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Escalating Gram Negative Infections And Carbapenem Resistance In Icus: A Critical Challenge In Tertiary Care

Dr A Kavinkumar[1], Dr Sunil Indernath[2], Dr Neelaveni Rajasekar[3], Kanimozhi D[4*]
[1]Assistant Professor, Dept of Microbiology, Government Medical College, Dindigul, Tamilnadu.
[2]Associate Professor, Dept of Microbiology, Vels Medical College and Hospital, Vels Institute of Science Technology and Advanced Studies (VISTAS), Tiruvallur.
[3]Associate Professor, Dept of Microbiology, Shri Sathya Sai Medical College and Research Institute, Sri Balaji Vidyapeeth (Deemed to be University) Chennai Campus.
[4]PhD Research Scholar, Dept of Microbiology, Mahatma Gandhi Medical College and Research Institute. Sri Balaji Vidyapeeth (Deemed to be University), Pondicherry.

Abstract:
Introduction: *Nosocomial infections in Intensive Care Units (ICUs) pose a significant healthcare challenge, primarily due to multidrug-resistant (MDR) Gram negative bacteria (GNB). These infections, often caused by Klebsiella spp., Pseudomonas spp., E. coli, and Acinetobacter spp., are exacerbated by the emergence of carbapenem resistance. This study aims to identify the prevalence of GNB in ICU-acquired infections and to explore the mechanisms of carbapenem resistance, including the detection of* bla_{NDM} *and* bla_{KPC} *genes.*
Methodology: *A total of 165 clinical samples from respiratory specimens, urine, blood, and pus were collected aseptically from ICU patients with nosocomial infections. The isolates were identified using standard microbiological techniques, and antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method. Carbapenem resistance was confirmed through the RAPIDEC CarbaNP test, and the presence of* bla_{NDM} *and* bla_{KPC} *genes was determined using multiplex PCR.*
Results: *The most common pathogen was Klebsiella spp. (35%), followed by Pseudomonas spp. (25%), E. coli (25%), and Acinetobacter spp. (15%). Respiratory samples constituted the majority (56%), with Klebsiella spp. as the predominant isolate. High levels of carbapenem resistance were observed. 85% in Acinetobacter spp., 68% in Klebsiella spp., 62% in E. coli, and 58% in Pseudomonas spp. The RAPIDEC CarbaNP test confirmed carbapenemase production in resistant isolates. PCR identified* bla_{NDM} *in six Klebsiella spp. isolates, while none were positive for* bla_{KPC}.
Conclusion: *The study highlights the alarming prevalence of carbapenem-resistant GNB in ICUs, particularly Klebsiella spp. and Acinetobacter spp. These findings emphasize the urgent need for antibiotic stewardship, infection control measures, and regular surveillance to combat MDR pathogens. Collaborative efforts between clinicians and microbiologists are crucial to mitigating this growing threat.*
Keywords: *carbapenem resistance, gram negative bacteria, Intensive Care Unit, Nosocomial Infection, CarbaNP*

INTRODUCTION:

The concept of Intensive Care Unit (ICU) started in 1854 by Florence Nightingale in the Crimean war where she separated the seriously wounded soldiers from the less seriously wounded, and in 1953 the first Intensive care unit was established in Copenhagen by Ibsen. Though an Intensive Care Unit is the place where special attention and care is given to the patient, it is also the one where they are 5-10 times more likely to acquire nosocomial infections than other hospitalized patients. Nosocomial infection can be defined as those occurring within 48 hours of hospital admission, 3 days of discharge or 30 days of an operation[1]. The risk factors include prolonged hospitalization, advanced age, severity of co-morbid illness, immunosuppression associated with chemotherapy and organ transplantation, invasive monitoring techniques and mechanical ventilation.[2] The most frequent types of infection acquired in ICU are Ventilator Associated Pneumonia (VAP), Surgical Site Infections (SSI), Urinary tract infections and Blood stream infections. Sepsis can be due to VAP, central line catheters, endotracheal tubes, wound infections and urinary tract infections.3 It has been studied that Gram-Negative Bacteria are the most common organisms that cause these infections. They primarily are *Pseudomonas aeruginosa, Escherichia coli, Klebsiella species* and *Acinetobacter species.*[4] In the past 20 years, changes in health care, infection control practices, and anti-microbial use and resistance have influenced the frequency of infection by these gram negative organisms[3]. The treatment being used against

