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Evaluation of Rapid Methods of Carbapenemase Genes Detection Compared to Automated and Phenotypic Methods of Determining Antimicrobial Susceptibility

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Abstract

Background: Carbapenem-resistant Gram-negative bacteria, particularly Enterobacteriaceae, pose a major public health challenge due to limited therapeutic options and increased morbidity and mortality. Rapid detection of carbapenemase-producing organisms is essential for timely and appropriate antimicrobial therapy.

Objectives: To determine the prevalence of carbapenemase-producing Gram-negative bacilli and to evaluate rapid, cost-effective methods-lateral flow immunochromatographic assay and PCR-for detecting carbapenemase genes in comparison with phenotypic and automated susceptibility testing.

Material and Methods: A prospective study was conducted on 100 non-duplicate clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* collected over three months. Identification and antimicrobial susceptibility testing were performed using the VITEK 2 system and E-test. Carbapenemase detection was carried out using lateral flow immunochromatographic assay and real-time PCR targeting KPC, NDM, OXA-48, VIM, and IMP genes.

Results: Out of 100 isolates, 32% showed carbapenem resistance. The prevalence was highest in *P. aeruginosa* (47.61%), followed by *K. pneumoniae* (38.88%) and *E. coli* (18.6%). Among resistant isolates, 31/32 were positive for carbapenemase genes by both rapid methods. NDM was the most prevalent gene (90.62%), followed by OXA-48 (43.75%) and VIM (3.13%), with co-existence observed in several isolates. Both lateral flow assay and PCR demonstrated high diagnostic accuracy with sensitivity of 96.88% and specificity of 100%. Most carbapenem-resistant isolates were also resistant to ceftazidime-avibactam, except those harboring only OXA-48.

Keywords: Carbapenem resistance; NDM; OXA-48; Lateral flow immunochromatographic assay

Introduction

Antibiotic resistance places significant pressure on Indian healthcare systems. (R1) The World Health Organization has highlighted the Carbapenem-resistant Enterobacteriaceae (CRE) particularly among drug-resistant bacteria as one of the most critical bugs that urgently need new antibiotics to treat effectively. (R2) Enterobacteriaceae are frequent causes of serious infections, including pneumonia, bloodstream infections, complex urinary tract infections, and complex intra-abdominal infections. (R3) Therefore, increasing resistance in this class of bacteria leads to increased mortality, longer hospital stays, and significantly higher medical expenses compared to bacterial infections responsive to carbapenems. (R4)

In India, carbapenem resistance, particularly in ICU settings, has increased drastically over the last decade with CR- *E. coli* and CR- *Klebsiella pneumoniae* being the dominant cause, accounting for 90% of all CRE strains (R5). The common resistance mechanisms for carbapenem resistance in Enterobacteriaceae are

enzyme production, efflux pumps and porin mutations which often co-exist. (R3) In India, enzyme production due to presence of NDM and OXA-48 is the main resistance mechanism. (R5)

The choice of therapy in critically ill patients is increasingly limited due to the dissemination of CREs in the ICUs. It is crucial to decide empirically whether to start critically ill patients on ceftazidime avibactum, carbapenems or colistin. To identify appropriate empirical therapy for critically ill patients, this study set out to ascertain the prevalence of Carbapenemases and assess quick and affordable methods for identifying the carbapenemase genes from gram negative bacilli.

Materials and methods

A total of 100 consecutive clinically confirmed isolates of *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were collected from non-duplicate culture specimens obtained from Department of Microbiology, Pacific institute of Medical Sciences, Udaipur. The study period was from June 2025 to August 2025. Prospective study was carried out. The study was approved by Institutional Ethical Committee and Ethical clearance was obtained.

The isolates were obtained from urine, rectal swabs/stools, blood, respiratory specimens, wounds, sterile fluids and tissue samples. The isolates were subjected to Automated identification and sensitivity by Vitek2- system (make-bioMérieux, France) (identification by GNID and antibiotic susceptibility by N405/N406 cards) and E-tests for Ceftazidime-avibactum. The Clinical Laboratory Standards Institute (CLSI) interpretation guidelines were followed. Isolates other than *E. coli, K. pneumoniae,* or *P. aeruginosa* were not included in the study.

All strains were tested by Lateral flow chromatography assay and genotypic methods (PCR) for the identification of the genes KPC, IPM, VIM, NDM, and OXA-48.

Lateral flow chromatography assay

The lateral flow chromatography assay used was a commercially available rapid and visual multiplex immunochromatographic assay that detected one or more of the five common types of carbapenemase enzymes (KPC (K), OXA-48-like (O), IMP (I), VIM (V), NDM (N)) in bacterial colonies.

Principle: Monoclonal antibodies that individually recognize each of the five carbapenemases are immobilized on a nitrocellulose membrane. Free monoclonal antibodies are present in the conjugate pad and labeled with colloidal gold. Upon addition of colonies mixed with extraction buffer to the sample pad, the capillary action of the nitrocellulose draws the sample through the mobile antibodies and immobile antibodies on the test strip. The immobilized control antibodies capture any mobile antibodies that run through the sample pad and nitrocellulose without binding to other test lines.

