Original Article

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Microbiology Section

Rapid Characterisation of Carbapenem-resistant *Klebsiella pneumoniae* using the OKNVI RESIST-5 Multiplex Lateral Flow Assay and Evaluation of the Synergistic Activity between Aztreonam and Ceftazidime-avibactam: A Cross-sectional Study

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ABSTRACT

Introduction: Carbapenemase-Producing *Klebsiella pneumoniae* (CP-KP) is a major public health concern, since such isolates are inherently resistant to most available antibiotics. A newer therapeutic option, namely Ceftazidime-Avibactam (CAZ-AVI), is effective against Extended-Spectrum Beta-Lactamase (ESBL) producers and carbapenemase-producing organisms. However, CAZ-AVI is not active against strains bearing class B Metallo-Beta-Lactamases (MBLs) such as NDM, VIM, and IMP. The monobactam antibiotic Aztreonam (ATM) is stable against all MBLs (NDM, VIM, IMP) and hence serves as a good therapeutic choice for MBL-producing Enterobacterales. There are no established standards (CLS I/EUCAST) for evaluating synergy of CAZ-AVI and ATM. The RESIST-5 OKNVI (O-Oxacillinase (OXA)-48, K-*Klebsiella pneumoniae* Carbapenemase (KPC), N-New Delhi Metallobetalactamase (NDM, V-Verona Integron-encoded Metallobetalactamase (VIM), I-Imipenemase (IMP) assay enables rapid and accurate detection of five major carbapenemases, supporting timely and appropriate antimicrobial treatment.

Aim: To detect carbapenemases using the RESIST-5 (OKNVI) test and to determine the in vitro activity of CAZ-AVI alone or in combination with ATM against carbapenemase-producing *Klebsiella pneumoniae* isolates.

Materials and Methods: A cross-sectional study was conducted over four months (February 2024-May 2024) in the Department of Microbiology, KLE's Prabhakar Kore Hospital, Belagavi, Karnataka, India. A total of 123 nonduplicate *Klebsiella pneumoniae* isolates from various clinical specimens, identified using standard biochemical methods during the study period, were included. The most common clinical samples were wound swabs, urine, and blood. Antibiotic susceptibility was tested by the disc diffusion method, with an ertapenem disc (10 µg) used to screen for carbapenem resistance. Carbapenem-resistant *Klebsiella pneumoniae* were subjected to the Modified Carbapenemase Inactivation Method (mCIM) to confirm carbapenemase production. *Klebsiella pneumoniae* isolates were further tested using the OKNVI RESIST-5 test for rapid detection of carbapenemases and were also evaluated for synergy between CAZ-AVI and ATM using modified disc diffusion and E-test methods. Statistical significance was assessed using the Chi-square test, with a p-value <0.05 considered significant.

Results: Ceftazidime-avibactam (CAZ-AVI) and aztreonam (ATM) synergy testing showed that synergy between CAZ-AVI and ATM was observed in 24 out of 30 *Klebsiella* spp. isolates (80%) using both the E-test and ATM disc diffusion methods. Fifteen isolates (50%) were resistant to CAZ-AVI and 18 (60%) to ATM, while 6 (20%) isolates were resistant to CAZ-AVI plus ATM by disc diffusion and E-test methods. Among 33 CP-KP isolates, 94% showed one or more carbapenemases, with 42% coproducing NDM and OXA-48. NDM and OXA-48 were detected in 26% and 32%, respectively, while 6% were negative for all five carbapenemases.

Conclusion: CAZ-AVI in combination with ATM is effective against carbapenemase-producing *Klebsiella pneumoniae*. This study suggests that further commercialisation of ATM-AVI as a pharmaceutical preparation could be an attractive option to treat infections caused by MBL producers.

Keywords: Antimicrobial resistance, Disk diffusion, Metallo-β-lactamases, Modified carbapenemase Inactivation

INTRODUCTION

According to the World Health Organisation (WHO) and the Centers for Disease Control and Prevention (CDC), Antimicrobial Resistance (AMR) poses a serious danger to global health and is responsible for more than 35,000 fatalities in the United States alone each year. Because carbapenems are frequently used to treat multidrug-resistant (MDR) Gram-negative infections [1], resistance to these antibiotics has rapidly developed with their increased use. Carbapenem-resistant Enterobacterales (CRE) have emerged as an important source of healthcare-associated infections, often resistant to conventional therapeutic agents. Since these isolates are essentially resistant to the majority of current antibiotics, such as β-lactams, fluoroquinolones, and aminoglycosides, CP-KP represents a serious public health concern. A 30% fatality rate is linked to CRE-induced bloodstream infections [2]. AMR determinants are frequently acquired and shared by organisms, contributing to the rapid global spread of CRE. Problematic organisms are those that produce MBL which render them resistant to carbapenems. With the exception of monobactams like ATM these enzymes can inactivate the majority of β-lactams used in clinical settings.

