Original Article

Investigating Gram-negative bacilli isolates' sensitivity to ceftazidime/avibactam

Sunali[1,2†], Mithilesh Kumar Jha[3†], Mukesh Kumar[2], Maneesh Kumar[4], Nishant Ranjan[5]

[1]Department of Microbiology, Geetanjali Medical College and Hospital, Udaipur, Rajasthan, India, [2]Department of Microbiology, Netaji Subhas Medical College and Hospital, Bihta, Patna, Bihar, India, [3]Department of Microbiology, All India Institute of Medical Sciences, Deoghar, Jharkhand, India, [4]State VRDL, Department of Microbiology, All India Institute of Medical Sciences, Deoghar, Jharkhand, India, [5]Department of General Surgery, Geetanjali Medical College and Hospital, Udaipur, Rajasthan, India

[†]Consider both authors as first author.

Abstract

Background: Multidrug resistant (MDR) Gram negative organisms are becoming increasingly common. Carbapenem resistant Enterobacterales (CRE) pose a major threat and necessitate the development of new antibiotics. MDR and carbapenem resistant infections, which are common in intensive care units and hospitals, lead to increased morbidity, mortality, prolonged hospital stays, and higher healthcare costs. New antimicrobials such as ceftazidime avibactam offer potential alternatives to conventional treatments such as tigecycline and colistin, which have significant side effects and limitations. **Aim:** This study focuses on the antibiotic susceptibility of ceftazidime/ avibactam to Gram negative bacilli found in a large number of clinical samples collected from a tertiary care facility in Netaji Subhas Medical University and Hospital, Bihta, India. **Methodology:** The study included 81 Gram negative bacteria isolated from patient samples. Based on the Clinical Laboratory Standards Institute guidelines mentioned in the Kirby Bauer disc diffusion method. **Result and Conclusion:** the results showed that ceftazidime avibactam inhibited 89.9% of the Enterobacteriaceae isolates, which was higher than the 80.3% of amikacin and the 85.1% of meropenem. Ceftazidime avibactam was effective against CRE isolates in 69.9% of cases and against MDR isolates in urine in 94% of cases, which was higher than the 40% of ceftriaxone and 94% of nitrofurantoin. The results show that ceftazidime avibactam can cure MDR and CRE infections, especially urinary tract infections, better than conventional antibiotics, which is a great help in the fight against increasing antibiotic resistance.

Keywords: Carbapenem-resistant, ceftazidime-avibactam, enterobacterales, gram-negative bacilli, Kirby-Bauer disc diffusion, multidrug-resistant

Introduction

The pathogens with the highest priority for the discovery of new antibiotics are multidrug-resistant (MDR) Gram-negative organisms such as carbapenem-resistant Enterobacterales (CRE). As there are a few alternatives for the treatment of Gram-negative infections, these pathogens pose a serious problem in hospitals.[1,2] Aerobic Gram-negative bacteria (GNB) are the most common cause of nosocomial infections and infections in intensive care units (ICUs). MDR and CRE infections are associated with higher morbidity and mortality rates, longer hospital stays, and higher healthcare costs. Timely initiation of appropriate antibiotic treatment is crucial for the diagnosis of MDR organisms as it can lead to better therapeutic outcomes and survival rates.[3-5] Colistin

Address for correspondence: Dr. Mithilesh Kumar Jha, Department of Microbiology, All India Institute of Medical Sciences, Deoghar - 814 152, Jharkhand, India.
E-mail: dr.mithileshjha7@gmail.com

