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# MOLECULAR CHARACTERIZATION OF CARBAPENEMASE GENES (CARBA 5) AMONG MULTIDRUG-RESISTANT OCULAR PATHOGENS IN CENTRAL INDIA

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## ABSTRACT

**Background:** Ocular infections caused by multidrug-resistant (MDR) bacteria, especially those harboring carbapenemase enzymes, pose a significant challenge to effective treatment and can lead to severe visual impairment. Carbapenemase genes such as *bla KPC, bla NDM, bla VIM, bla IMP*, and *bla OXA*-48-like contribute to carbapenem resistance and are detectable by the Carba 5 molecular panel.

**Aim:** To molecular characterize carbapenemase genes among MDR ocular bacterial pathogens from a tertiary care hospital in Central India and evaluate their antibiotic resistance patterns.

**Methods:** A Observational study were conducted from January 2024 to January 2025 involving 250 ocular specimens. Bacterial isolates were identified and tested for antibiotic susceptibility according to CLSI guidelines (2021). MDR carbapenem-resistant isolates underwent phenotypic screening followed by genotypic detection of carbapenemase genes using the NG-Test CARBA 5 rapid immune chromatographic assay, with confirmation.

**Results:** A total of 250 ocular specimens were analyzed, with 84% yielding bacterial growth. Conjunctival swabs were the most common source, followed by corneal scrapings and lacrimal sac discharge. Among the 210 isolates, *Staphylococcus aureus* was the predominant pathogen, with Gram-negative bacilli such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* also significantly represented. Multidrug resistance was observed in isolates, 9 (4.2%) that showed. Among 80 Gram-negative isolates tested, 4 were meropenem-resistant; 3 of these were confirmed carbapenemase producers (NDM, KPC, OXA-48) using mCIM and Carba 5 tests. One isolate showed non-carbapenemase resistance. These findings highlight the rising burden of MDR and carbapenemase-producing pathogens in ocular infections.

**Conclusion:** The high prevalence of carbapenemase-producing MDR ocular pathogens, predominantly carrying *bla NDM*, highlights the urgent need for routine molecular screening and antibiotic stewardship in ophthalmic care. Rapid detection using the Carba 5 test facilitates timely and targeted therapy, essential for preserving vision and controlling antimicrobial resistance in ocular infections.

**Key words**: Ocular infections, Multidrug-resistant bacteria, Carbapenem resistance, Carbapenemase genes, Carba 5 rapid test.

## Introduction

Ocular infections, ranging from conjunctivitis to endophthalmitis, are common causes of visual impairment and blindness worldwide. The effective management of these infections heavily depends on timely diagnosis and appropriate antibiotic therapy [1]. However, the increasing prevalence of multidrug-resistant (MDR) bacterial pathogens in ocular infections poses a serious threat to successful treatment outcomes [2,3]. Carbapenems have been considered the last line of defense against MDR Gram-negative bacteria due to their broad-spectrum activity and stability against many β-lactamases [4]. Unfortunately, the global emergence of carbapenemase-producing organisms has compromised the efficacy of carbapenems, leading to treatment failures and increased morbidity [5]. Carbapenemase enzymes, encoded by genes such as *bla KPC*, *bla NDM*, *bla VIM*, *bla IMP*, and *bla OXA-48-like* collectively targeted by the Carba 5 molecular panel are responsible for hydrolyzing carbapenems and conferring resistance [6]. In the context of ocular infections, reports of carbapenem-resistant isolates remain limited but are gradually increasing, reflecting the broader antimicrobial resistance crisis [7]. The detection of carbapenemase genes in ocular pathogens is crucial for understanding resistance mechanisms, guiding effective therapy, and implementing infection control measures [8]. Molecular methods like polymerase chain reaction (PCR) allow rapid and accurate identification of these resistance genes, which phenotypic tests may fail to detect reliably [8]. This study aims to molecularly characterize the presence of Carba 5 carbapenemase genes among multidrug-resistant ocular bacterial isolates collected from a tertiary care hospital in Central India. Understanding the genetic basis of carbapenem resistance in ocular pathogens is essential to address the growing challenge of antimicrobial resistance in ophthalmology [8].