ATM is stable against all MBLs (NDM, VIM, IMP) and hence serves as a good therapeutic option for MBL-producing Enterobacterales,

which are otherwise resistant to β-lactams and β-lactam–β-lactamase inhibitor (BL-BLI) combinations. However, ESBL and AmpC enzymes, which are frequently coproduced in organisms that produce MBLs, can hydrolyse ATM. AVI is a β-lactamase inhibitor (diazabicyclooctane or DBO) that exhibits in vitro activity against class A and class D β-lactamases that are carbapenemases [3]. In order to combat these resistant isolates, AVI with CAZ has been used; however, this combination exhibits no efficacy against MBLs such as blaNDM, blaVIM, and blaIMP. AVI can neutralise ESBL and AmpC enzymes as well as OXA-48-like enzymes. Aztreonam and ceftazidime-avibactam (ATM-CAZ-AVI) combination therapy is considered a prudent option against MBL-producing organisms. ATM remains active, while CAZ is hydrolysed by MBLs and other enzymes. Alternatively, the ATM-AVI combination would be ideal, but it is not commercially available [4].

Rapid and precise detection of carbapenemase genes is crucial for limiting the spread of infection and choosing appropriate treatment modalities. The gold standard technique for accurately and specifically detecting carbapenemase genes is genotypic identification using Polymerase Chain Reaction (PCR). Widespread use is limited by cost and the need for qualified professionals in a dedicated setting. There are various rapid phenotypic methods available for identifying carbapenemase-producing Enterobacterales. The MASTDISCS Combi Carba Plus disc system is used to detect OXA-48, MBL, and KPC enzymes, and inhibitory-based phenotypic confirmatory tests also exist for the confirmation of class A (KPC) and class B (NDM, VIM, IMP) carbapenemases [5]. The OKNVI (OXA-48, KPC, NDM, VIM, IMP) RESIST-5 test (3B BlackBio Biotech's TRUERAPID RESIST) is a diagnostic tool for AMR that uses membrane technology and immunochromatographic lateral-flow assays with colloidal gold nanoparticles to identify carbapenemases. These tests are ready to use and can detect carbapenemases in Enterobacteriaceae growth on agar plates. The kit contains two cassettes: one for detecting KPC, OXA-48, and NDM in one cassette and another for IMP and VIM in the other cassette [6].

Clinicians intending to initiate ATM-CAZ-AVI therapy often want to know the in vitro synergy between the two agents. Considering that there are no established standards (CLSI-EUCAST) for evaluating synergy, broth dilution, gradient strip methods (E-test), gradient strip–disc diffusion (DD) methods, and disc diffusion have all been suggested as potential screening techniques for synergy between ATM and CAZ-AVI for MBL-producing Enterobacterales [7]. Using a screening approach based on disc diffusion and (E-test/disc) methodologies, present study investigated synergy.

This study aimed to strengthen infection control and preventive efforts, antibiotic stewardship, and diagnostic stewardship, making it easier to identify and contain multidrug-resistant organisms (MDROs). Diagnostic stewardship plays a key role in the management of infectious diseases by enabling clinicians and clinical microbiologists to select appropriate tests for diagnosis and prognosis. Therefore, phenotypic techniques were employed to identify isolates that produced carbapenemase, which is crucial for clinical treatment decision-making, empirical antibiotic selection, framing antibiotic policies, and updating local antibiotic guidelines for physicians [8].

the aim of the present study was to assess the role of the OKNVI RESIST-5 test for rapid detection of carbapenemases and the synergy effect of CAZ-AVI and ATM on CP-KP. The primary objective was to determine the in vitro activity of CAZ-AVI alone or in conjunction with ATM against a *Klebsiella pneumoniae* isolate that produces carbapenemase. The secondary objective was to detect carbapenemase using the OKNVI RESIST-5 test.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Microbiology, KLE's Prabhakar Kore Hospital, Belagavi, Karnataka, India. The study period spanned four months (February 2024 to May 2024). Clearance for the study was obtained from the Institutional Ethics Committee (IEC) before commencing the study (MDC/JNMCIEC/433/date: 20.01.2024).