these bacteria were penicillins, cephalosporins and carbapenems. But due to resistance, the bacilli have been able to evade killing.[5] Of these, carbapenems have been the most powerful antibiotics for treating infections, and as the emergence of β-lactamases with carbapenem-hydrolyzing activity (carbapenemases) is on the rise, the antimicrobial resistance is of great concern[6].

This study was taken up to know the types of nosocomial infections and the various gram-negative etiological agents in our ICU and to study the carbapenem resistance and various enzymatic mechanisms of drug resistance in the gram-negative isolates.

METHODOLOGY:

The study was conducted in the Microbiology department, of a tertiary care hospital. A total of 150 samples including Respiratory samples like sputum, Endotracheal Tube (ET) tip, ET aspirate, pleural fluid, blood, Urine, Pus and sterile body fluids Intravenous catheter tips were obtained from the patients admitted in the ICU of the tertiary care center. The samples were collected aseptically from patients who developed infections 48 hours after admission, transported to the laboratory and processed immediately.

Specimens were examined with gram stain those containing inflammatory cells and bacteria were considered for the study and cultured on to 5%Sheep blood agar, MacConkey agar, and Chocolate agar. Quantitative culture technique was used for ET aspirates/secretions by standard loop method.[7] A growth of more than 10^5 CFU/ml were considered significant and was included in the study. Maki's rolling tip technique is used for IV catheter tip and more than 15 colonies of growth is considered significant[7]. In urine more than 10^5 CFU/ml was considered significant.[8] Single or mixed growth isolated after culture were identified by colony morphology and using standard biochemical reactions[9]. Antibiotic susceptibility testing for carbapenems was performed on all the isolated strains by the standard Kirby Bauer's disc diffusion method as per the CLSI guidelines[10], and Carbapenemase detection was done using RAPIDEC CarbaNP test (BioMerieux, India). It is based on the detection of carbapenem hydrolysis by the carbapenemase- producing bacteria.[11] Quality control: Positive control strain: *Klebsiella pneumonia* ATCC® BAA-1705™

Negative control strain: *Klebsiella pneumonia* ATCC® 700603™. Genotypic detection of the resistant genes, bla_{NDM} and $bla_{KPC,}$ was done using conventional multiplex PCR. *Klebsiella pneumonia* ATCC® BAA-1705™ (positive control), *Escherichia coli* ATCC 25922 (negative control)[12]

This study was approved by the institutional ethics committee. Data analysis was done using descriptive statistics in Microsoft Excel

RESULTS:

A total of 165 clinical isolates were analyzed, comprising samples from respiratory specimens, urine, blood and body fluids, and pus or wound swabs. The data in Table 1 revealed notable variations in the distribution of bacterial species across different sample types. Respiratory specimens constituted the largest sample group (n = 100). Among these, *Klebsiella* spp. was the most prevalent organism, accounting for 40% of the isolates. This was followed by *Pseudomonas* spp at 27%, *Acinetobacter* spp. at 19%, and *E. coli* at 14%. Urine samples (n = 36) demonstrated a different distribution, with *E. coli* being the dominant isolate (41%), followed by *Pseudomonas* spp (36%) and *Klebsiella* spp (19%), while *Acinetobacter* spp was rarely encountered (2%). In blood and body fluid samples (n = 20), *E. coli* and *Klebsiella* spp were equally prevalent, each contributing 45%, while *Acinetobacter* spp accounted for 10%. Interestingly, *Pseudomonas* spp was not detected in this category. Pus and wound swabs (n = 9) showed a relatively even distribution of isolates, with *E. coli* comprising 33%, and *Klebsiella* spp., *Pseudomonas* spp., and *Acinetobacter* spp each contributing 22%. Overall, *Klebsiella* spp was the most frequently isolated organism, representing 35% of the total isolates (n = 58), followed by *Pseudomonas* spp (25%, n = 42), *E. coli* (25%, n = 41), and *Acinetobacter* spp (15%, n = 24). The proportion of isolates resistant to carbapenems was strikingly high across all bacterial species, as shown in Figures 1 and 2. Among the tested isolates, *Acinetobacter* spp exhibited the highest resistance rate, with 85% of isolates resistant to carbapenems. This was followed by *Klebsiella spp.,* with 68% resistance, *E. coli* with 62%, and *Pseudomonas* spp with 58%. These findings indicate a significant prevalence of carbapenem resistance among Gram-negative pathogens, posing serious challenges to clinical management. The Rapidec CarbaNP test further confirmed carbapenemase production in the resistant isolates. This test demonstrated a positive result for

