Original Article

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An Innovative Laboratory Technique Showcasing the Synergy among Ceftazidime- Avibactam and Aztreonam in Combating Infections caused by Enterobacterales producing Metallo-beta-lactamases: A Prospective Study

Microbiology Section

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ABSTRACT

Introduction: Combination therapy with Ceftazidime-Avibactam (CAZ-AVI) and Aztreonam (ATM) has been studied in the context of infections caused by Enterobacterales that produce Metallo-Beta-Lactamases (MBL). The development of combination therapy is a crucial factor in combating MBL-producing Enterobacterales. As most isolates from Intensive Care Unit (ICU) patients produce a variety of beta-lactamases, offering resistance to a broad range of antibiotics, they need to be treated with the CAZ-AVI and ATM combination. This study addresses a pressing public health issue-the rise of multidrug-resistant bacteria, particularly MBL-producing Enterobacterales. By investigating the synergy between CAZ-AVI and ATM, the study aims to provide valuable insights to guide clinical practice, improve patient outcomes, and contribute to the global effort to combat antibiotic resistance.

Aim: To demonstrate the synergy between CAZ-AVI and ATM in patients with infections caused by MBL-producing Enterobacterales.

Materials and Methods: A prospective study was conducted in the Department of Microbiology at Symbiosis Medical College for Women (SMCW) and Symbiosis University Hospital and Research Centre (SUHRC), Symbiosis International (Deemed University), Lavale, Pune, Maharashtra, India. The study duration was six months, from January 2023 to June 2023. All isolates meeting the inclusion criteria were processed to demonstrate synergy between CAZ-AVI and ATM. Isolates from the Enterobacteriaceae family with resistant breakpoints for carbapenemase, ceftazidime-avibactam, and aztreonem, as determined by the Phoenix automated system, were included in the study. Restoration of the ATM breakpoint was observed following the addition of CAZ/AVI to ATM. Breakpoints provided by the BD Phoenix system were compared to the results obtained from CAZ/AVI and ATM disc diffusion/stacking, E-strip/disc methods, in terms of susceptibility and resistance. Results were simultaneously compared with the broth disc elution test, considered the gold standard.

Results: Sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) were calculated for the E-test/disc and disc stacking methods. The disc stacking synergy test demonstrated a PPV of 96.88% and an NPV of 62.5%. By using the E-test/disc, the ATM/CAZ-AV synergy test revealed a PPV of 97.22% and an NPV of 83.33% when CAZ/AVI was added to ATM. Comparatively, disc stacking was less sensitive than the broth disc elution.

Conclusion: In the majority of MBL-producing Enterobacterales that are ATM-resistant, the CAZ/AVI+ATM combination showed strong synergy. In the microbiology laboratory, the E-test/disc and disc stacking approaches are rapid, repeatable, and reliable methods for checking clinically significant synergy.

Keywords: Carbapenemases, Combination therapy, Disc elution, Disc stacking

INTRODUCTION

The existence of Enterobacterales producing Class B MBL enzyme constitutes a substantial menace to medical treatment. MBLs, such as the New Delhi MBL (NDM), are appearing in clinical isolates all over the world at an increasing rate [1,2]. Beside from other Extended-Spectrum Beta-Lactamase (ESBL) and non-beta lactamase resistance mechanisms, MBLs are typically plasmid expressed and captured. As a result, the presence of MBLs provides resistance to almost all beta-lactam antibiotic therapy, which includes carbapenems [2,3]. Additionally, isolates frequently exhibit resistance to fluoroquinolones and aminoglycosides, preventing the development of effective substitutes for drugs like colistin that have extremely high toxicity and unpredictable Pharmacokinetics (PK) [2,3]. The development of combination therapy is crucial in combating MBL-producing Enterobacterales. The monobactam ATM is susceptible to breakdown by serine Class A but resistant to MBLs. Third-generation cephalosporin CAZ-AVI, combined with a beta-lactamase inhibitor, consumes significant movement against serine beta-lactamases but is hydrolysed by MBLs. The combination of ATM and CAZ-AVI has been shown to work synergistically with MBLs in-vitro employing supplementary experimental methods, demonstrating that ATM-AVI is efficacious in-vivo [4-10]. In order to support the medical usage of CAZ/AVI and ATM moving forward, routine test bed methods to identify patients who might benefit from starting combination therapy are needed.