Sampling procedure: Universal sampling. All *Klebsiella pneumoniae* isolates from clinical samples during the study period were included.

Inclusion criteria: The study population included all *Klebsiella pneumoniae* isolates from clinical samples, including blood, pus, respiratory specimens (e.g., sputum, bronchoalveolar lavage), and other body fluids, identified by conventional methods were included in the study.

Exclusion criteria: Duplicate isolates from the same patients were excluded from the study.

Study Procedure

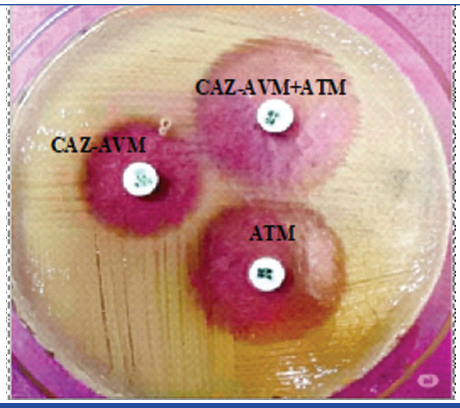
A total of 123 non duplicate *Klebsiella pneumoniae* isolates from specimens received from inpatients and outpatients for culture and sensitivity testing in the bacteriology section, belonging to various clinical departments during the study period, were included. *Klebsiella pneumoniae* was identified using standard biochemical techniques. Antibiotic susceptibility testing (AST) was performed by the disc diffusion method. An ertapenem (10 µg) disc was used as a screening test for carbapenem resistance. Fifty-four (44%) isolates were carbapenem-resistant and were subjected to a confirmatory test for carbapenemase production using Modified Carbapenemase Inactivation Method (mCIM).

Phenotypic detection of carbapenemase by the Modified Carbapenemase Inactivation Method (mCIM): mCIM was used for detection of carbapenemase, as recommended by Clinical and Laboratory Standards Institute (CLSI) [9]. Two milliliters of trypticase soy broth were inoculated with fresh colonies of the isolate that were resistant to ertapenem (a loopful). After a brief vortexing, a 10 µg meropenem disc was placed into the tube. After four hours of incubation at 37°C in ambient air, the meropenem discs were removed using a sterile loop, and any excess inoculum was decanted by pressing the disc against the test tube's sides. The disc was then placed on a lawn of *Escherichia coli* American Type Culture Collection (ATCC) 25922. The isolate was considered to have produced carbapenemase if the area around the meropenem disk showed growth of the indicator organism (i.e., reduced or absent zone of inhibition). In this study, an inhibitory zone size of 6–15 mm on mCIM was interpreted as carbapenemase production (indicating meropenem hydrolysis), while a zone size of ≥19 mm was considered negative (no carbapenemase), and a diameter of 16–18 mm was considered indeterminate [10]. Of the 54 (44%) carbapenem-resistant *Klebsiella pneumoniae*, 33 were carbapenemase-producing as confirmed by mCIM and were subjected to synergy testing by the modified disc diffusion method and the modified E-test/disc diffusion method.

Combination Testing

1 Modified Disk diffusion method

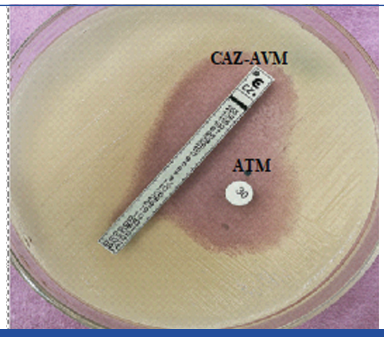
For the disk diffusion procedure, a suspension of the tested isolate (0.5 McFarland) was inoculated onto a Mueller-Hinton Agar (MHA) plate. Two CAZ-AVI disks (30 µg/20 µg) (Oxoid, Basingstoke, England) provided by Pfizer, New York, USA, and one ATM disk were initially applied to the MHA plates inoculated with the test organism and incubated at 37°C for one hour. Later, one of the CAZ-AVI disks was replaced with an aztreonam disk at the same site. Plates were re-incubated overnight and then observed for zones of inhibition the next day. Synergistic activity of CAZ-AVI and ATM was reported with an increase in the inhibition-zone diameter of ≥21 mm. This is in agreement with the susceptibility criterion of a ≥21 mm zone size recommended for CAZ-AVI according to CLSI guidelines [Table/Fig-1] [11].