carbapenemase activity in isolates of *E. coli*, *Klebsiella spp.*, *Pseudomonas spp.*, and *Acinetobacter spp.*, supporting the findings from molecular testing. The molecular analysis of resistance genes provided further insights into the mechanisms driving carbapenem resistance. Among the tested isolates, the bla_{NDM} gene was detected in six isolates of *Klebsiella spp.*, confirming the role of this carbapenemase in mediating resistance. None of the tested isolates, including *E. coli* and *Klebsiella spp.*, were positive for the bla_{KPC} gene, suggesting that other mechanisms, such as efflux pumps or other beta-lactamase genes, may contribute to carbapenem resistance in these bacteria. Polymerase chain reaction (PCR) results, visualized via agarose gel electrophoresis, validated the presence of the bla_{NDM-1} gene in Sample 4, as indicated by the amplified product in Lane 7. Positive controls for bla_{NDM-1} and bla_{KPC} genes (Lanes 2 and 3, respectively) further confirmed the accuracy of the assay. Other tested samples (Lanes 4–6 and Lane 8) were negative for both genes, consistent with the absence of amplification products in these lanes. The high correlation between phenotypic and molecular results underscores the reliability of these diagnostic approaches.

Table 1: Distribution of isolates in different samples

	E coli	*Klebsiella* spp	*Pseudomonas* spp	*Acinetobacter* spp	Total number of samples
Respiratory samples	14(14%)	40(40%)	27(27%)	19(19%)	100
Urine	15(41%)	7(19%)	13(36%)	1(2%)	36
Blood and body fluids	9(45%)	9(45%)	-	2(10%)	20
Pus and swabs	3(33%)	2(22%)	2(22%)	2(22%)	9
TOTAL	41	58	42	24	165