Prospective techniques of selection for in-vitro synergy between CAZ-AVI and ATM for Enterobacterales expressing MBL have been suggested, and these include disc diffusion/stacking, E-strip/disc and broth disc dilution methods [2,4-6,9-12]. However, there is a lack of data on whether this combination therapy can be applied in clinical practice. To establish a laboratory procedure for monitoring a therapeutically suitable combination effect between CAZ-AVI and ATM in NDM-expressing isolates, a disc stacking/diffusion and E-strip/disc method were developed and compared with the disc elution method, considered equivalent to the traditional broth dilution method. These novel, conventional, time-saving, and economical techniques demonstrated the synergy between CAZ-AVI and ATM.

The combination of CAZ-AVI and ATM is more effective than each antibiotic alone in treating infections caused by MBL-producing Enterobacterales. This study aims to test and validate this hypothesis through laboratory experiments and antimicrobial susceptibility testing. The Minimum Inhibitory Concentration (MIC) of CAZ-AVI and ATM will be measured individually against MBL-producing Enterobacterales to establish baseline susceptibility. The study will also consider the potential clinical implications of the observed synergy, such as improved treatment outcomes and reduced antibiotic resistance development.

The rationale for this study lies in addressing a pressing public health issue-the rise of multidrug-resistant bacteria, particularly MBL-producing Enterobacterales. By investigating the synergy between CAZ-AVI and ATM, the study aims to provide valuable insights to guide clinical practice and contribute to the global effort to combat antibiotic resistance. The study will demonstrate the synergy between CAZ-AVI and ATM in patients with infections caused by Enterobacterales and assess the presence of a synergistic antimicrobial effect when combining these antibiotics against MBL-producing Enterobacterales.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Microbiology at Symbiosis Medical College for Women and SUHRC, Symbiosis International (Deemed University), Lavale, Pune, Maharashtra, India, from January 2023 to June 2023. The study was conducted with the waiver obtained from the Institutional Ethics Committee (IEC) to conduct the research using patient samples collected for routine diagnostic purposes. A blanket consent policy was followed for patient participation in the study and the use of their data for research and educational purposes.

Inclusion criteria: Bacterial isolates from the Enterobacteriaceae family with resistant breakpoints for carbapenemase, cefta avibactam, and aztreonem by Phoenix were included in the study.

Exclusion criteria: Isolates sensitive to carbapenemases, cefta-avibactam, and ATM were excluded from the study.

Sample size: All samples received during the study period (N=48) were included using the convenience sampling method.

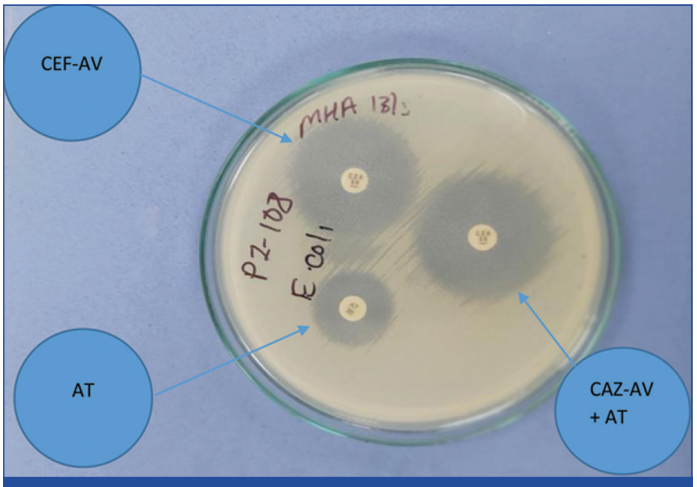
Study Procedure

Isolates with high-level resistance to carbapenemase, ATM, and CAZ-AVI were further studied. A total of 48 MBL-producing isolates were examined. The restoration of the ATM breakpoint was investigated after the inclusion of CAZ/AVI. The synergy test using the disc diffusion and E-strip/disc methods was compared with the gold standard disc elution method. The Clinical and Laboratory Standards Institute (CLSI) recommended disc diffusion, Phoenix identification techniques, and phenotypic ESBL confirmation with ceftazidime (10 µg) with and without clavulanic acid were used to assess the antimicrobial susceptibility of the isolates [13].

