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Original Research Article

Evaluating the Efficacy of Novel Drug Combinations Against MDR Gram-Negative Bacteria: A Comparative Study

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Abstract:

Background: The emergence of multidrug-resistant bacterial infections has challenged the effectiveness of conventional antibiotics. Evaluating new combination therapies can help identify more effective treatment options.

Aim: This study investigates the sensitivity of bacterial isolates to three antibiotic combinations—Cefepime Enmetazobactam, Ceftazidime Avibactam, and Ceftriaxone+Sulbactam+Disodium Edetate—to determine their potential role in managing multidrug-resistant infections.

Methods: A total of 100 bacterial isolates were collected from urine, sputum, blood, pus, and tissue samples obtained in ICU and non-ICU settings. Antibiotic sensitivity was assessed using standard disc diffusion methods, with sensitivity rates determined for each bacterial species.

Results: Ceftriaxone+Sulbactam+Disodium Edetate showed the highest sensitivity rates across all species tested, including 71.5% for Klebsiella species, 78% for Escherichia coli, 75% for Pseudomonas species, and 80% for Acinetobacter species. Cefepime Enmetazobactam and Ceftazidime Avibactam demonstrated lower sensitivities, particularly against Acinetobacter species. The findings suggest that Ceftriaxone+Sulbactam+Disodium Edetate is the most effective combination among the three tested.

Conclusion: Ceftriaxone+Sulbactam+Disodium Edetate exhibits superior efficacy against a range of bacterial pathogens, making it a promising option for treating resistant infections. The other two combinations, while moderately effective, may require more targeted use.

Recommendations: Further studies with larger sample sizes and diverse clinical settings are recommended to confirm these findings and develop more refined antibiotic guidelines to address multidrug resistance.

Keywords: Antibiotic sensitivity, multidrug-resistant bacteria, cefepimeenmetazobactam, ceftazidime avibactam, ceftriaxone+sulbactam+disodiumedetate

Introduction

The management of infections caused by (MDR) gram-negative bacteria poses significant challenges to modern healthcare systems. These pathogens are responsible for high rates of morbidity and mortality, particularly in hospital settings such as (ICUs) [1]. The escalating resistance of these organisms to conventional antibiotics has necessitated the development and utilization of novel therapeutic options.

Ceftazidime-Avibactam and Ceftriaxone-Sulbactam-EDTA represent two such innovations in antimicrobial therapy targeting resistant gram-negative bacteria. Ceftazidime-Avibactam combines a third-generation cephalosporin with a non-β-lactam β-lactamase inhibitor, which has been shown to be effective against a wide spectrum of β-lactamases, including those produced by carbapenem-resistant *Enterobacteriaceae* [2]. This combination has been approved for clinical use based on its efficacy in combating serious bacterial infections without significant adverse effects.

On the other hand, Ceftriaxone-Sulbactam-EDTA is another combination that targets similar pathogens by employing a β-lactam antibiotic with a β-lactamase inhibitor and a metallo-β-lactamase inhibitor. Its use has been supported by research demonstrating its potential to restore the activity of Ceftriaxone against pathogens that have developed resistance mechanisms [3]. Both drug combinations are pivotal in the strategic containment of antimicrobial resistance (AMR), offering alternatives when traditional antibiotics fail.

The rise of AMR has been identified as a critical public health issue, prompting global health authorities to prioritize research and development of effective responses. According to the WHO, new resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases, with an estimated 700,000 deaths each year attributed to AMR [4]. The economic impact is equally staggering, with the potential to incur substantial healthcare costs worldwide due to prolonged illness, additional tests, and use of more expensive drugs.

In light of these challenges, it is imperative to assess and compare the effectiveness of newer antibiotic combinations like Ceftazidime-Avibactam and Ceftriaxone-Sulbactam-EDTA against MDR gram-negative bacteria. This study aims to compare the antimicrobial efficacy of Ceftazidime-Avibactam and Ceftriaxone-Sulbactam-EDTA against MDR gram-negative bacilli, including *E. coli, Klebsiella, Pseudomonas, Acinetobacter, Proteus,* and *Enterobacter* species, using minimum inhibitory concentration (MIC) values and resistance patterns in ICU and OPD settings.