Liquid extraction buffer was used as a cell lysing solution when mixed with colonies. A 1 µL loopful of bacteria was mixed with five drops of extraction buffer, and 100 µL of the mixture was dispensed into the cassette. Results were interpreted after 15 min of incubation at room temperature. A positive result was interpreted when a red line appeared on the control region (C) and one or more lines appeared in the test regions (K, O, V, I, or N) and indicated that the sample contained one or more carbapenemases. A negative result was noted when only the control line was observed and indicated that the sample did not contain any of the 5 carbapenemases. If the control line did not appear, the test result was considered invalid.

Genotypic methods (RT-PCR)

Commercially available Carbapenem Resistance Detection Kit was used as a nucleic acid amplification assay for the qualitative detection and differentiation of the KPC (K), OXA-48-like (O), IMP (I), VIM (V), NDM (N)gene sequences associated with carbapenem-non-susceptibility on Real-Time PCR.

To isolate genomic DNA, a pure culture of bacterial isolates was cultured overnight on nutrient agar culture plates. PCR amplification was used to detect carbapenemase-encoding genes in all isolates. PCR reaction mix was of 15 ul master mix (consisting of Hot-start DNA polymerase Reaction Buffer, dNTPs (dATP, dCTP, dGTP, dTTP) MgCl2 and stabilizers) and 5 µl of primer probe mix in two tubes (Tube1 for bla_{KPC}, bla_{NDM} and Internal Control detection and Tube 2 for bla_{OXA-48}, bla_{VIM} and bla_{IMP} detection). 20 µl of the PCR reaction Mix was added to 5 µl of DNA or Positive control or Negative control to make up the

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final volume 25 µl. Cycle conditions were 10 min hold at 95°C 38 cycles of amplification consisting of 15 sec at 94°C, 60 sec at 45°C and 15 sec at 72°C. Amplification confirmed the presence of bla_{KPC}, bla_{NDM} ,bla_{OXA-48}, bla_{VIM} and bla_{IMP} genes.

Results

Among the 100 consecutive isolates collected, 43 were *Escherichia coli*, 36 were *Klebsiella pneumoniae*, 21 were *Pseudomonas aeruginosa* as confirmed by VITEK results. Out of 100 isolates, 68 were sensitive to carbapenems and 32 isolates showed phenotypic resistance to carbapenems. 70 were sensitive to ceftazidime-avibactam and 30 isolates were resistant to ceftazidime-avibactam. Prevalence of Carbapenem resistance in *Escherichia coli* was 18.6 %, in *Klebsiella pneumoniae* was 38.88% and *Pseudomonas aeruginosa* was 47.61%.

The carbapenem susceptible strains (68) were negative for presence of carbapenemases on the immunochromatographic assay as well as PCR. These strains were also susceptible to ceftazidime avibactam.

The 32 carbapenem resistant isolates were subjected to immunochromatographic assay and nucleic acid amplification assay. Of these, 31 were detected as having carbapenemase genes by lateral flow assay and PCR. The most common genes were NDM (29 isolates), OXA-48 (14 isolates) and VIM (1 isolate), found in combination in 13 isolates. Overall, the prevalence of NDM was 90.62%, OXA-48 was 43.75% and VIM was 3.13%. One carbapenem resistant isolate of *Pseudomonas aeruginosa* was missed by both methods. (Table 2).

Considering the phenotypic test as reference method, Sensitivity 96.88%, Specificity 100.00%, Positive Predictive Value 100.00%, Negative Predictive Value 98.68% was obtained for the immunochromatographic assay as well as the PCR test. Of these 32 carbapenem resistant isolates, 30 were resistant to ceftazidime-avibactam. MIC for 2 isolates was in the sensitive range. These isolates corresponded to having only OXA-48 as the carbapenemase. (Table 3)

Table 1: Pathogen distribution and Susceptibility to carbapenems and ceftazidime-avibactam

		Carbapenems		**Ceftazidime-avibactam**	
Organism	**Number**	**Susceptible**	**Resistant**	**Susceptible**	**Resistant**
E. coli	43	35	8	36	7
Klebsiella pneumoniae	36	22	14	23	13
Pseudomonas aeruginosa	21	11	10	11	10
Total isolates	100	68	32	70	30

Table 2: Results of ICT and PCR

Gene combinations	**No. of isolates (ICT)**	**No. of isolates (PCR)**
KPC	0	0
IMP	0	0
VIM+NDM	1	1
NDM	**16**	**16**

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OXA-48	**2**	**2**
NDM and OXA-48	**12**	**12**
All neg	1	1
Total isolates	**32**	**32**

Table 3: Correlation of ceftazidime-avibactam with presence of Carbapenem genes

	Carbapenem Resistant	**Ceftazidime avibactam Sensitive**	**Ceftazidime avibactam Resistance**	**NDM**	**OXA-48**	**VIM**	**KPN**	**IMP**
E. coli	8	1	7	7	4	0	0	0
Klebsiella pneumoniae	14	1	13	13	9	1	0	0
Pseudomonas aeruginosa	10	0	10	9	1	0	0	0
Total isolates	32	2	30	29	14	1	0	0

Discussion

Gram negative bacilli remain the leading cause of infections causing a wide range of infections from pneumonia, urinary tract infections and gastrointestinal infections. They are implicated as a major cause in hospital acquired infections. However, carbapenem resistance in gram negative bacilli has emerged as a significant international public health problem with no immediate solution. (R2, R4) Carbapenems are the treatment of choice for extended-spectrum beta-lactamase (ESBL) and *AmpC* producers. With the global transmission of carbapenem resistant bacteria and the lack of new effective antibiotics, the rapid and accurate diagnosis with targeted therapy is of essence for minimizing mortality (R4).