Table 2: Gene detection of Bla_{NDM} and Bla_{KPC} genes

ORGANISM	Bla_{NDM} gene	bla_{KPC} gene
E coli	-	-
Klebsiella spp	6	-

Figure 1: Percentage of organisms resistant to Carbapenems

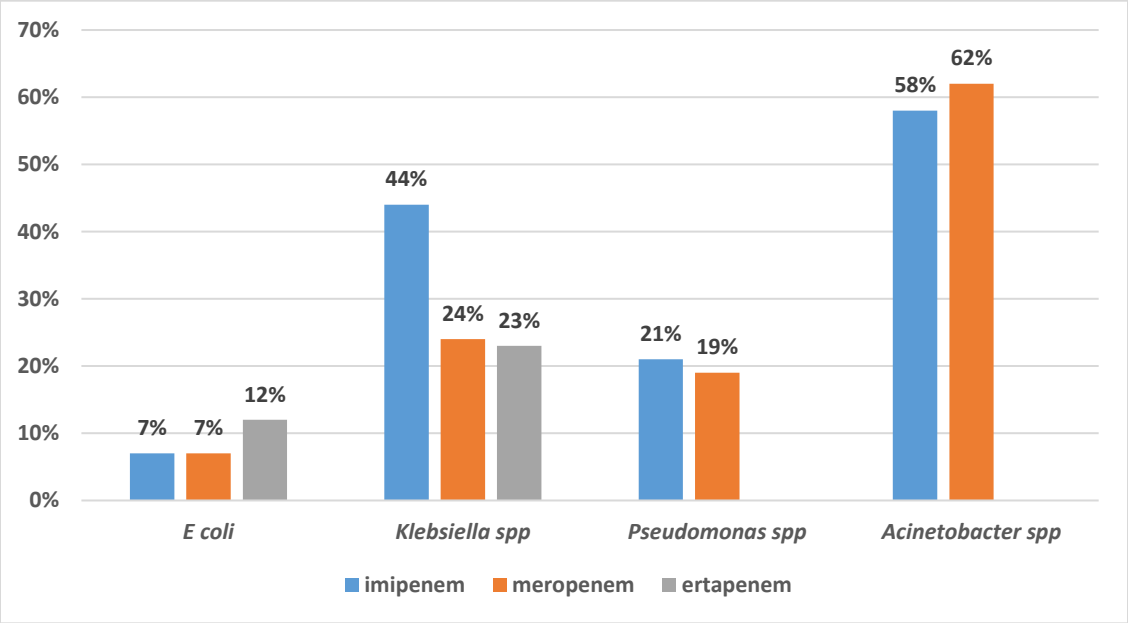


Figure 2: Percentage of the organisms resistant to Carbapenems

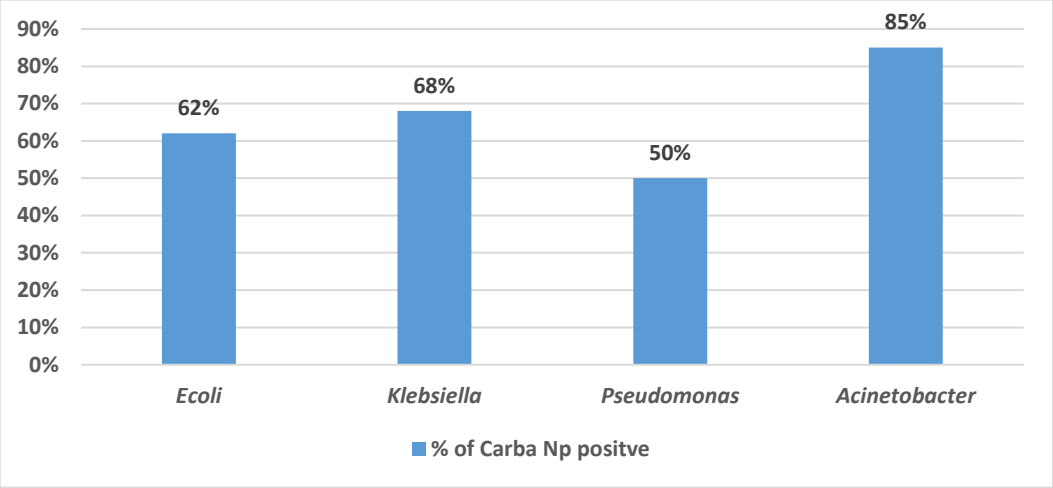


Figure 3: Rapidec CarbaNP test.