Testing for susceptibility:

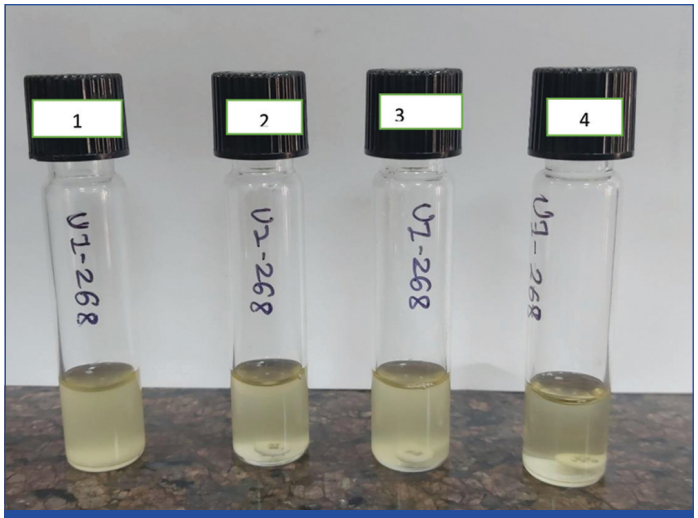
1. **E-Strip/disc method [1]:** The CAZ/AVI E-test/ATM disc diffusion method was used to determine the CAZ/AVI+ATM synergy as shown in [Table/Fig-1]. On Muller Hinton agar innocula with turbidities of 0.5 McFarland, as assessed by spectrophotometry were plated. On the agar plate was a CAZ/AVI E-test with a preset AVI concentration of 4 mg/L. An ATM disc (30 µg) was positioned 15 mm from the E-Strip's center to the disc's center. It was positioned at the 8 mg/L CAZ/AVI breakpoint. For 11 of the 48 isolates (22.92%), duplicate plates were prepared to assess the method's repeatability. At 16 to 18 hours, plates were incubated and read. Two definition of synergy were looked at an estimated disc diameter for ATM alone or CAZ/AVI+ATM was calculated by measuring the zone radius on each side of the disc. This was done using a quantitative technique first. For ATM with synergy, defined as the restoration of an estimated zone diameter congruent with observed breakpoints, zone diameters were compared to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) zone size. Second, a qualitative strategy was used, with an inverse-D defined as a synergy demonstration. This qualitative method was generalised as the opposite observation of inducible clindamycin resistance, which is frequently evaluated for as part of standard laboratory antimicrobial susceptibility testing. [Table/Fig-1] shows the E-test/disc set-up with specified observations dependent on synergy's presence or absence.
2. **Disc diffusion/disc stacking [2]:** Pfizer supplied the CAZ-AVI discs (30 µg/20 µg) (BD Diagnostic Systems Sparks, MD, USA). In this method, ATM disc and CAZ-AVI disc were stacked together and moistened with a small drop of saline before being incubated at 35.2°C overnight. The following day, as per standard CLSI recommendations for disc diffusion testing, a zone of inhibition was then looked for as shown in [Table/Fig-2].
3. **Broth disc elution test [1,2]:** Now-a-days, the disc elution method has been put out and has developed a strong relationship with the broth micro dilution method. ATM, CAZ, AVI, or a mixture of both of these are added to tubes holding 2 mL of sterile Muller Hinton broth and allowed to elute drug material to broth for 30 minutes. A 12 µL of the freshly made bacterial inoculum, which contains 0.5 McFarland, are added to the broth with the disc. Incubate test tubes for 16 to 20 hours, then check for turbidity the following day. When turbidity is present on a single disc but not present on both discs in a test tube, the disc is susceptible as shown in [Table/Fig-3].

[Table/Fig-1]: E-test/disc test showing synergy between CAZ-AVI and ATM.
AT: Aztreonam; CAZ-AVI: Ceftazidime avibactam



[Table/Fig-2]: Synergy test by disc diffusion/stacking.

These methods were used to test and validate the synergy between CAZ-AVI and ATM in MBL-producing Enterobacterales. The MIC



[Table/Fig-3]: Synergy test by disc elution.
1-control, 2-Aztreonam, 3- Ceftazidime-Avibactam, 4- Ceftazidime- Avibactam+Aztreonam

of CAZ-AVI and ATM against these bacteria was also measured to establish baseline susceptibility and assess the potential clinical implications of the observed synergy.