[Table/Fig-1]: Modified disc diffusion test showing synergy.

2 Modified E test/disc diffusion method

The CAZ-AVI and ATM synergy for the 30 isolates was determined using the CAZ-AVI E-test (bioMérieux, Marcy-l'Étoile, France) that contained CAZ (0.016–256 µg/mL) and AVI (4 µg/mL) and ATM disk (30 µg). The tested bacterial suspension was uniformly streaked over the Mueller-Hinton agar plates after being adjusted to 0.5 McFarland. A CAZ-AVI E-test strip was positioned 15 mm from the disk so that the strip's center was parallel to the sensitivity breakpoint of the CAZ-AVI E-test (8 µg/mL). The plates were incubated at 37°C for 16–18 hours, and a threefold reduction in MIC was reported as synergy [Table/Fig-2] [12].



[Table/Fig-2]: Modified E test/disc diffusion test showing between Ceftazidime-Avibactam and Aztreonam synergy between Ceftazidime-Avibactam and Aztreonam.

Phenotypic detection of carbapenemase genes by OKNVI RESIST-5 [13]

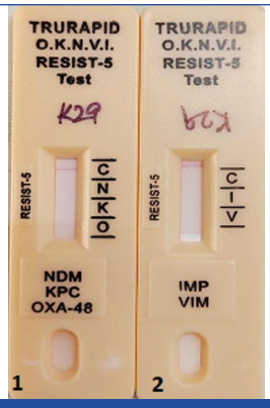
Two lateral-flow cassette immunochromatography tests were used to identify carbapenemase genes NDM, OXA-48, VIM, KPC, and IMP. A monoclonal antibody against OXA-48 carbapenemase and its variants (except OXA-163-like enzymes) ("O" line), KPC ("K" line), NDM ("N" line), VIM ("V" line), IMP ("I" line), and a control capture reagent ("C" line) are used on the nitrocellulose membrane.

Sample preparation: Three bacterial colonies from fresh cultures were suspended in buffer in a collection tube and vortexed to homogenise the preparation. Add three drops (approximately 100 µL) of the sample to a sample well. The immobilised antibodies on the nitrocellulose strip capture complexes formed with the sample conjugates. If the sample contains NDM, VIM, OXA-48, KPC, or IMP carbapenemase, the respective lines (NDM: "N" line, VIM: "V" line, OXA-48: "O" line, KPC: "K" line, IMP: "I" line) will appear, along with the control line (C).

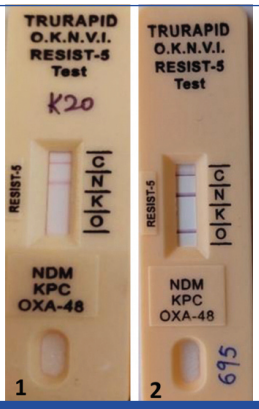
Interpretation [14]:

Negative test result: A reddish-purple line appears only at the control line ("C") [Table/Fig-3].

Positive test result: In addition to the control line, a visible reddish-purple line appears at the test line position corresponding to the detected carbapenemase (N for NDM, K for KPC, O for OXA-48, or I/V for IMP/VIM). Combinations of positive lines can occur [Table/Fig-4].



[Table/Fig-3]: Cassette 1 and 2 all 5 genes tested negative only 'C' control bands produced.



[Table/Fig-4]: Cassette 1: NDM positive. Cassette 2: NDM and OXA-48 positive.

Invalid test results: Absence of a control line indicates a test failure.

STATISTICAL ANALYSIS

The data were analysed using Microsoft Excel and IBM Statistical Package for the Social Sciences (SPSS) Statistics, Version 29.0. The Chi-square test was used to determine statistical significance, and p-values were calculated accordingly.

RESULTS

A total of 123 *Klebsiella pneumoniae* isolates were collected from various clinical samples, including blood, pus, respiratory specimens such as sputum and bronchoalveolar lavage fluid, and other body fluids.