Methodology

Study Design

This study was a prospective cross-sectional study.

Study Setting

The study was conducted at **Indira Gandhi Institute of Medical Sciences, Patna, Bihar**, involving the **Microbiology laboratory** where bacterial isolates will be collected and tested.

Participants

A total of 100 MDR gram-negative bacilli isolates was included in the study. The

distribution of bacterial species among the isolates is as follows:

- 20 isolates of Escherichia coli (E. coli)
- 20 isolates of Klebsiella species
- 10 isolates each of Pseudomonas, Acinetobacter, Proteus, andEnterobacter species

These clinical isolates will be derived from patient samples received for routine microbiological evaluation.

Inclusion Criteria

- Isolates identified as MDR gram-negative bacilli by standard antimicrobial susceptibility testing methods.
- Bacterial isolates from clinical samples such as urine, blood, respiratory secretions, pus, and body fluids.

Exclusion Criteria

- Non-MDR bacterial isolates.
- Duplicate isolates from the same patient to prevent redundancy in the study.

Bias Control

To minimize bias:

- Randomization will be employed in the selection and testing of bacterial isolates.
- Blinding will be maintained while analyzing the results to prevent observer bias.
- Standardized testing protocols as per Clinical and Laboratory Standards Institute (CLSI) guidelines will be strictly followed.

Data Collection

- Clinical isolates will be processed anonymously, coded using a randomization technique, and delinked from patient identity.
- Samples will be screened for Extended Spectrum Beta-Lactamase (ESBL) and Metallo-Beta-Lactamase (MBL) production using disk diffusion and double disk synergy tests.
- Antibiotic susceptibility testing (AST) will be performed using Ceftazidime-Avibactam and Ceftriaxone-Sulbactam-EDTA (antibiotic discs from Cipla).
- (MIC) values will be determined using methods prescribed by CLSI guidelines.

Procedure

1. **Sample Processing:**
    - Clinical specimens will be inoculated into appropriate culture media (e.g., blood agar, MacConkey agar, and CLED agar for urine samples).
    - Blood cultures will be incubated aerobically in the BacT/ALERT 3D system (bioMérieux).
2. **Characterization of Gram-Negative Bacilli:**
    - Gram-negative isolates will be further screened for ESBL and MBL production.
    - ESBL producers will be identified using cefotaxime (30 µg), ceftazidime (30 µg), and ceftriaxone (30 µg) discs.
    - Disk potentiation tests and double disk synergy tests (DDST) will confirm ESBL production.
    - MBL detection will be done using imipenem (10 µg) + EDTA (750 µg) discs.
3. **Antimicrobial Susceptibility Testing:**
    - Ceftazidime-Avibactam and Ceftriaxone-Sulbactam-EDTA will be tested on all MDR isolates using the Kirby-Bauer disc diffusion method.
    - MIC values will be determined using E-test or broth microdilution methods.

Statistical Analysis

The statistical analysis will be conducted using SPSS version 23.0. The chi-square test will assess the significance of the results, with a 95% confidence interval

determining the strength of any association. Additionally, one-way ANOVA followed by Tukey's multiple comparison test will evaluate the differences in MIC values between the drug combinations, with a p-value of less than 0.05 considered statistically significant.

Results

The study includes 100 bacterial isolates from various clinical samples, which are tested for sensitivity against three antibiotics:

1. **CefepimeEnmetazobactam**
2. **Ceftazidime Avibactam**
3. **Ceftriaxone+Sulbactam+DisodiumEdetate**

Table 1: Antibiotic Sensitivity of CefepimeEnmetazobactam

Bacterial Species	Total (n=100)	Sensitive n (%)	χ^2 value	p-value
Klebsiella species	22	12 (55%)	4.21	0.04*
Escherichia coli	34	12 (35.3%)	6.87	0.01**
Pseudomonas species	28	15 (53%)	3.95	0.047*
Acinetobacter species	16	4 (25%)	8.21	0.004**

(*Significant at $p < 0.05$, **highly significant at $p < 0.01$)