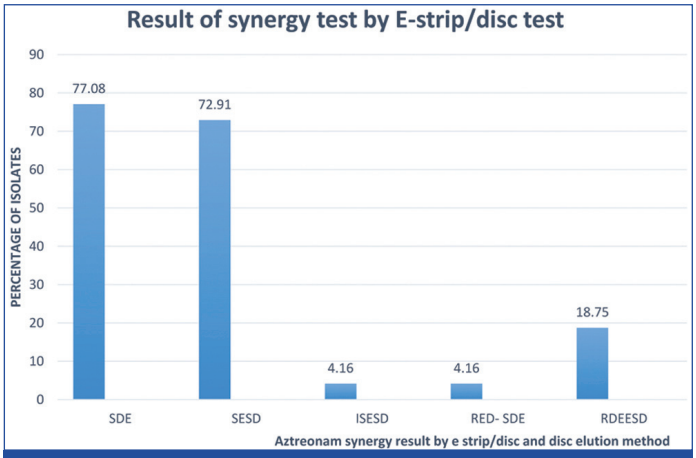
STATISTICAL ANALYSIS

The data collected in this study were subjected to statistical analysis. Descriptive statistics were used to summarise the data, and the findings from the disc diffusion synergy test and E-strip/disc method were obtained. The results obtained from the BD Phoenix test and the gold-standard disc elution test, which is used as a benchmark, were compared for the same strain. Descriptive statistics were used to report all the data. A 2x2 table was used to calculate the sensitivity, specificity, PPV, and NPV of the E-strip/disc method, using the broth dilution method as the gold standard. The statistical analysis will provide insights into the performance and accuracy of the E-strip/disc method in detecting synergy between CAZ-AVI and ATM in MBL-producing Enterobacterales. The sensitivity, specificity, PPV, and NPV values will help evaluate the diagnostic accuracy of the E-strip/disc method compared to the gold-standard method.

RESULTS

The study included a total of 48 confirmed carbapenemase-producing isolates. *Escherichia coli (E.coli)* accounted for the largest portion of the isolates at 37.5%, followed by *Klebsiella* species at 41.7%, *Enterobacter cloacae* and *Citrobacter freundii* at 8.3% each, *Proteus rettgeri* contributed very less 4.2% among all isolates. Routine antimicrobial susceptibility testing using MICs (Phoenix) revealed that these isolates displayed resistance to a wide range of antibiotics. However, minocycline and doxycycline showed the highest susceptibility rates among these isolates.

1. Results of testing the synergy of ATM and CAZ-AVI using disc diffusion/disc stacking- The disc diffusion synergy test was subsequently employed to assess the interaction between ATM and CAZ-AVI. Among the isolates, 31 out of 48 (64.58%) exhibited an inhibitory zone diameter of less than 21 mm for Aztreonam. This observation aligns with the susceptibility criteria outlined in the CLSI guidelines, which specify a zone size of 21 mm as indicative of susceptibility to CAZ-AVI. In the case of these 31 isolates, the introduction of CAZ-AVI alongside ATM resulted in an expansion of the inhibitory zone size. Conversely, for 17 out of 48 (35.41%) isolates, there was no significant increase in the inhibitory zone size.
2. Results of ATM/CAZ-AVI synergy testing by E-strip/disc stacking method was also used to test the synergy between ATM and CAZ-AVI. The results showed that in 35 of 48 cases (72.91%), the disc radius measurement for ATM alone was concordant with the breakpoint estimations.
3. Results of testing the synergy of ATM, CAZ, and AVI by disc elution. In 37/48 (77.08%) isolates using disc elution, synergy was seen when CAZ/AVI was added to ATM.
4. A comparison of methods e-test/disc techniques and broth disc elution. Comparisons between the E-test/disc techniques and broth disc dilution are shown in [Table/Fig-4]. The disc radius measurement for ATM alone showed breakpoint estimations to be concordant in 35 of 48 cases (about 72.91%) when compared to broth synergy. A 13/48 (27.08%) showed discordant results by this method. Eleven isolates were totally resistant by broth disc elution as well as E-strip/disc method. The qualitative E-test/disc technique revealed synergy in 35 of 48 (72.91%) isolates. In 13 of 48 (27.08%) isolates, there was no evidence of synergy. One sample showed false positive synergy. Sensitivity of this test was found to be 94.59% (CI 81.81%to 99.34%), specificity 90.91% (CI 58.72% to 99.77%, PPV 97.22% (CI 84.36% to 99.56%) and NPV 83.33% (CI 56.18% to 95.12%).

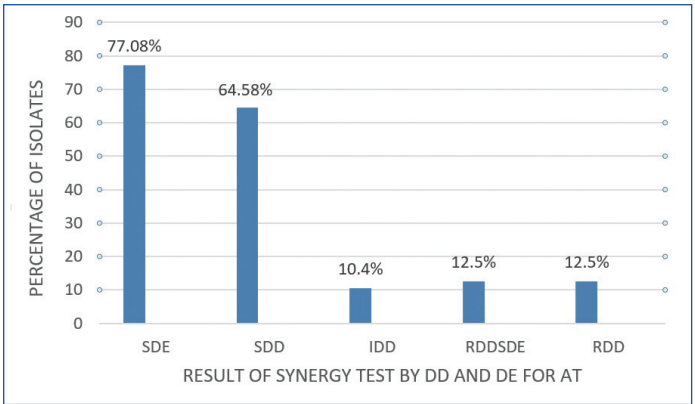


[Table/Fig-4]: Comparison of synergy tests by E-strip/disc and broth disc elution.
ISESD: Intermediate sensitive by E-Strip/disc; RDEESD: Resistant by disc elution as well as E-strip/dis; RED-SDE: Resistant by E-Strip/disc and sensitive by disc elution; SDE: Sensitive by broth disc elution; SESD: Sensitive by e-strip/disc