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and tigecycline are the drugs of first choice for the treatment of CRE and MDR infections. However, both drugs have serious side effects. While tigecycline has limitations due to its lower plasma concentrations that may reduce its therapeutic benefit, colistin is known for its nephrotoxicity and neurotoxicity.[6-8] These difficulties have led to the development of alternative antimicrobials such as ceftolozane-tazobactam, imipenem-cilastatin-sulbactam, plazomicin, meropenem-vaborbactam, ceftazidime-avibactam, eravacycline, and cefiderocol.[9,10] In this respect, ceftazidime-avibactam represents a significant breakthrough. It combines the new β-lactamase inhibitor avibactam with the broad-spectrum antibiotic ceftazidime.[11] Avibactam is a reversible covalent inhibitor of diazabicyclooctanes (DBOs), a non-β-lactam β-lactamase inhibitor. Compared to conventional inhibitors, DBOs have a different mode of action, are more potent, and have a broader spectrum of activity. Due to its reversible binding ability, avibactam can enhance its efficacy by inhibiting multiple molecules of the β-lactamase enzyme.[12-14] Avibactam effectively inactivates certain class D (such as OXA-48) class A (such as ESBLs and KPC) and class C (AmpC) β-lactamases.[15,16] The European Medicines Agency (EMA) and the Food and Drug Administration (FDA) have approved ceftazidime-avibactam for the treatment of complex urinary tract infections (cUTIs), including pyelonephritis. Ceftazidime-avibactam is often combined with metronidazole, has also been tested in children, and is approved in Europe for the treatment of nosocomial pneumonia. Clinical studies around the world have shown that ceftazidime-avibactam is safe and effective in the treatment of Gram-negative MDR infections found in pediatrics.[17-19] In addition, several real-world studies have confirmed the efficacy of ceftazidime-avibactam in the treatment of these difficult conditions and supported its use in Indian patients.[2,20] The development and use of ceftazidime-avibactam represents a significant breakthrough in the fight against MDR Gram-negative bacteria and provides physicians with a powerful weapon to improve treatment outcomes despite increasing antibiotic resistance.[21]

In this study, we aim to highlight the antimicrobial susceptibility profile of ceftazidime/avibactam against Gram-negative bacilli isolated from a variety of clinical samples. This study is of critical importance as it aims to provide comprehensive data on the effectiveness of ceftazidime/avibactam in the control of infections caused by these pathogens. Given the increasing prevalence of MDR organisms, including those producing extended- spectrum β-lactamases (ESBL) and carbapenem-resistant enterobacterales (CREs), it is crucial to evaluate the potential of new antimicrobial agents.

Material and Methods

The laboratory-based, time-bound study was conducted in the Department of Microbiology, Netaji Subhas Medical College and Hospital (NSMCH), Bihta, for a period of 5 months from July 2022 to December 2022. Ethical clearance was obtained from NSMCH, Bihta Institutional Scientific and Ethics Committee. A total of 2250 samples for aerobic bacterial cultures including sputum, pus, urine, and fluid samples were analyzed as part of the study. All clinical samples received by the microbiology laboratory were cultured on routine culture media and identified according to standard microbiological procedures by examination of colony morphology, motility, Gram-staining, and biochemical reactions. Further antibiotic susceptibility testing for clinically relevant isolates was performed using the Kirby-Bauer disc diffusion method, and results were evaluated according to Clinical Laboratory Standards Institute guidelines (CLSI 2022, M100). The antibiotic discs (HiMedia Laboratories Pvt. Ltd, India) used were ceftazidime-avibactum, amikacin, amoxicillin clavulanic acid, ceftazidime, ceftazidime clavulanic acid, ceftriaxone, cefepime, meropenem, tobramycin, tetracycline, nitrofurantoin, and ciprofloxacin. Eighty-one MDR GNB isolates were identified from 220 samples received at the laboratory for routine testing. Of these MDR GNB isolates, 58% were *E. coli* isolates, 37% were Klebsiella pneumonia, and 5% were other bacterial pathogens.[22,23]

Results

The research study investigated the efficacy of ceftazidime-avibactam, a novel antibiotic combination, against 81 MDR bacterial isolates, with a focus on the Enterobacteriaceae family. The results were much impressive and showed that ceftazidime- avibactam successfully inhibited 89.9% of all Enterobacteriaceae isolates at a susceptibility breakpoint above 21 μg/ml [Figure 1]. This result underscores the potent activity of ceftazidime-avibactam against MDR pathogens, including the notoriously difficult-to-treat CRE [Figure 2]. For comparison, two other antibiotics were also examined in the study: amikacin and meropenem. In accordance with the standards set by the Clinical and Laboratory Standards Institute (CLSI), amikacin had a sensitivity rate of 80.3%, while meropenem had a slightly higher performance rate of 85.1%. Although these antibiotics remain viable options for the treatment of Enterobacteriaceae infections, ceftazidime-avibactam performed better than both in this particular study.