Table 2: Antibiotic Sensitivity of Ceftazidime Avibactam

Bacterial Species	Total (n=100)	Sensitive n (%)	χ^2 value	p-value
Klebsiella species	28	10 (36%)	5.18	0.02*
Escherichia coli	32	14 (44%)	4.91	0.027*
Pseudomonas species	16	10 (62.5%)	2.88	0.09
Acinetobacter species	24	8 (33.3%)	6.35	0.012*

(*Significant at $p < 0.05$)

Table 3: Antibiotic Sensitivity of Ceftriaxone+Sulbactam+DisodiumEdetate

Bacterial Species	Total (n=100)	Sensitive n (%)	χ^2 value	p-value
Klebsiella species	28	20 (71.5%)	10.67	0.001**
Escherichia coli	32	25 (78%)	12.45	0.0004**
Pseudomonas species	16	12 (75%)	7.98	0.005**
Acinetobacter species	24	19 (80%)	9.34	0.002**

(**Highly significant at $p < 0.01$)

Additional Data: Sample Types and Locations

Samples	Total number
Urine	22
Sputum	45
Blood	10
Pus	15
Tissue	8

Location Distribution:

- ICU: 34 samples
- Non-ICU: 64 samples

This distribution highlights the source of the bacterial isolates, indicating a higher number of sputum samples, mostly from non-ICU settings. This could suggest a

prevalence of respiratory tract infections in the general patient population more than critical care areas.

Discussion

In this study assessing the sensitivity of 100 bacterial isolates to three different antibiotics across various clinical samples, the results demonstrate a variable response based on the type of antibiotic and bacterial species involved.

Antibiotic Sensitivity of CefepimeEnmetazobactam

CefepimeEnmetazobactam demonstrated moderate effectiveness against multidrug-resistant Gram-negative bacteria. The sensitivity rates varied across species, with Klebsiella species showing the highest sensitivity at 55%, while Acinetobacter species exhibited the lowest sensitivity at 25%. The chi-square test revealed significant associations for all species except Pseudomonas, with highly significant resistance patterns observed in Escherichia coli ($p=0.01$) and Acinetobacter species ($p=0.004$). These findings indicate that CefepimeEnmetazobactam may have limited clinical effectiveness against Acinetobacter infections and should be used cautiously in treating such cases.

Antibiotic Sensitivity of Ceftazidime Avibactam

Ceftazidime Avibactam showed variable sensitivity patterns, with Pseudomonas species displaying the highest response at 62.5%, though this association was not statistically significant ($p=0.09$). The sensitivity rates for Klebsiella (36%), Escherichia coli (44%), and Acinetobacter species (33.3%) were relatively low but statistically significant ($p<0.05$). These results suggest that Ceftazidime Avibactam may be a suitable option for treating Pseudomonas infections; however, its overall efficacy against other multidrug-resistant pathogens remains moderate, necessitating further evaluation based on local resistance patterns.

Antibiotic Sensitivity of Ceftriaxone+Sulbactam+DisodiumEdetate

Ceftriaxone+Sulbactam+DisodiumEdetate emerged as the most effective antibiotic combination in this study, demonstrating high sensitivity rates across all bacterial species tested. The sensitivity ranged from 71.5% in Klebsiella species to 80% in Acinetobacterspecies. Statistical analysis showed highly significant associations for all species ($p<0.01$), reinforcing its superior efficacy against multidrug-resistant Gram-negative bacteria. These findings suggest that Ceftriaxone+Sulbactam+DisodiumEdetate could be considered a first-line treatment for infections caused by these pathogens, particularly in settings with high antimicrobial resistance.

Sample Distribution and Clinical Implications

Among the 100 bacterial isolates analyzed, the highest number of samples were obtained from sputum (45%), followed by urine (22%), pus (15%), blood (10%), and tissue (8%). The majority of these samples (64%) were collected from non-ICU settings, while 34% originated from ICU patients. This distribution suggests that multidrug-resistant bacterial infections are prevalent in both critical care and general hospital settings, with a notable presence in respiratory tract infections.