5. Comparison of the disc diffusion/stacking techniques and broth disc elution. An analysis of the disc elution test to the disc diffusion test is presented in [Table/Fig-5]. For CAZ/AVI+ATM, disc elution test evaluation of the 31 (100%) isolates that were sensitive on disc diffusion corresponded with synergy. In the broth disc elution test, 6/17 isolates showed sensitivity, while in the CAZ-AVI disc diffusion synergy test, they showed intermediate sensitivity. Six of the 11 isolates were completely resistant to disc diffusion and disc elution, while five of the isolates were intermediately sensitive. Disc elution testing revealed that one isolate had tested mistakenly positive by disc diffusion. Compared to disc elution in broth concordance in synergy was observed in 31/48 (64.58%) cases. Comparatively, disc diffusion was found to have sensitivity of 83.78% (CI 67.99% to 93.81%) when compared to broth disc elution. There has been 90.91% specificity (CI 58.72% to 99.77%). The disc synergy test was shown to have a PPV of 96.88% (CI 82.63% to 99.51% and NPV of 62.5% (CI 43.90% to 78.02%). Sensitivity, specificity, PPV and NPV of disc stacking/disc diffusion and E-strip/Disc method when compared with disc elution for CAZ-AV-ATM combination shown in [Table/Fig-6]. Results of synergy test by disc diffusion/stacking and disc elution among different isolates shown in [Table/Fig-7].

DISCUSSION

Gram-negative bacteria's multidrug resistance has become a major concern in recent years [14]. Many people are concerned about the rise in antimicrobial resistance rates in the COVID-19 pandemic as the increased use of antibiotics for observational therapy [15]. Following that, managing these multidrug resistant bacteria requires the proper,



[Table/Fig-5]: Comparison of synergy tests by disc diffusion and disc elution.
AT: Aztreonam; DD: Disc diffusion/disc stacking; DE: Disc elution; IDD: Intermediate sensitive by disc diffusion; RDD: Resistant by disc diffusion; RDDSDE: Resistant by disc diffusion and sensitive on disc elution; SDE: Sensitive by disc elution; SDD: Sensitive by disc diffusion

Laboratory techniques	Sensitivity	Specificity	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)
Disc stacking/ disc diffusion	83.78%	90.91%	96.88%	62.5%
E-strip/disc	94.59%	90.91%	97.22%	83.33%

[Table/Fig-6]: Sensitivity, specificity, PPV, and NPV of disc stacking/disc diffusion and E-strip/Disc methods when compared with disc elution for CAZ-AVI and ATM combination.
NPV: Negative predictive value; PPV: Positive predictive value

optimal administration of antibiotics. Serine carbapenemases are dominant in other regions of the world, while MBLs are the main carbapenemases in South East Asia, particularly in India [16]. The only medications that exhibit good action against MBL are monobactams, hence a combination of CAZ-AVI and ATM can be used to counteract this activity. Marshall S et al., did an original investigation using the same combination in 2017. In their study, they found that 21 MBL positive isolates were independently resistant to CAZ-AVI, but when ATM was added, 17 out of 21 isolates proved to be susceptible to the disc diffusion approach [16]. Sekar R et al., documented the MIC range for carbapenems, prevalence and mechanisms of carbapenem resistance among Enterobacteriaceae in rural South India. It substantiated NDM as a leading mechanism of carbapenem resistance [17]. Another study by Karlowsky JA et al., gathered 267 MBL positive Enterobacterales from 40 different countries and found that the combination of ATM-AVI was inhibitory to 99.9% of isolates and did not exhibit any regional differences [18]. In another study, Rawson TM et al., findings indicate that out of 43 isolates, 33 (77%) exhibited resistance to ATM. The introduction of CAZ/AVI successfully lowered the ATM breakpoint in 29 out of the 33 resistant isolates, representing an 89% restoration rate. In the broader context, study showed that E-test/disc method was in agreement with the results obtained through broth dilution in 35 out of 48 instances (72.91% correlation). The sensitivity of the E-test/ disc method was 77%, and its specificity was 85%. The PPV stood at 92%, while the NPV was 61% [1]. On comparison, the authors found