The age group most commonly affected was 1–30 years, accounting for 44 (35%), followed by 31–60 years. Among 18 (14%) neonates (<23 days), 16 (22%) had hospital-acquired neonatal infection. *Klebsiella pneumoniae* was commonly isolated from wound swabs (n = 43, 35%), with 27 (38%) samples mainly linked to Surgical Site Infection (SSI), followed by 34 (27%) urine samples and 16 (23%) from Catheter-Associated Urinary Tract Infection (CAUTI). The majority of the blood samples (12, 17%) were from neonatal bloodstream infections.

Higher resistance was seen with CP-KP compared to non carbapenemase-producing *Klebsiella pneumoniae* (non-CP-KP). Highest susceptibility was observed with colistin (88%), tigecycline (58%), followed by fosfomycin (53%), whereas non-CP-KP showed higher sensitivity to carbapenems and aminoglycosides. These differences were statistically significant ($p<0.001$; Fisher's exact test where applicable) except for amikacin [Table/Fig-5].

Among the 54 (36%) carbapenem-resistant *Klebsiella pneumoniae* (CR-KP), 33 were carbapenemase-producing as confirmed by mCIM and were subjected to synergy testing by the Modified Disk Diffusion Method and Modified E-test/Disc Diffusion Method. Of the 33 isolates, 28 (85%) showed synergy between CAZ/AVI and ATM discs, 18 (55%) were resistant to CAZ/AVI, and 16 (49%) were resistant to ATM alone, according to the E-test/disc diffusion method

Antibiotics	CP- KP (33)		Non CP-KP (90)		Chi-square	p-value
	Resistance n (%)	Sensitive n (%)	Resistance n (%)	Sensitive n (%)		
Ampicillin	33 (100)	0	75 (83)	15 (16)	-	0.0106* FT
Tobramycin	27 (82)	6 (18)	24 (27)	66 (73)	28.03	<0.001*
Gentamycin	24 (73)	9 (27)	24 (27)	66 (73)	21.48	<0.001*
Amikacin	21 (64)	12 (36)	51 (57)	39 (43)	0.46	>0.5
Cefotaxime	30 (91)	3 (9)	44 (49)	46 (51)	17.88	<0.001*
Imipenem	33 (100)	0	14 (16)	76 (84)	72.75	<0.001*
Meropenem	33 (100)	0	12 (14)	78 (86)	78.2	<0.001*
Piperacillin-Tazobactam	33 (100)	0	33 (37)	57 (63)	38.95	<0.001*
Tigecycline	13 (42)	20 (58)	17 (19)	73 (81)	5.50	<0.001*
Colistin	3 (9)	30 (88)	0	90 (100)	-	0.0036*FT
Levofloxacin	20 (62)	13 (38)	34 (38)	56 (62)	5.11	0.024*
Nitrofurantoin	19 (60)	14 (40)	28 (32)	62 (68)	7.16	0.0075*
Fosfomycin	15 (47)	18 (53)	12 (14)	78 (86)	14.56	<0.001*

[Table/Fig-5]: Antibiotic sensitivity of Carbapenemase and Non-Carbapenemase Producing *Klebsiella pneumoniae* (CP-KP).
*p-value <0.001 FT- Fisher's-exact test, Chi-square test

and the Modified Disk Diffusion Method. A total of 5 (15%) isolates showed no signs of synergy. Combining CAZ with aztreonam resulted in a 4–9 mm increase in zone size [Table/Fig-6].

Synergy testing	No. of isolates -33	
	Sensitive	Resistant
CAZ- AVI	15 (46%)	18 (55%)
ATM	17 (51%)	16 (49%)
CAZ-AVI + ATM	28 (85%)	5 (15%)

[Table/Fig-6]: CAZ-AVI/ATM synergy testing.

NDM, OXA-48, KPC, VIM, and IMP carbapenemases were detected by the OKNVI RESIST-5 rapid diagnostic kit. Of the 33 CP-KP isolates, 31 (94%) exhibited one or more carbapenemases, and 2 (6%) were negative for all five carbapenemases. Co-production NDM plus OXA-48 two carbapenemases was found in 13 (42%) isolates. NDM was detected in 8 (26%) and OXA-48 carbapenemase was detected in 10 (32%). [Table/Fig-7].