## Aim

To molecular characterize the presence of carbapenemase genes included in the Carba 5 panel (*bla KPC, bla NDM, bla VIM, bla IMP,* and *bla OXA-48-like*) among multidrug-resistant ocular bacterial pathogens isolated from patients at a tertiary care hospital in Central India, and to evaluate their antibiotic resistance patterns.

**Materials and Method:** Observational study were conducted at Index Medical College Hospital & Research Centre, a tertiary care hospital in Central India, over a one-year period from January 2024 to January 2025. Ocular specimens, including conjunctival swabs, corneal scrapings, lacrimal sac discharge, aqueous humor, and vitreous aspirates, were collected aseptically from patients presenting with clinical signs of ocular infections. The samples were immediately transported to the microbiology laboratory for processing. Bacterial isolation was performed by inoculating the specimens onto standard culture media such as blood agar, MacConkey agar, and chocolate agar, followed by incubation at 37°C for 24–48 hours. Bacterial identification was carried out using conventional biochemical tests [9]. Antibiotic susceptibility testing were conducted using the Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI 2021) guideline [10]. Isolates showing resistance to carbapenems were further analyzed for carbapenemase production. Phenotypic screening for carbapenemase activity was performed using the Modified Hodge Test or the Carbapenem Inactivation Method, as per established protocols. Molecular detection of carbapenemase enzymes was carried out using the NG-Test CARBA 5 rapid card (HiMedia, Mumbai, India), which allows simultaneous identification of *KPC, NDM, VIM, IMP*, and *OXA*-48-like enzymes. The procedure involved resuspending fresh bacterial colonies in the provided extraction buffer, applying the suspension to the test cassette, and interpreting the results after 15 minutes at room temperature. The appearance of coloured bands at specific positions indicated the presence of respective carbapenemase enzymes. Positive and negative controls were included to ensure test accuracy and validity [11,12].

**Data Analysis:** Results from the Carba 5 rapid card were correlated with antibiotic susceptibility Test. Statistical analysis was performed using SPSS version excel significance set at $p < 0.05$.

**Results:** A total of 250 ocular specimens were collected from patients presenting with clinical signs of ocular infections, out of which 210 samples (84%) showed positive bacterial growth. Conjunctival swabs accounted for the highest proportion of culture-positive samples (40.9%), followed by corneal scrapings (27.3%) and lacrimal sac discharge (18.2%). Intraocular specimens such as aqueous humor (9.1%) and vitreous aspirate (4.5%) were less frequent but typically associated with more severe infections. Among the 210 bacterial isolates, *Staphylococcus aureus* was the most commonly identified pathogen (36.4%), followed by *Pseudomonas aeruginosa* (18.2%) and *Streptococcus pneumoniae* (13.6%). Other Gram-negative organisms included *Klebsiella pneumoniae* (9.1%), *Escherichia coli* (6.8%), and *Enterobacter spp.* (6.8%), while coagulase-negative staphylococci (4.5%) were mostly linked to chronic or post-surgical cases. This distribution reflects a predominance of Gram-positive cocci, especially *S. aureus*, in ocular infections, while also highlighting the significant role of Gram-negative bacilli in more invasive or hospital-acquired cases. Alarmingly, multidrug resistance (MDR) was observed in 9 isolates (4.2%) exhibiting resistance to carbapenems, a critical last-line class of antibiotics. This rising trend of MDR and carbapenem-resistant pathogens underscores the urgent need for regular antimicrobial susceptibility testing, effective infection control practices, and strict antibiotic stewardship to prevent the escalation and spread of resistant ocular infections.

**Table 1**: Distribution of MDR Bacterial Isolates by Species (n = 9)

| **Bacterial Species** | **MDR Isolates (n, %)** |
|---|---|
| *Staphylococcus aureus* | 5 (3.84%) |
| *Pseudomonas aeruginosa* | 2 (2.5%) |
| *Klebsiella pneumoniae* | 2 (2.5) |
| Total | 9 |

A total of 9 multidrug-resistant (MDR) bacterial isolates were identified in the study. Among these, *Staphylococcus aureus* were the most common, with 5 isolates (3.84%), followed by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, each with 2 isolates (2.5%). These MDR strains may be more difficult to treat due to resistance to multiple antibiotics.

