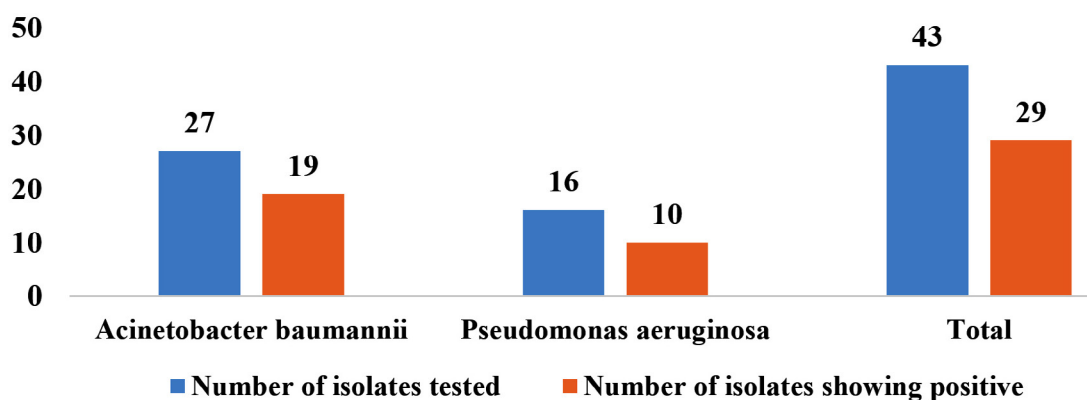

Figure 7. Screening for carbapenemase detection among non-fermenters by Modified Hodge Test
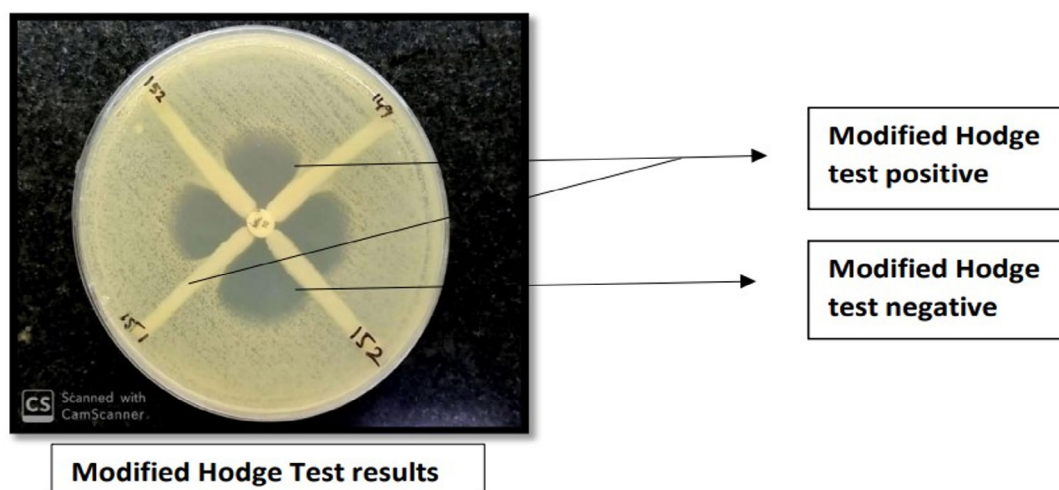

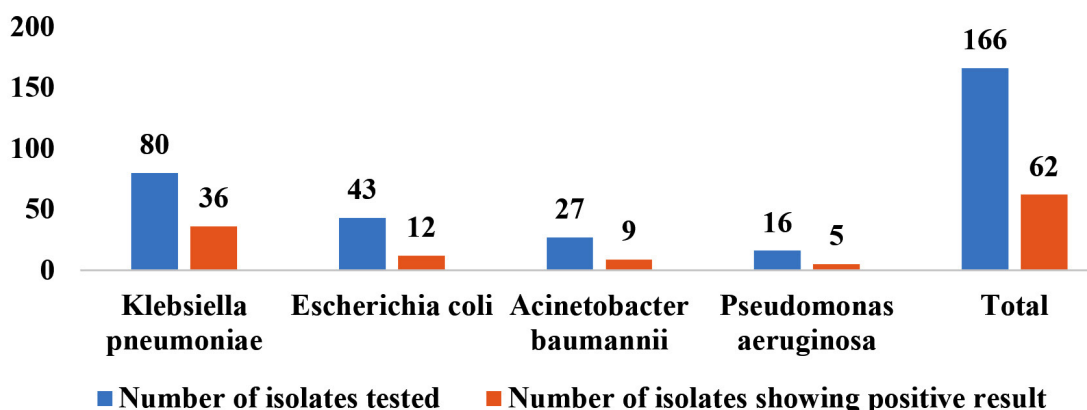**Figure 8.** Modified Hodge Test results