(MDR) Gram-negative bacteria pose a significant global health challenge, necessitating the development of novel therapeutic strategies. A meta-analysis evaluating β-lactam/β-lactamase inhibitor combinations found that these regimens provide enhanced efficacy against MDR Gram-negative pathogens, though existing guidelines remain limited due to the low quality of available evidence [5]. Similarly, new antibiotics such as plazomicin, eravacycline, and cefiderocol have

demonstrated potent activity against carbapenem-resistant *Enterobacterales*, *Acinetobacterbaumannii*, and *Pseudomonas aeruginosa*, though resistance has been noted in some cases [6].

Combination therapies incorporating polymyxins have been extensively studied due to their role as last-line agents. Studies indicate that combinations such as meropenem/vaborbactam, ceftazidime/avibactam, and ceftolozane/tazobactam exhibit strong activity against MDR pathogens, though their use requires careful pharmacokinetic optimization [7]. A study investigating polymyxin B in combination with 13 other antibiotics against MDR *Pseudomonas aeruginosa* found significant synergistic effects with aztreonam, cefepime, fosfomycin, and minocycline, indicating promising treatment avenues [8].

Recent studies have explored the efficacy of novel antibiotic combinations against multidrug-resistant (MDR) Gram-negative bacteria, focusing on Cefepime-Enmetazobactam, Ceftazidime-Avibactam, and Ceftriaxone+Sulbactam+Disodium EDTA (CSE). A randomized controlled trial (RCT) compared CSE with best alternative treatments (BAT), including Colistin, Polymyxin B, Meropenem, and Ceftazidime-Avibactam for treating complicated urinary tract infections (cUTI) caused by metallo-beta-lactamase (MBL) producing *Enterobacterales.* The study concluded that CSE did not meet the non-inferiority criteria compared to BAT, indicating limited efficacy in MBL-producing infections [9].

The antibacterial activity of Cefepime/Enmetazobactam (FPE) was assessed against U.S. *Enterobacterales*isolates collected between 2019 and 2021. FPE exhibited potent in vitro activity, with an MIC90 of 0.12 µg/ml, comparable to Meropenem (0.06 µg/ml) and Ceftazidime-Avibactam (0.25 µg/ml). Notably, susceptibility to FPE was ≥98.4%, whereas susceptibility to third-generation cephalosporins and piperacillin-tazobactam was ≤85.8%, highlighting the potential of FPE as a viable alternative for treating ESBL-producing pathogens [10].

A retrospective study compared the efficacy of CSE and Meropenem in the management of Gram-negative bacterial sepsis. It found that 81.42% of isolates were susceptible to CSE, compared to 64.28% for Meropenem. Furthermore, 54.05% of patients treated with CSE achieved clinical cure, whereas only 33.33% of those receiving Meropenem were cured without the need for additional therapy. These findings suggest that CSE may serve as an effective alternative to Meropenem in the treatment of Gram-negative sepsis [11].

Another study analyzed the in vitro activity of Ceftazidime-Avibactam against MDR *Enterobacterales* and *Pseudomonas aeruginosa* collected in Latin America. The results indicated that 96.7% of *Enterobacterales* isolates were susceptible to Ceftazidime-Avibactam, surpassing the efficacy of other tested agents. Additionally, the susceptibility rate for MDR *Pseudomonas aeruginosa* was 45.8%, making Ceftazidime-Avibactam a promising treatment option for MDR infections in Latin America [12].

The efficacy of Cefepime-Enmetazobactam was further demonstrated in an in vitro study that tested its activity against *Enterobacteriaceae*and *Pseudomonas aeruginosa*. The study found that*Enmetazobactam* significantly enhanced Cefepime's potency, lowering MIC90 values across multiple bacterial species. The findings suggest that Cefepime-Enmetazobactam could serve as a potent carbapenem-sparing alternative for treating MDR infections caused by ESBL-producing *Enterobacterales*[13].

Conclusion

The study findings indicate that Ceftriaxone+Sulbactam+DisodiumEdetate is the most effective antibiotic combination for treating multidrug-resistant Gram-

negative infections. Ceftazidime Avibactam exhibited moderate effectiveness, particularly against Pseudomonas species, while CefepimeEnmetazobactam showed the least efficacy, especially against Acinetobacter species. These results highlight the importance of selecting antibiotics based on local resistance patterns to optimize treatment outcomes and minimize the risk of antimicrobial resistance

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