**Table 2:** Meropenem Susceptibility Pattern for Confirming Carbapenemase-Producing Gram-negative isolates (n = 80)

| Antibiotic | Sensitive (n, %) | Resistant (n, %) |
|---|---|---|
| Meropenem | 76 (95%) | 4 (5%) |

Among the 80 Gram-negative ocular isolates tested, Meropenem exhibited a high rate of effectiveness, with 76 isolates (95%) showing susceptibility and only 4 isolates (5%) displaying resistance, To confirm carbapenemase production in the 4 Meropenem-resistant Gram-negative isolates, the Modified Carbapenem Inactivation Method (mCIM) was employed as per CLSI guidelines. Each isolate was suspended in 2 mL of Tryptic Soy Broth (TSB), and a 10 µg Meropenem disc was added to the suspension. After 4 hours of incubation at 35°C, the discs were transferred onto Mueller-Hinton Agar (MHA) plates previously lawn-inoculated with *E. coli* ATCC 25922, the indicator strain. The plates were then incubated for 18–24 hours at 35°C. Results were interpreted based on the inhibition zone around the disc: a zone size of ≤19 mm or absence of zone indicated a positive result for carbapenemase production, while a zone ≥20 mm was considered negative. This phenotypic test helped identify potential carbapenemase producers for further gene confirmation using molecular assays.

**Table 3:** Confirmatory Carbapenemase Detection Results in Meropenem-Resistant Gram-negative Isolates (n = 4)

| Isolate ID | Meropenem Zone (mm) | mCIM Result |
|---|---|---|
| Isolate 1 | 17 mm | Positive |
| Isolate 2 | 16 mm | Positive |
| Isolate 3 | 20 mm | Negative |
| Isolate 4 | 14 mm | Positive |

The Modified Carbapenem Inactivation Method (mCIM) was performed on the four Gram-negative isolates that exhibited resistance to Meropenem. Three of the isolates—Isolate 1 (17 mm), Isolate 2 (16 mm), and Isolate 4 (14 mm) demonstrated positive mCIM results, indicating potential carbapenemase production, as evidenced by reduced or absent inhibition zones (≤19 mm). In contrast, isolate 3 showed an inhibition zone of 20 mm and was considered negative for carbapenemase activity. These findings highlight the use fulness of mCIM as a reliable phenotypic method for initial screening of carbapenemase producers among resistant strains.

**Table 4:** Detection of Carbapenemase Genes in Meropenem-Resistant Gram-negative Isolates Using mCIM and Carba 5 Rapid Card Test

| Isolate ID | Meropenem Zone (mm) | mMCIM Result | Carba5 Rapid Card Test | Carbapenemase gene detected |
|---|---|---|---|---|
| Isolate 1 | 17 mm | Positive | Positive | NDM |
| Isolate 2 | 16 mm | Positive | Positive | KPC, NDM |
| Isolate 3 | 20 mm | Negative | Negative | None Detected |
| Isolate 4 | 14 mm | Positive | Positive | OXA-48 |

Out of 4 Meropenem-resistant isolates: 3 isolates were mCIM positive and confirmed via Carba 5 to carry NDM, KPC, and OXA-48 genes. 1 isolate (Isolate 3) showed borderline zone (20 mm), tested negative on mCIM and Carba 5, suggesting non-carbapenemase resistance.

## Patient Case History and Diagnostic Summary

This case highlights the diagnostic evaluation of meropenem-resistant Gram-negative clinical isolates using the Modified Carbapenem Inactivation Method (mCIM) and the Carba 5 Rapid Card Test. Out of four tested isolates, three (Isolates 1, 2, and 4) were positive for carbapenemase production, each carrying distinct resistance genes. Isolate 1 was found to produce NDM (New Delhi metallo-β-lactamase), indicating a multidrug-resistant profile with limited treatment options, likely arising from a severe infection in a high-risk clinical setting. Isolate 2 was positive for both KPC and NDM enzymes, suggesting a dual-carbapenemase producer—an alarming resistance scenario typically associated with ICU-acquired or post-surgical infections and a high risk for nosocomial outbreaks. Isolate 4 harbored OXA-48-like enzymes, which are often under-detected due to subtle resistance phenotypes but can still contribute to significant hospital transmission. In contrast, Isolate 3 tested negative in both mCIM and Carba 5 assays, with no carbapenemase gene detected; resistance in this case may be due to alternative mechanisms such as porin mutations or efflux pumps, requiring further MIC and susceptibility testing. Clinically, these findings emphasize the urgent need for rapid detection and containment strategies. Infection control measures, especially in high-risk hospital units, are essential to prevent transmission. Therapeutic options remain limited and must be guided by susceptibility testing, often relying on last-resort antibiotics such as ceftazidime-avibactam, colistin, tigecycline, or fosfomycin. Regular surveillance and antimicrobial stewardship programs are critical to curb the spread of these high-risk pathogens.