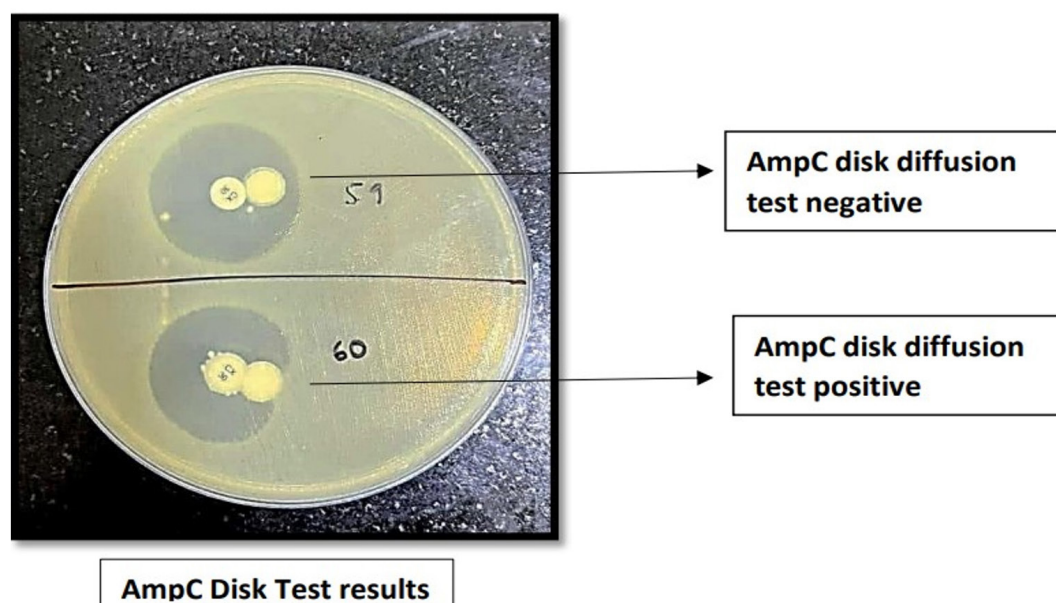**Figure 9.** Detection of AmpC β-lactamases by Disk diffusion method

Figure 10. AmpC disk test results

antibiotic stewardship program is in place resulting in lesser rate of MDR GNB.

In a study conducted by Nicolas Francisco Fernandez-Martinez *et al.*, at Reina Sofᵤa University Hospital, Spain have reported a higher isolation rate of MDR GNB among male patients (61.5%) compared to the female patients (38.5%)[22] and this is found consistent with the current study where we have also reported the higher isolation rate of MDR GNB among males (72.89%) compared to females (27.10%).

In our study, the maximum number of MDR GNB were reported in the patients aged between 61-70 years (30.7%) which is consistent with the study by Aurora E Pop-Vicas, *et al.*, at Beth Israel Deaconess Medical Center (Boston, MA) who have also reported that patients aged >=65 years contributed to the highest MDR GNB.[23] Same study mentioned above[23] has also reported that urine samples have the highest contribution (55%) to the total MDR GNB which correlates with our study where urine samples (31.05%) has contributed to highest number of MDR GNB.

In a study conducted by Silpi Basak, Wardha, *et al.*, the highest number of MDR GNB strains were isolated from surgery wards (32.3%) followed by different ICUs (18.3%),[19] however in the current study, we have reported a slightly higher rate in ICUs (30.4%) than Surgical wards (14.45%). This high incidence of MDR isolation in ICUs in the present study can be justified by the fact that MDR isolates are a common nosocomial pathogen which are present in ICU environment and also, ours being a tertiary care hospital where patients admitted to ICUs are referred from other hospitals and are previously exposed to antibiotics.