The study looked more closely at the efficacy of ceftazidime-avibactam against CRE, a subset of particularly resistant bacteria. Of the 13 CRE isolates tested, ceftazidime-avibactam inhibited growth in 69.9% of cases. This result is significant as it positions ceftazidime-avibactam as a promising treatment option for infections caused by these highly resistant pathogens. The study also included an analysis of 70 urine samples to evaluate the antibiotic's efficacy in treating urinary tract infections (UTIs) [Figure 3]. The results were astounding: 94% of the isolates from these samples were sensitive to ceftazidime-avibactam. Nitrofurantoin, another antibiotic commonly used for UTIs, only surpassed this high sensitivity rate. Ceftriaxone, a widely used broad-spectrum antibiotic, had a much lower sensitivity rate of only 40%.

Disc diffusion method

The Kirby-Bauer diffusion method was used to test each isolate for ceftazidime, aztreonam, and ceftazidime-avibactam. We also evaluate the efficacy of a combination of ceftazidime- avibactam and aztreonam. After incubating the ceftazidime-avibactam disc for 1 hour, we replaced it with an aztreonam disc and left it to incubate overnight at a temperature of 35°C. All isolates exhibited susceptibility as indicated by an inhibition zone width of ≥22 mm. According to the CLSI standards [Figure 4], the sensitivity criterion for ceftazidime-avibactam is thus fulfilled. When ceftazidime-avibactam and aztreonam were administered together, the diameters of the zones of inhibition increased significantly. Specifically, 27 of the isolates tested showed an increase of 5–10 mm, 29 showed an increase of 10–15 mm, and 11 showed an increase of more than 15 mm. Four isolates did not show a large increase in inhibitory zone size as they already had a larger inhibitory zone when treated with ceftazidime-avibactam alone [Figures 5 and 6]. This study shows that the combination of ceftazidime-avibactam and aztreonam significantly improves the ability of the antibiotics to kill bacteria and achieves the size required by the CLSI guidelines for ceftazidime-avibactam. Overall, these results underscore the great potential of ceftazidime-avibactam as an effective treatment for infections caused by MDR and CRE organisms. Of particular note is the performance of ceftazidime-avibactam in UTIs, where it outperformed some conventional antibiotics. This study provides valuable evidence for the use of ceftazidime-avibactam in clinical settings where antibiotic resistance is a major concern.