Carbapenemase	*Klebsiella pneumoniae* (n-33)	
	Positive (%)	CAZ-AVI/AZT Synergy Positive
NDM	8 (26)	5 (62)
KPC	0	--
VIM	0	--
IMP	0	--
OXA-48	10 (32)	10 (100)
NDM and OXA-48	13 (42)	13 (100)
Total	31 (94)	28 (90)

[Table/Fig-7]: Distribution of carbapenemase producing genes among *Klebsiella pnuemoniae* isolates by RESIST-5 OKNVI test.

DISCUSSION

Resistance to widely used broad-spectrum antibiotics like carbapenems presents a significant challenge, particularly with bacteria that produce MBL. Early detection of CRE is crucial to ensure appropriate antibiotic therapy and to implement timely and effective infection control measures, as CRE poses a serious global health threat [15]. For the treatment of Enterobacterales that produce NDM, the Infectious Diseases Society of America (IDSA) and the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommend ATM in combination with ceftazidime/avibactam (CZA); however, there is a lack of evidence supporting the efficacy of this antibiotic combination [16].

The prevalence of carbapenemase-producing *Klebsiella pneumoniae* (CPKP) isolates in the present study was 27% among the 123 clinical isolates. Present study findings were consistent with Kumari N et al., at a tertiary care centre in North India, who reported a CP-KP prevalence of 16.8% [17], while Pawar S et al., showed a prevalence of 31.77% CR-KP in rural western India [18].

The current study found that *Klebsiella pneumoniae* is resistant to most commonly used antibiotics, which may be related to the duration and type of drug exposure, as well as the prevalence of antibiotic-resistance genes. In present study, *Klebsiella pneumoniae* showed notable antibiotic resistance, consistent with the findings of Wang N et al., [19]. Similar to other studies, in present study isolates were entirely resistant to ampicillin [20]. Furthermore, 88% of CP-KP isolates were susceptible to colistin and 58% to tigecycline, indicating good in vitro activity against our CP-KP isolates; nevertheless, resistance concerns persist during therapy, posing a substantial risk to public health. Delays in appropriate antibiotic therapy for severe infections are strongly associated with worse outcomes and higher mortality rates in patients with severe sepsis and septic shock [21].

The optimal treatment of infections owing to CRKP is uncertain, and none of the currently available antibiotics used as single therapy may be effective against all strains of carbapenemase producers. Hence, CAZ in conjunction with ATM can be used to counteract their effects. Although other novel beta-lactamase inhibitors, such as relebactam or vaborbactam, can be used in conjunction with ATM, they have demonstrated less activity against class A serine beta-lactamases, making the avibactam–aztreonam combination a promising option for the treatment of MBL-producing organisms.

Synergy between aztreonam discs and CAZ-AVI (E-test) was observed in 28 of 33 (85%) CP-KP isolates. A study by Marshall S et al., showed that 21 MBL-positive isolates were individually resistant to CAZ-AVI, yet all 21 demonstrated a decrease in MICs in the agar dilution test with this combination, and 17 of the 21 were susceptible by disk diffusion when aztreonam was added [22]. Wenzler E et al., used two approaches—the agar E-test method and the MIC:MIC ratio method—to investigate synergy for several antibiotic combinations (CAZ + ATM, CAZ + CAZ-AVI, and CAZ-AVI + ATM) against seven isolates containing serine and MBL enzymes. The greatest synergy was with CAZ-AVI + ATM, observed in five of seven isolates with at least a 128-fold reduction in MIC [23].

The efficacy of this combination has been demonstrated in vivo in prospective observational studies, case reports, and series, with treatment durations ranging from 10 days to more than six weeks. For example, a *Klebsiella pneumoniae* isolate producing both blaOXA-48 and blaNDM-1 carbapenemases, and a *Pseudomonas*

aeruginosa strain producing blaNDM-1 and AmpC beta-lactamase, were treated with CAZ-AVI/ATM for 10 days and were infection-free at the end of therapy [24].

Mobile genetic elements such as plasmids and transposons frequently carry carbapenemase genes circulating within *Klebsiella pneumoniae*, allowing transfer to other members of the Enterobacteriaceae family. The frequency of carbapenemase genes varies by geographic location and has been linked to severe outbreaks that pose a major therapeutic obstacle [25]. Therefore, identifying Carbapenemase-Producing Enterobacterales (CPE) producers by rapid testing may be the most effective strategy to curb their spread. Although PCR is the gold standard for detecting carbapenemase genes, many laboratories in developing countries lack access to PCR instrumentation due to cost. Compared with molecular techniques like PCR, the RESIST-5 OKNVI assay, which is based on multiplex immunochromatographic lateral-flow technology, offers several advantages and is one of the few tests capable of identifying five clinically significant carbapenemases. It provides easy and clear interpretation, requires no special equipment, and offers a shorter turnaround time.