S. No.	Isolate	Phoenix MIC ATM S/I/R	Phoenix CAV-AV MIC (S/I/R)	Aztreonem Disc (S/I/R) S=21 I=18-20 R=<17	CAZ-AVI Disc (S/I/R) S=>21 R=<20 (mm)	CAZ-AVI+ATM (mm)	Synergy by disc ellution	Synergy by disc stacking	Synergy by modified E-test/disc diffusion method. (Reverse D)
1	*Escherichia coli*	>16R	>16/4	6 R	6	23	P	P	P
2	*Escherichia coli*	>64R	>16/4	6R	10	25	P	P	P
3	*Escherichia coli*	>16R	>16/4	6R	12	21	P	P	P
4	*Escherichia coli*	>16R	>16/4	10	12	23	P	P	P
5	*Escherichia coli*	>16R	>16/4	12	10	25	P	P	P
6	*Escherichia coli*	>16R	>16/4	10	8	26	P	P	P
7	*Escherichia coli*	>16R	>16/4	6	13	17	A	A	A
8	*Escherichia coli*	>16R	>16/4	17	15	25	P	P	P
9	*Escherichia coli*	>16R	>16/4	16	16	22	P	P	P
10	*Escherichia coli*	>16R	>16/4	15	10	23	P	P	P
11	*Escherichia coli*	>16R	>16/4	14	18	22	P	P	P
12	*Escherichia coli*	>16R	>16/4	10	6	18	A	A	A
13	*Escherichia coli*	>16R	>16/4	6	6	25	P	P	P
14	*Escherichia coli*	>16R	>16/4	9	6	26	A	A	A
15	*Escherichia coli*	>16R	>16/4	10	10	26	P	P	P
16	*Escherichia coli*	>16R	>16/4	13	8	26	P	P	P
17	*Escherichia coli*	>16R	>16/4	12	6	25	P	P	P
18	*Escherichia coli*	>16R	>16/4	17	10	23	P	P	P
19	*Klebsiella pneumoniae*	>16R	>16/4	15	6	21	P	A	P
20	*Klebsiella ozaenae*	>16R	>16/4	11	6	22	P	P	P
21	*Klebsiella pneumoniae*	>16R	>16/4	10	10	19	P	A	P
22	*Klebsiella pneumoniae*	>16R	>16/4	6	12	23	P	P	P
23	*Klebsiella pneumoniae*	>16R	>16/4	8	14	23	P	P	P
24	*Klebsiella pneumoniae*	>16R	>16/4	15	18	22	P	P	P
25	*Klebsiella pneumoniae*	>16R	>16/4	12	16	21	P	P	P
26	*Klebsiella pneumoniae*	>16R	>16/4	13	10	22	P	A	p
27	*Klebsiella pneumoniae*	>16R	>16/4	15	10	23	P	P	P
28	*Klebsiella pneumoniae*	>16R	>16/4	13	10	24	A	A	A
29	*Klebsiella pneumoniae*	>16R	>16/4	12	10	25	P	P	P
30	*Klebsiella pneumoniae*	>16R	>16/4	15	12	24	P	P	P
31	*Klebsiella pneumoniae*	>16R	>16/4	14	14	18	P	A	A
32	*Klebsiella pneumoniae*	>16R	>16/4	12	13	17	P	A	P

33	*Klebsiella pneumoniae*	>16R	>16/4	13	15	23	P	A	P
34	*Klebsiella pneumoniae*	>16R	>16/4	14	13	21	P	P	P
35	*Enterobacter cloacae*	>16R	>16/4	10	12	24	A	A	A
36	*Enterobacter cloacae*	>16R	>16/4	6	13	18	A	P	A
37	*Enterobacter cloacae*	>16R	>16/4	16	10	25	P	P	P
38	*Enterobacter cloacae*	>16R	>16/4	12	10	23	P	P	A
39	*Proteus rettgeri*	>16R	>16/4	10	15	21	P	P	P
40	*Proteus rettgeri*	>16R	>16/4	6	14	22	A	A	A
41	*Klebsiella pneumoniae*	>16R	>16/4	9	14	26	P	A	p
42	*Klebsiella pneumoniae*	>16R	>16/4	13	12	23	A	A	A
43	*Klebsiella pneumoniae*	>16R	>16/4	12	10	24	P	P	P
44	*Klebsiella pneumoniae*	>16R	>16/4	10	10	26	P	P	P
45	*Citrobacter freundii*	>16R	>16/4	15	15	25	A	A	A
46	*Citrobacter freundii*	>16R	>16/4	`2	14	23	P	P	P
47	*Citrobacter freundii*	>16R	>16/4	6	12	21	A	A	A
48	*Citrobacter freundii*	>16R	>16/4	6	10	22	A	A	A