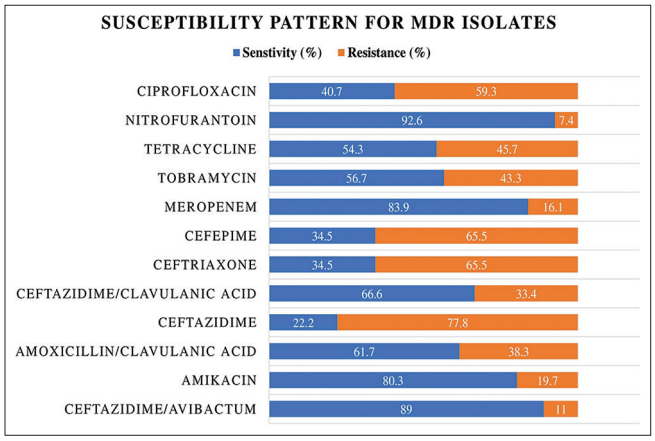

Figure 1: Graphical representation of MDR isolates

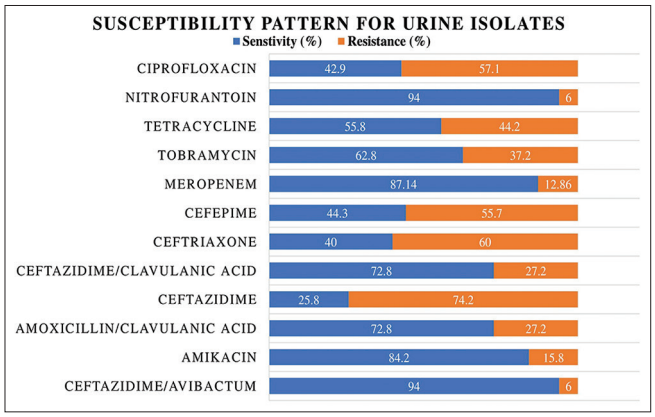

Figure 3: Graphical representation of urine isolates

Discussion

For a long time, there was limited clinical evidence for the efficacy of many combination products in the treatment of infections caused by GNB that are extensively drug-resistant (XDR) and multidrug-resistant (MDR). *In vitro* studies have shown the efficacy of colistin, tigecycline, fosfomycin, and certain aminoglycosides against these isolates. However, each of these drugs has limitations that preclude their use as empiric therapy for life-threatening infections.[24] The emergence of CRE has raised considerable concern due to their widespread resistance and potential for rapid spread.[25,26] The study examined 81 MDR isolates, and ceftazidime-avibactam inhibited 89.9% of all Enterobacteriaceae isolates. These data are consistent with the findings of Sader e*t al.*, who reported a meropenem susceptibility rate of 98.5% in the United States from 2013 to 2016.[27-29] The smaller sample

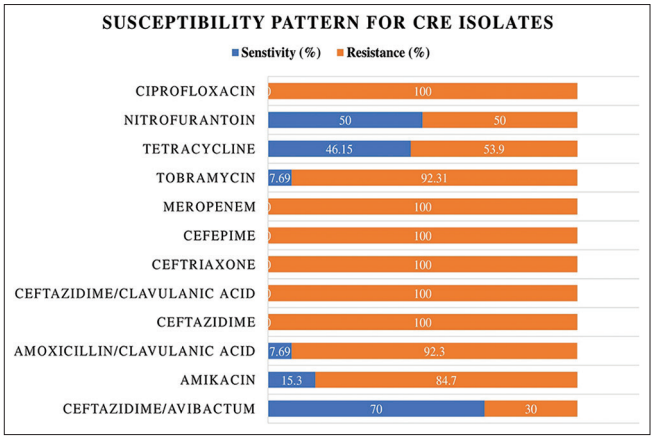

Figure 2: Graphical representation of CRE isolates

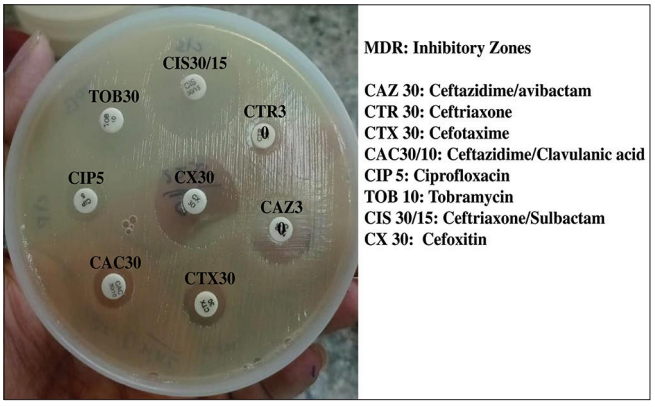

Figure 4: Susceptibility testing by disk diffusion showing ceftazidime-avibactam, including many other antibiotics to validate the MDR

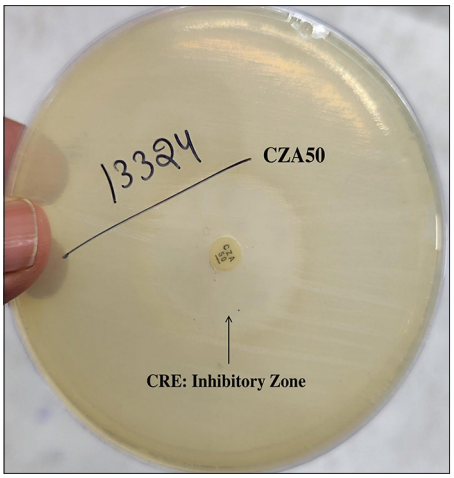

Figure 5: Susceptibility testing by disk diffusion showing ceftazidime-avibactam to validate the CRE