In the present study, 24 (73%) of the bacterial strains had one or more carbapenemases, while 9 (27%) were negative for all five carbapenemases. This suggests that some isolates have additional carbapenem resistance mechanisms, including efflux pumps or porin loss. NDM and OXA-48 carbapenemases were the most prevalent, and the co-existence of NDM and OXA-48 was found in 13 (42%) of our isolates; however, KPC, VIM, and IMP were not detected in any of the isolates. Similar findings were noted in another study conducted in Mangalore [26].

In a study conducted in South Korea by Hong J et al., the effectiveness of the RESIST-5 OKNVI assay for detecting the five common carbapenemases (OXA-48-like, KPC, NDM, VIM, and IMP) compared with conventional PCR was evaluated. The RESIST-5 OKNVI test precisely identified KPC and OXA-48-like carbapenemases with 100% sensitivity (51/51) and 100% sensitivity (49/49), respectively. IMP, NDM, and VIM carbapenemases had sensitivity values of 100% (46/46), 97.6% (59/60), and 93.3% (42/45), respectively [14].

MBLs constitute the majority of carbapenemases in Southeast Asia, particularly in India, whereas serine carbapenemases are more common in other regions of the world [27]. Since monobactams such as ATM are the only beta-lactams active against MBLs, the combinations of CAZ-AVI with ATM are a promising option against these enzymes. Although they are less effective against class A serine beta-lactamases, newer beta-lactamase inhibitors like vaborbactam and relebactam can also be used in conjunction with ATM. Consequently, the CAZ-AVI plus ATM combination is recommended for treating bacteria that produce KPC or OXA-48-type carbapenemases [28]. Although the CAZ-AVI–ATM combination has shown enhanced activity against MBL-producing organisms, emerging resistance to this regimen has been reported [29]. This resistance is primarily attributed to structural alterations in the PBP3 protein, specifically the insertion of four amino acids (such as YRIN or YRIK), or co-production of CMY-42 beta-lactamase. These factors collectively contribute to reduced susceptibility to the drug combination and a consequent increase in MICs. Prior research found that isolates producing only KPC or OXA-48-like enzymes remained susceptible to CZA, but the presence of any MBL renders the combination less effective due to the activity of KPC and OXA-48-like enzymes [30]. This finding aligns with avibactam's spectrum of activity against different carbapenemases, which was also noted in this study.

The RESIST-5 OKNVI test results would assist clinical microbiologists and treating physicians in selecting antibiotics for the treatment of NDM, OXA-48 KPC-producing *Klebsiella* spp. Diagnostic stewardship through the introduction of the RESIST-5 OKNVI test would be a good option to strengthen antimicrobial stewardship for treating carbapenemase-producing Enterobacterales in hospital settings. The combination of CAZ-AVI and ATM is considered an effective therapeutic option, particularly against *Klebsiella pneumoniae* producing more than one carbapenemase gene, including MBLs.

Limitation(s)

This study lacks information on the clinical application of this drug combination, as in vitro susceptibility does not always correlate with in vivo effectiveness due to factors such as pharmacodynamics and the host's immune response. The role of other co-existing mechanisms, such as porin loss or efflux pumps, in the development of carbapenem resistance was not evaluated. Comparisons with standard methods such as checkerboard assays, time-kill assays, PCR for gene confirmation, broth microdilution (BMD) for MIC determination, and whole-genome sequencing were not performed.

CONCLUSION(S)

The ATM in combination with CAZ-AVI should be considered for use in Enterobacterales that produce MBLs. Susceptibility testing should guide whether to employ this combination in patients. Reliable methods include gradient diffusion and strip-based approaches, as well as BMD MIC testing for aztreonam/avibactam. The RESIST-5 OKNVI assay has excellent performance in detecting five targeted carbapenemase genes and is a rapid, easy, and efficient tool to apply in the clinical microbiology laboratory.

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Authors' contribution: All authors listed have made substantial, direct, and intellectual contributions to the work and approved it for publication.

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