[Table/Fig-7]: Results of synergy tests by disc diffusion/stacking and disc elution among different isolates.
P: Present; A: Absent

that, by using disc diffusion techniques, authors attempted to confirm a quick, useful laboratory procedure for observing for clinically relevant synergy among CAZ/AVI+ATM in MBL expressing Enterobacterales. In the present study for ATM resistant isolates, CAZ/AVI combination resulted in clinically significant synergy with upgrading of ATM breakpoint (MIC 4 mg/L) in 35/48 (72.91%) of resistant isolates by E-test /disc method with sensitivity 94.59%, specificity 90.91%,and PPV 97.22% as compared to 31/48 (64.58%) by disc stacking with sensitivity 83.78%, specificity 90.91% PPV 96.88%. Sahu C et al., observed the greatest level of synergy in *Klebsiella pneumoniae* when using the Disc-E-Strip and E-Strip-Agar methods, with percentages of 86% and 84%, respectively [11]. Sreenivasan P et al., observed that in individual tests, all isolates displayed complete resistance to both CAZ-AVI and ATM, indicating a 100% resistance rate (60/60). When employing the disc diffusion method, a consistent inhibition zone size of 21 mm was observed across all isolates, with 16 of them showing an increase in the inhibition zone size exceeding 16 mm. In the E-test fixed ratio method, when CAZ-AVI and ATM were tested separately, their Minimum Inhibitory Concentrations (MICs) ranged from 8/4 µg l-1 to ≥256/4 µg l-1 and 16 µg l-1 to 256 µg l-1, respectively. However, when these antibiotics were used in combination, the MICs significantly decreased, ranging from 0.016/4 µg l-1 to 2/4 µgl-1, and the Fractional Inhibitory Concentration (FIC) consistently remained below 0.5 for all isolates. So, findings of the study done by Sreenivasan P et al., are consistent with present study [15].

Previous studies have also investigated the synergy between CAZ-AVI and ATM in MBL-producing isolates. Marshalls et al., found that the addition of ATM to CAZ-AVI resulted in susceptibility in 17 out of 21 MBL-positive isolates [16]. Another study by Karlowsky JA et al., showed that the combination of ATM-AVI was inhibitory to 99.9% of MBL-positive isolates. These findings support the use of CAZ-AVI and ATM as a combination therapy for MBL-producing isolates.

In this study, different methods were used to test the synergy between CAZ-AVI and ATM. The E-strip/disc method showed concordant results with the broth disc dilution method in 72.91% of cases. The sensitivity of the E-strip/disc method was 77%, and its specificity was 85%. The disc diffusion/stacking method showed sensitivity of 83.78% and specificity of 90.91%. These results indicate that both methods can be useful for detecting synergy between CAZ-AVI and ATM in MBL-producing isolates.

The study also compared the results of the different methods and found that the E-strip/disc method had higher sensitivity, specificity, and PPV compared to the disc stacking method. The E-strip/disc method had a sensitivity of 94.59%, specificity of 90.91%, and PPV of 97.22%. These findings suggest that the E-strip/disc method is a reliable and accurate screening tool for detecting synergy between CAZ-AVI and ATM in MBL-producing Enterobacterales.

Other studies have also reported high levels of synergy between CAZ-AVI and ATM in MBL-producing isolates. Sahu C et al., observed the greatest level of synergy in *Klebsiella pneumoniae* when using the Disc-E-Strip and E-Strip-Agar methods [11]. Sreenivasan P et al., found that the combination of CAZ-AVI and ATM resulted in a significant decrease in MICs and consistently low Fractional Inhibitory Concentrations (FIC) for all isolates [15].

The correlation between the disc elution method and the disc diffusion method suggests that the disc diffusion method can be a useful screening strategy for detecting synergy between CAZ-AVI and ATM. The study also emphasises the importance of considering clinical breakpoints when determining appropriate treatment for infections caused by MBL-producing isolates.

With 85% to 95% categorical agreement, the study showed that gradient strip-based synergy testing performed well overall. Comparatively an inferior agreement of 64.58% was seen with disc stacking [12]. When thinking about how to treat infections that produce MBL, taking into account the organism break point is becoming increasingly crucial [19-22]. Breakpoints consider the chance of reaching therapeutic medication concentrations inside the patient, in-vitro measurements (or correlates) of MIC, and clinical proof of results from infection treatment [23].