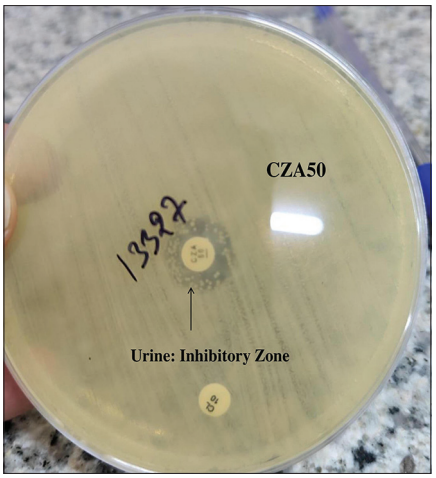

Figure 6: Susceptibility testing by disk diffusion showing ceftazidime-avibactam to validate the inhibition in the bacterial culture using urine sample

size likely contributed to the slightly lower meropenem susceptibility in this study (83.9%). However, 70% of CRE isolates were sensitive to ceftazidime-avibactam. This suggests that ceftazidime-avibactam may be able to reduce the side effects of colistin treatment while preventing GNB from becoming more resistant to colistin. According to Tandogdu e*t al.,* ceftazidime-avibactam has a higher clinical cure rate and a lower rate of acute kidney injury (AKI) than colistin. The use of ceftazidime-avibactam against MDR isolates found in urine was 94, significantly higher than the 40% efficacy of third-generation cephalosporins in this study.[30] In severe UTIs, Gram-negative organisms are resistant to third-generation cephalosporins to varying degrees (30–50%). These findings suggest that hospitalized patients with complex UTIs may benefit from ceftazidime-avibactam as an alternative treatment.[31] In India, the Indian Council of Medical Research (ICMR) found a high level of carbapenem resistance in GNB, with resistance rates of 30% in *Escherichia coli* and 50% in *Klebsiella pneumoniae*.[32] The increasing incidence of antibiotic resistance and MDR-negative bacterial infections jeopardizes current therapeutic strategies. The treatment of infections with MDR bacteria is becoming increasingly difficult due to the lack of antibiotic alternatives. According to Swaminathan S *et al.*, ceftazidime-avibactam, a combination of a third-generation cephalosporin and a non-lactam lactamase inhibitor, has shown therapeutic efficacy in both pivotal phase III trials and practice.[2,32] Ceftazidime-avibactam is associated with lower mortality rates with early and appropriate treatment of infections caused by susceptible organisms.

Conclusion

As antibiotic resistance is increasing worldwide, choosing the right antimicrobial agent for empirical treatment is crucial. In our study, there is evidence that ceftazidime and avibactam together could be an alternative for the treatment of Gram-negative bacteria resistant to carbapenem and other drugs (MDR). Antimicrobial resistance is on the rise, and conventional antibiotics are not always up to the task of treating infections caused by MDR and carbreloxerogenic bacteria. We need to explore new and effective therapeutic alternatives to overcome this obstacle. As an effective replacement for conventional antibiotics, our research shows that ceftazidime/avibactam is very effective against these resistant bacteria. When we looked at the AST profiles, we found that ceftazidime/avibactam was quite effective against many different types of GNB and MDR bacteria. This discovery highlights the ability of ceftazidime/avibactam to fill the treatment gap created by other antibiotics losing efficacy due to resistance. Ceftazidime/avibactam will likely play an important role in empirical treatment protocols due to its ability to inhibit these resistant bacteria. Rapid and effective intervention is critical in severe and potentially fatal diseases.

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Ethical

The Netaji Subhas Medical College and Hospital Institutional Ethics Committee has approved this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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