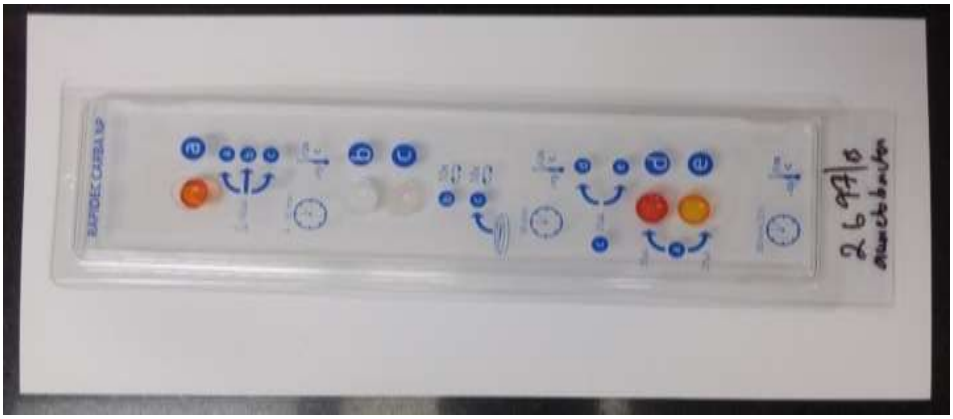
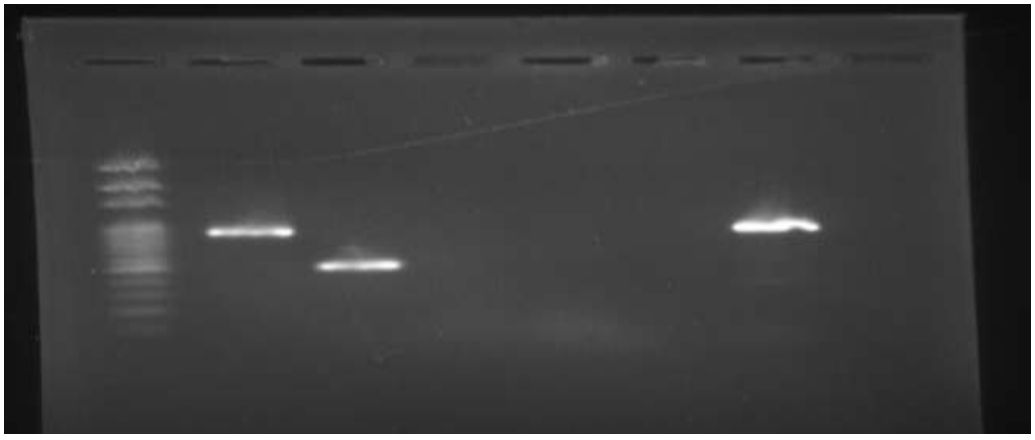


Figure 4: Polymerase chain reaction of bla_{NDM-1} and bla_{KPC} gene



Lane 1: DNA ladder (100 bp)
Lane 2: Positive control for bla_{NDM-1} gene
Lane 3: Positive control for bla_{KPC} gene
Lane 4: Sample 1- Negative for bla_{NDM-1} and bla_{KPC}
Lane 5: Sample 2- Negative for bla_{NDM-1} and bla_{KPC}
Lane 6: Sample 3- Negative for bla_{NDM-1} and bla_{KPC}

Lane 7: Sample 4- Positive for bla_{NDM-1}
Lane 8: Sample 1- Negative for bla_{NDM-1} and bla_{KPC}

DISCUSSION:

The results of this study support the notion that the rate of new ICU-acquired Gram-negative infections is still high amongst a cohort of medical-surgical patients, and is mostly due to MDR bacteria. During our study period a total of 165 Gram negative bacteria was isolated from the specimens received from patients in ICU. Among the gram negative bacteria, *Klebsiella* spp was found to be the most commonest infection causing organism (35%) followed by *Pseudomonas* spp(25.3%), *E coli* (24.8%) and finally *Acinetobacter* spp(14.5%). The results cumulated contradicts the 12 year period study from1993-2004 conducted in the ICUs of multiple hospitals in the US conducted by Merck Research Laboratories, the most common organisms being *P.aeruginosa* (22.2%), *E.coli* (18.8%), *K.pneumoniae* (14.2%), *Acinetobacter* spp (6.2%)[13]. A study conducted in a neurological ICU of a tertiary hospital in central India described *Pseudomonas* to be the highest number isolated (33.20%), followed by *Klebsiella* (31.22%).[14] This shows that the bacterial trends in this region is different as *Klebsiella* species is found to be most prevalent in the ICU our hospital.