In the current study, the prevalence of Carbapenem resistance in 100 consecutive clinical Gram-negative isolates comprising *Escherichia coli* (43%), *Klebsiella pneumoniae* (36%), and *Pseudomonas aeruginosa* (21%) was overall determined to be 32%. The carbapenem resistance rate varied between species with *E. coli* showing 18.6%, *K. pneumoniae* showing 38.9%, and *P. aeruginosa* having the highest at 47.6%. These results closely reflect the findings of AMR Surveillance Network, Indian Council of Medical Research (R6) which showed increasing carbapenem resistance in *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Results from nearby regions also show prevalence of CRE as 29.07% over a period of five years. (R7, R8)

Majority of the tested strains (68%) were carbapenem susceptible on phenotypic testing. This was also reflected on genotypic as well as the lateral flow immunochromatographic assay. These strains were susceptible to ceftazidime avibactam. Therefore, absence of carbapenemase genes on testing can be considered a reliable indicator for the effectiveness of carbapenems for empiric treatment. This is reflected by multiple studies which reflect the high positive predictive value of the lateral flow assay. (R5, R9, R10).

Out of the resistant isolates, 31 were detected accurately as having carbapenemase genes by lateral flow as well as genotypic assay. NDM was the most prevalent followed by OXA-48 and VIM. This is

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consistent with earlier research showing that NDM is the most common in India, particularly in combination with OXA-48. (R1, R5, R11) Both methods overlooked one strain of *Pseudomonas aeruginosa* that was resistant to carbapenem. This may be due to the presence of other carbapenem resistance mechanisms such as efflux pumps, porin loss or hyper expression of *AmpC*. (R9, R10). Presence of only OXA-48 correlated with sensitivity to Ceftazidime avibactam. These findings are consistent with those reported in previous studies. (R1, R5)

The diagnostic performance of the lateral flow immunochromatographic assay and PCR were excellent when compared to the phenotypic reference standard. Both methods yielded a sensitivity of 96.88%, specificity of 100%, a positive predictive value (PPV) of 100%, and a negative predictive value (NPV) of 98.68%. These values are consistent with previously published data supporting the high reliability of rapid molecular and immunochromatographic techniques for carbapenemase detection. (R9, R10, R12, R13)

Numerous studies have demonstrated the high diagnostic accuracy of the lateral flow immunochromatographic assay. (R9, R10, R12, R13) With high sensitivity and specificity across the main carbapenemase families, it has shown reliable results in *Enterobacterales* and other gram-negative bacteria such as *Pseudomonas aeruginosa*. Compared to molecular methods like multiplex PCR, the test is much faster, less demanding technically and more affordable, with results available within 20 minutes.

Rapid and accurate detection of Carbapenemase resistance in gram negative bacilli is of great necessity for both treatment decision making and implementation of infection control. Implementation of lateral flow immunochromatographic assay can reduce the time to initiating specific therapy by a day, which has a great impact of patient mortality and morbidity in ICUs, especially when treating invasive infections by carbapenem resistant gram-negative bacteria. (R1, R12, R13)

Limitations

In this study, small sample size from a single centre is a limitation of this study. In this study, bla_{ndm} and $bla_{OXA-48}$are the predominant carbapenemases and therefore, the results may not be replicable in areas where bla_{VIM} and bla_{IMP} are the predominant carbapenemase. Sequencing to determine the false negatives was not done to rule out subtypes or mutations in the carbapenemases.

Conclusion

The high frequency of carbapenem resistance among clinically relevant Gram-negative bacteria, which is largely mediated by NDM and OXA-48 carbapenemases, is emphasized in this study. Prompt and precise identification of these resistance mechanisms using PCR and immunochromatographic assays enables prompt treatment choices and infection prevention strategies. The poor efficacy of ceftazidime-avibactam against isolates that produce NDM highlights the pressing need for improved antimicrobial stewardship and new therapeutic approaches.

In situations like intensive care unit admissions, bloodstream infections, and surgical site infections where prompt decision-making is necessary, the lateral flow immunochromatographic assay test is particularly beneficial. Targeted antibiotic therapy, suitable isolation techniques, and early contact precautions are made feasible by it. Additionally, incorporating it into antimicrobial stewardship initiatives improves treatment efficacy overall and contributes to a decrease in the usage of broad-spectrum antibiotics.

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