

EMERGING SUPER BUGS IN BLOOD STREAM INFECTIONS AND ITS MOLECULAR CHARACTERIZATION IN INTENSIVE CARE UNITS AT A TERTIARY CARE HOSPITAL

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Received : 10/06/2026
Received in revised form : 30/07/2026
Accepted : 13/08/2026

Keywords:
BSI, ICU, MDRO, blaKPC, Carbapenems.

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DOI: 10.47009/jamp.2026.8.4.275

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2026; 8 (4); 1626-1628



ABSTRACT

Background: Blood stream infections (BSIs) followed by sepsis, the second leading cause of death is due to infections. Intensive care units (ICUs) are having the highest burden of treating the patients with BSI and nosocomial infections compared to other areas of hospital. Our objective was to identify the bacteriological profile and emerging drug resistant pattern of blood stream infections & molecular detection of bla- KPC gene in isolates of carbapenem resistant *Klebsiella pneumoniae* causing BSI in ICUs. **Materials and Methods:** Totally, 102 blood samples were collected from patients with clinically suspected sepsis. All samples were processed by conventional methods & growth were identified by Standard guidelines. Antimicrobial sensitivity testing and molecular study were done for the identification of resistant gene. **Result:** Resistance were high among Gram negative organisms (n = 33) compared to Gram positive isolates (n = 17). Among Enterobacteriaceae, *Klebsiella pneumoniae* showed more resistance and blaKPC gene (40%) plays a major role in *Klebsiella pneumoniae* resistance. **Conclusion:** Enterobacteriaceae family was the leading cause among Gram negative bacilli and multi drug resistance was noticed in this family. *Klebsiella* was the most common multi drug resistant bacteria and even showed resistant to last resort of the medical field that is carbapenems which was confirmed by both disc diffusion and minimum inhibitory concentration methods. All the ICUs should do molecular method for identification of these type of isolates and start early treatment depending upon the resistant pattern and to have a better outcome.

INTRODUCTION

Superbugs are nothing but multidrug- resistant organisms (MDROs) have been increasingly reported from health care facilities during the last decade. The clinical outcome of patients infected with MDROs can be more dreadful, compared to the susceptible strains.^[1]

Despite the advances in conditioning, supportive care and prevention, blood stream infection is playing a major role in morbidity and mortality in intensive care unit patients due to continued emergence of superbugs along with new antimicrobial resistance patterns.^[2]

Klebsiella pneumoniae carbapenemase (KPC) is one of the main cause of resistance to carbapenems in *Klebsiella pneumoniae*, and it can widely spread due to its location on various plasmids. Dissemination of KPC around the world highlights the role of blaKPC gene in the spread of antimicrobial resistance. Thus,

strains harbouring blaKPC gene are a major cause of concern for healthcare systems around the world.^[3] In this study, out of 102 blood samples collected, 54 samples turned out to be positive. Therefore, stringent strategies should be developed to prevent and control the spread of MDROs and to minimize the risk of cross infection to other patients, staff and visitors.

MATERIALS AND METHODS

Study design, place, and population: This study was conducted at SRM Medical College and Hospital, Kattankulathur, India, and was prospective and cross-sectional. The subjects of this study were symptomatic hospitalized patients who had been admitted in intensive care units between March 2019 and February 2020.

Sample collection: In this study, blood samples were collected from intensive care unit patients suspected

of having blood stream infection in brain heart infusion (BHI) broth.

Sample rejection criteria: Blood samples received in an inappropriate container, incorrectly labeled or unlabeled samples were summarily rejected after discussing with clinical team in an appropriate way.

Sample processing: 5 ml of blood was collected from each adult patient using strict aseptic precautions, and inoculated immediately into 50 ml of 'Brain Heart Infusion' (BHI) broth with 0.025% of sodium polyanethol sulfonate as anticoagulant (HI media). In paediatric cases 1 - 2 ml of blood was inoculated in 5 - 10 ml of BHI broth. The broths were observed on daily basis for seven days. A negative result was followed-up by examining the broth daily and doing a final blind subculture at the end of seventh day. Positive growth was identified by Gram staining, colony characteristics, and standard biochemical tests. Antimicrobial susceptibility testing were done according to clinical and laboratory standards institute guidelines [Figure 1-3]. Molecular analysis was done to detect blaKPC gene in *Klebsiella pneumoniae* isolates only.

Ethical considerations: This study protocol was reviewed and approved by the SRM Medical College Hospital & Research Centre (approval number: 1585/IEC/2019). All participants provided informed consent prior to their inclusion in this study. Confidentiality of personal information was maintained at all parts of this study, and participants were informed of their right to withdraw from the study at any point of time without any compromise in the quality of their medical care.