Also, the most common sample that we obtained from the ICU which showed the growth of GNB was respiratory samples (56%), followed by urine (36%), blood and body fluids (14%) and lastly pus and swabs (6%). A study done by K. Sharma et al. in Chandigarh shows that urine 25% was the most common, followed by respiratory samples 24%, blood 23%, then pus and swabs13%, which differs from our study slightly as respiratory samples were the most common.[15]

Carbapenems, the last line of therapy, are now frequently needed to treat resistant gram negative infections, and increasing resistance to this class of β-lactams leaves the health care system with almost no effective drugs. Among the Enterobacteriaceae, *E coli* found to be the most sensitive with 93% each for imipenem and meropenem and 83% sensitivity for ertapenem. *Klebsiella spp* had a lower sensitivity with 56%, 59% and 61% for the three drugs respectively. Among the non-fermenters *Pseudomonas spp* showed a higher sensitivity of 79% and 81% for imipenem and meropenem and *Acinetobacter spp* showed a sensitivity of 42% and 38% respectively. Ghafur *et al* showed almost the same results with 93%, 93%, 90% sensitivity in *E coli* for imipenem, meropenem and ertapenem while *Klebsiella* had a slightly lower sensitivity with 92%, 91% and 84 % respectively.[16] Among the non-fermenters *Pseudomonas spp* had a sensitivity of 70.1% and 69.1% for imipenem and meropenem and *Acinetobacter spp* showed a low sensitivity of 27% and 25% respectively. This study has similar findings and it can be commented that due to the same geographical area (South India) the resistance patterns may be similar.

The Rapidec CARBANP test done in this study showed that 85% of *Acinetobacter* spp, 76% of *Klebsiella* spp, 71% of *E. coli* and 53% of *Pseudomonas* spp showed positive. The study investigates the carbapenem resistance in ICU isolates of *Klebsiella* and *E. coli* by Budak S *et al.*, out of 108 of 196 patients,130 *E.coli* and *Klebsiella pneumonia*e related nosocomial infections was determined. The carbapenemase resistance was determined at 22% for *K.pneumoniae* and 3% in *E.coli* strains.[17] Garg *et al* published a study where the same kit was used. They found 23 out of 26 (88%) *E coli*, 2 out of 3(66%) *Klebsiella spp*, and 10/10(100%) *Pseudomonas spp* and 5/5(100%) *Acinetobacter spp* showed a positive result for the test.[18]

One of the methods of resistance to carbapenems is Enzyme-mediated, which is due to the production of beta-lactamases that render the carbapenem inactive. This type of resistance is the most important clinically because these enzymes hydrolyze beta-lactams, confer high levels of minimum inhibitory concentrations (MICs). The other mechanisms of carbapenem resistance are reduced outer membrane permeability by loss of porins and overexpression of efflux pumps that expel carbapenems, mostly meropenem, may lead to carbapenem resistance.

PCR for bla_{NDM} and bla_{KPC} was performed on 65 Enterobacteriaceae that were resistant to any one of the carbapenems (imipenem, meropenem, ertapenem) and which was subject to the RAPIDEC CarbaNP test. We found 6 isolates, all *Klebsiella spp* species, containing the NDM gene, we did not find any positive result for *E coli*. A study by Shenoy *et al* from Bangalore found similar results.[19] (34 NDM positive among 74 MDR *Klebsiella spp* isolates), whereas Karabay *et al* from Turkey found no NDM positive strains in 100 isolates which contradicts our study.[20] The study by Azzab *et al* contradicts ours as they found 6 positives of KPC out of 26

imipenem resistant isolates, whereas in our study we did not find any.[32] This is synonymous with the fact that NDM has been reported to be found in this part of the world and KPC still has not made its way to South Asia. The reason for negative result for PCR for bla $_{NDM}$ and bla$_{KPC}$ in the carbapenem resistant isolates may be the presence of other carbapenemase producing genes such as bla $_{VIM}$, bla $_{IMP}$ and bla $_{OXA}$

CONCLUSION:

Our study shows that Gram Negative Isolates commonly cause nosocomial infection with Klebsiella spp being the most common, followed by Pseudomonas, Ecoli and finally Acinetobacter, and though the number of Acinetobacter organisms were the least, they showed the most resistance to the important drugs used. There is an alarming increase in the rate of MDR Gram negative superbugs in ICUs thus increasing the morbidity, mortality and additional cost to the hospital. Constant evaluation on the use of antibiotics should be done, antibiotic stewardship should be practiced, and also there should be good rapport between microbiologist and the clinicians in order to curb this alarming situation.

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