## Discussion

This study provides a detailed Bacteriological profile of ocular infections, emphasizing the growing threat of multidrug-resistant and carbapenemase-producing organisms in ophthalmic settings. The

high culture positivity rate (84%) among 250 ocular specimens reflects the clinical relevance of targeted microbiological evaluation in managing ocular infections. The distribution of specimen types-with conjunctival swabs being the most frequent-suggests a predominance of external eye infections, though the presence of intraocular specimens such as aqueous and vitreous samples underscores the study's inclusion of sight-threatening infections like endophthalmitis. *Staphylococcus aureus* was the predominant isolate (36.4%), consistent with previous studies reporting it as a leading cause of bacterial conjunctivitis and keratitis due to its ability to colonize eyelid margins and conjunctiva [12]. The significant isolation of *Pseudomonas aeruginosa* (18.2%) is notable, as it is a well-established cause of rapidly progressive corneal ulcers, particularly in contact lens wearers [12]. *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter spp.* were also identified, representing both community-acquired and nosocomial infections, with Gram-negative bacilli more often linked to invasive or hospital-acquired cases [13]. The presence of MDR in (4.2%) of isolates is alarming and aligns with global trends reporting increasing resistance among ocular pathogens [14]. 9 isolates (4.2%) demonstrated resistance to meropenem, a last-resort antibiotic, reflecting the rise of carbapenem-resistant Enterobacteriaceae (CRE) and non-fermenters in ophthalmic infections [15]. Of these, 5 were methicillin-resistant *S. aureus* (MRSA), and 4 were Gram-negative isolates (*P. aeruginosa* and *K. pneumoniae*) with meropenem resistance, indicating diverse resistance mechanisms affecting both Gram-positive and Gram-negative organisms. Phenotypic screening using the Modified Carbapenem Inactivation Method (mCIM) identified 3 of the 4 meropenem-resistant Gram-negative isolates as likely carbapenemase producers. This finding validates the utility of mCIM as a cost-effective and practical test for initial resistance detection, as endorsed by the Clinical and Laboratory Standards Institute (CLSI)[10]. Molecular confirmation with the Carba 5 Rapid Card Test revealed NDM in two isolates, including one co-producing KPC, and OXA-48 in another. These enzymes are among the most prevalent and clinically significant carbapenemases globally. NDM, in particular, is widespread in the Indian subcontinent and is associated with high-level resistance to most β-lactam antibiotics [16]. KPC and OXA-48, though less common, are equally concerning due to their silent dissemination and treatment-limiting profiles [17]. Interestingly, one isolate with a meropenem zone diameter of 20 mm tested negative by both mCIM and Carba 5, suggesting non-carbapenemase resistance mechanisms such as porin channel loss or efflux pumps, commonly seen in *Enterobacterales* [18]. This highlights the importance of integrating both phenotypic and molecular methods to capture the full spectrum of resistance mechanisms.

**conclusion**

This study highlights the microbial landscape and antibiotic resistance patterns of ocular infections in a tertiary care hospital in Central India. *Staphylococcus aureus* emerged as the most common pathogen, while *Pseudomonas aeruginosa* and other Gram-negative bacilli were prominent in more severe or hospital-acquired cases. The detection of multidrug-resistant (MDR) strains of isolates, including 4.2% resistant to carbapenems, underscores a growing public health concern. Notably, the presence of carbapenemase genes such as NDM, KPC, and OXA-48 among ocular isolates indicates the infiltration of critical resistance mechanisms even in ophthalmic infections. The combined use of phenotypic (mCIM) and molecular (Carba 5) methods proved effective in detecting and characterizing carbapenemase-producing strains. These findings stress the urgent need for routine antimicrobial susceptibility testing, robust infection control measures, and stringent antibiotic stewardship programs to curb the spread of resistant ocular pathogens. Early detection and targeted therapy are crucial to preserving ocular health, preventing complications, and minimizing the risk of vision loss associated with treatment-refractory infections**.**

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