RESULTS



Figure 1: Colony characteristics

Out of 102 samples, 53% (n = 54) turned out to be positive for culture. [Figure 4] showed that among the 54 isolates, *Escherichia coli* (n = 15) remained at the top, followed by *Klebsiella pneumoniae* (n = 10), *Staphylococcus* other than *Staphylococcus aureus* (n = 9), *Staphylococcus aureus* (n = 6), *Candida* species (n = 4), *Acinetobacter* species (n = 3), *Pseudomonas* species (n = 2), *Enterococcus faecalis* (n = 2), *Proteus*

mirabilis (n = 1), *Salmonella Typhi* (n = 1), and *Citrobacter koseri* (n = 1). Out of 33 Gram negative isolates, 5 *Klebsiella pneumoniae* isolates showed resistance to carbapenems and subjected for molecular analysis. Among the five carbapenem resistant *Klebsiella pneumoniae* (CRKP) isolates, blaKPC gene detected for two isolates only.



Figure 2: Antimicrobial susceptibility testing in MHA plate

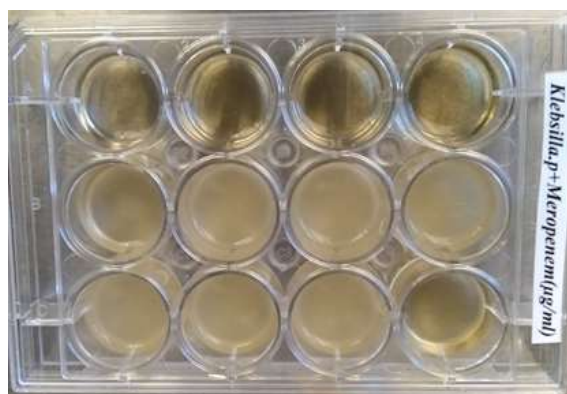


Figure 3: Minimum inhibitory concentration

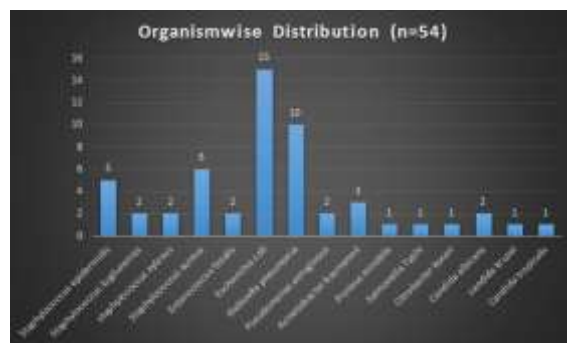


Figure 4: Organism wise distribution

DISCUSSION

Bloodstream infections (BSIs) are among the leading acquired infections in intensive care unit (ICU) patients. During these last few years, clinicians have witnessed a growing incidence of BSIs by bacteria with resistance against commonly used antimicrobials.^[4] In our study, major positive samples were from IMCU (42.6%) similarly higher

percentages are seen in other studies also, a study done by Calik Z had highest percentage with 81% of his samples were from IMCU. The variations in sample percentage from different ICUs depends on the health care catering of that hospital.^[5]

Enterobacteriaceae were the most commonly isolated organisms followed by non-fermenter. Among enterobacteriaceae, *Escherichia coli* is the commonest followed by *Klebsiella pneumoniae*. Similar pattern of bacteriological profile of organisms were isolated in some other studies like Samandika et al,^[6] In this study the percentage of resistance is high (24%) in gram negative bacteria than gram positive bacteria (14%). In a similar manner, high rate of multi-drug resistant gram-negative bacteria infections was 47% in Shreddha Siwakoti et al,^[7] Percentage of resistance among Enterobacteriaceae is high in *Klebsiella pneumoniae* (58%) than *Escherichia coli* (42%). Similar kind of high prevalence (84%) of *Klebsiella pneumoniae* resistance was seen in a tertiary care hospital in China.^[8]

Among 33 gram negative organisms, five *Klebsiella pneumoniae* isolates showed resistance to carbapenem by both phenotypic and genotypic methods (blaKPC gene). Other genes responsible for carbapenem resistance along with bla kpc gene are blaGES, blaIMI/NMC-A, blaIMP, blaVIM, blaGIM, blaSPM, blaSIM, blaNDM-1 and blaOXA-48 as per Jikun Du study.^[9] Therefore strong antibiotic policies and quality care in both hospitals and laboratories would help us to survive in these kind of multi drug resistance era.

CONCLUSION

In our study, intensive medical care unit had the maximum burden of BSI and Gram negative bacilli were the most common etiological agent isolated. Enterobacteriaceae family was the leading cause among Gram negative bacilli and multi drug resistance was noticed in this family. *Klebsiella* was the most common MDR bacteria and even resistant to last resort of treatment option for these that is carbapenems confirmed by both disc diffusion and minimum inhibitory concentration methods. 40% of our *Klebsiella* isolates were confirmed to carry blaKpc gene by molecular detection which has high transmissibility of MDR to other species and increase

the MDR nosocomial infection. So, we assumed that it is better that all the ICUs should do molecular method for identification of these type of isolates and start early treatment depending upon the resistant pattern and to have a better outcome.

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