Uncertainty exists regarding the best dosing approaches and Pharmacokinetic-Pharmacodynamic (PK-PD) targets for combination therapy, as well as the timing of when this combination should be considered for the management of MBL-producing Enterobacterales infections [3,7,10,24]. In a broad sample of 48 isolates, present study results using the disc diffusion approach showed equivalent promise for demonstrating clinically meaningful synergy, with a 72% promise compared to the disc elution method using CLSI-stated breakpoints. The issue of organism breakpoints is becoming increasingly important when treating infections that produce NDM [22]. The cut-off point takes into account MIC techniques used in-vitro, the potential for patient access to therapeutic medication concentrations, and definite evidence of the effects of initiating disease management [20,23,24]. Recently, disc stacking, gradient strip stacking, and gradient strip crossing were compared in a study using CLSI breakpoints in 10 MBL-producing Enterobacterales [15-17].

The study argues that the synergy of ATM with AVI can be effective in reducing the septicity of MBL-producing Enterobacterales, but there is debate over the best ways to administer medications. Expanding

applicable laboratory synergy screening techniques could help close this gap.

Clinical Implications

If the study demonstrates that the combination of CAZ-AVI and ATM is highly effective against MBL-producing Enterobacterales, it will likely lead to improved patient outcomes. This could include faster resolution of infections, reduced mortality rates, and shorter hospital stays. The findings may enable clinicians to tailor treatment strategies more precisely, especially for patients with MBL-producing Enterobacterales infections. This tailored approach could optimise therapy, reduce adverse effects, and minimise the development of resistance. Effective combination therapy could reduce the reliance on last-resort antibiotics like colistin. Preserving these antibiotics for cases where no alternatives exist is critical to manage highly drug-resistant infections. Positive study results may influence the development of clinical guidelines and recommendations for the treatment of MBL-producing Enterobacterales infections. This, in turn, could guide healthcare practices worldwide. The study's success may broaden the spectrum of treatment options for multidrug-resistant infections beyond MBL-producing Enterobacterales, potentially benefiting patients with other drug-resistant bacterial infections.

Future Perspectives

After the initial study, further clinical trials and real-world studies will be needed to validate the efficacy and safety of the CAZ-AVI and ATM combination. This may involve larger patient cohorts and longer-term follow-up. Future research may focus on optimising dosage regimens, treatment durations, and administration routes to maximise therapeutic benefits and minimise adverse effects. Understanding the potential for resistance development to this combination therapy will be essential. Future studies could investigate the genetic and molecular mechanisms underlying resistance and ways to mitigate it. Companion diagnostic tests could be developed to identify patients most likely to benefit from CAZ-AVI and ATM combination therapy. Such tests could guide treatment decisions.

Positive outcomes from this study could inspire research into combination therapies for other drug-resistant pathogens, expanding the toolbox of treatment options for challenging infections. If the combination therapy proves successful, its implementation in healthcare systems worldwide will be a critical future step. This will involve regulatory approvals, drug availability, and training for healthcare providers. This study provides further evidence for the synergy between CAZ-AVI and ATM in MBL-producing Enterobacterales. The E-strip/disc method and the disc diffusion method were found to be useful screening tools for detecting this synergy. Understanding the phenotypic characteristics of these organisms and considering clinical breakpoints are important for targeted therapy. Further research is needed to explore the clinical implications of this synergy and its impact on patient outcomes.

Limitation(s)

The small number of isolates used in the current investigation was a major flaw. Because the prevalence of carbapenem resistance and the types of beta-lactamases vary by geographic location, the findings of this study cannot be generalised to all organisms and hospital settings. Another drawback is the lack of information about the clinical application of this drug combination, as in-vitro susceptibility cannot always predict the in-vivo activity of the drugs in the patient due to the involvement of additional factors like pharmacodynamics and host immune response. For the investigation, only Enterobacterales with higher MICs for ATM, ceftazidime-avibactam, and carbapenemases were selected, and strains that produced NDM were not supported by molecular techniques. There were no clearly defined breakpoints for ATM/AVI or CAZ/AVI+ATM. Genotypic profiling was not performed on the isolates which were used. However, this laboratory phenotypic technique is highly helpful in determining the synergistic effect of CAZ/AVI+ATM.

CONCLUSION(S)

The E-test/disc and disc diffusion/stacking methods provide a quick, practical laboratory approach for assessing CAZ/AVI+ATM synergy and may potentially guide its targeted application in patients with MBL-producing Enterobacterales infection.

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