Silpi Basak *et al*,[19] Wardha, Vasant *et al.*,[21] Mumbai, reported *E. coli (*38%) to be the most prevalent MDR GNB followed by *K. pneumoniae (*28.4%), *A. baumannii (*19%), *P. aeruginosa (*19%), but, in the current study we have reported *Klebsiella pneumoniae* 80 *(*48.12%), as the most prevalent MDR GNB followed by *Escherichia coli* 43 *(*25.9%), *Acinetobacter baumannii* 27 (16.2%) *and Pseudomonas aeruginosa* 16 (9.6%). Our results were consistent with the study conducted by Mutasim *et al.*[13], at a Referral Hospital, Saudi Arabia in which the most prevalent MDR GNB reported was *K. pneumoniae* (34.69%) followed by *E. coli* (30.20%), *A. baumannii* (20%) and *P. aeruginosa* (15.10%). A study by Ching Jou, *et al.*, Australia, reported that the antimicrobial susceptibility of MDR GNB shows the most common three-drug resistance pattern to β-lactamases/β-lactamases inhibitors, third-generation cephalosporin and fluoroquinolones,[24] this correlates with the present study, also our isolates were found to be resistant to carbapenem drugs (98%).

We subjected 123 MDR *Enterobacterales* to Modified carbapenemase Inactivation method (mCIM) & EDTA carbapenemase Inactivation method (eCIM) to detect carbapenemase production, of which 83% of them were found to be Metallo β-lactamase (MBLs) producers which was slightly higher than that reported by Dalia El. Nobi, *et al.*, Assiut University, Egypt, who have reported 75.6% of MDR *Enterobacterales* to be Metallo β-lactamase (MBLs) producers by mCIM and eCIM method.[25]

In our study, the 43 MDR non-fermenters were screened for carbapenemase detection by Modified Hodge Test, of which 29 (67.4%) of them were found to be carbapenemase producers which includes, 70.39% of *A. baumannii* and 62.5% of *P.aeruginosa,* whereas in a study by Hala B. Othman, *et al.*, Ain Shams University Hospitals, Cairo, Egypt, have reported 54% of their *P. aeruginosa* isolates were MHT positive[26] and a study by Zahra Moulana, *et al.*, Babol University of Medical Sciences, Babol, Iran[27] showed 84% of *A. baumannii* isolates to be MHT positive.

Jennifer *et al.* at US Hospitals have reported 31% of their MDR GNB isolates to be AmpC β- lactamases producers,[28] which correlates with the current study where we have reported 29.51% of MDR GNB isolates to be AmpC producers by AmpC disk test. The present study showed that *A. baumannii* (33.33%) and *P. aeruginosa* (31.25%) were the most common AmpC β-lactamases producers followed by *K. pneumoniae* (28.75%) and *E. coli* (27.9%), this result correlates with a study conducted by Mutasim *et al.*, at a Referral Hospital, Saudi Arabia.[13]

Ours being a tertiary care hospital, patients would have been previously exposed to antibiotics there by facilitating the development of drug resistance including carbapenem resistance.

Therefore, it is imperative to develop a methodological approach that can expedite the comprehensive analysis and verification of resistance mechanisms within Multi-Drug Resistant (MDR) bacteria.

CONCLUSION

The increased cases of MDR GNB infections have become a global threat in hospitals and acute healthcare settings. Infections with MDR *E. coli*, *P. aeruginosa, K. pneumoniae and A. baumannii* contribute to the mortality of the most vulnerable populations.

The burden of drug resistance should be addressed with effective infection control practices like contact precautions to prevent further transmission of these MDR GNB within the hospital and in the community, implementation of evidence-based antibiotic stewardship programs, strict law for dispensing of antibiotics and awareness about rational use of antibiotics, public awareness program and hospital staff education.

Reliable phenotypic screening methods for carbapenemases and AmpC β-lactamases among MDR isolates will help in selecting appropriate antimicrobial therapy. This approach will not only advance our scientific understanding of antibiotic resistance but also have immediate clinical implications, enabling healthcare providers to facilitate timely therapeutic interventions and infection control strategies more effectively.

Limitations

In the present study, confirmation of different resistant mechanisms was not done using molecular methods, which makes way for future research directions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

RPM conceptualized and designed the study. VK and ANJ performed data collection. VK and RD performed the experiments. VK, RPM, RD and ANJ performed data analysis and interpretation of results. VK designed the figures. RPM supervised the project. VK, RPM, RD and ANJ drafted the manuscript. All authors read and approved the final manuscript for publication.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was apporoved by the Institutional Ethical Committee, JSS Medical College, JSS Academy of Higher Education & Research, Karnataka, India, with reference number JSS/MC/PG/36/2022-23.

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