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ORIGINAL ARTICLE

# Evaluating the *in vitro* activity of cefoperazone-sulbactam against Gram negative pathogens in blood stream infections using automated systems

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## ABSTRACT

**Background and Objectives:** The incidence of multidrug-resistant, Gram-negative organisms, isolated as the etiological agents of infections is ascending. The advent of novel antibiotics poses significant challenges, necessitating the optimization and utilization of extant antimicrobial agents. Cefoperazone, a third-generation cephalosporin and β-lactam antimicrobial, when combined with sulbactam, an irreversible β-lactamase inhibitor, mitigates the vulnerability of cefoperazone to β-lactamase-producing organisms. Nonetheless, regional data on the susceptibility patterns for this pharmacological combination remains scarce. The primary objective of this investigation was to assess the efficacy of the cefoperazone-sulbactam combination against prevalent Gram-negative bacteria isolated from blood cultures.

**Materials and Methods:** A total of 700 Gram-negative isolates, comprising *Escherichia coli, Klebsiella pneumoniae, Acinetobacter* species, and *Pseudomonas aeruginosa*, were procured using the BacT/Alert 3D system. The identification and susceptibility testing for cefoperazone-sulbactam were performed using the VITEK Compact ID and AST system. Comparative analysis was conducted against other tested antibiotics.

**Results:** The study revealed that cefoperazone-sulbactam exhibited commendable *in-vitro* activity against Gram-negative pathogens isolated from blood, surpassed only by colistin and tigecycline.

**Conclusion:** Cefoperazone-sulbactam demonstrates robust activity against the most frequently encountered clinical pathogens, suggesting its potential as an efficacious therapeutic agent. The findings underscore the imperative for ongoing surveillance of resistance patterns and trends among commonly used antimicrobials.

**Keywords:** Antimicrobial resistance; Cefoperazone/sulbactam; Gram negative isolates

## INTRODUCTION

The burgeoning menace of antimicrobial resistance (AMR) imperils the efficacious prevention and treatment of an expanding spectrum of infections instigated by bacteria, parasites, viruses, and fungi, thereby compromising our capability to manage high-burden infections effectively. AMR manifests when these microorganisms undergo genetic transformations over time, rendering them impervious to conventional therapeutics, thus exacerbating the difficulty in treating infections and amplifying the risks of disease proliferation, severe morbidity, and mortality (1). Consequently, these pharmacological agents become inefficacious, allowing infections to persist and propagate within the host, thereby heightening the potential for

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transmission to others. Since the inaugural Bacterial Priority Pathogens List (BPPL) was promulgated in 2017, the AMR threat has escalated, undermining the potency of numerous antibiotics and jeopardizing the advancements of contemporary medicine. On 17 May 2024, the World Health Organization (WHO) released an updated BPPL, categorizing *Acinetobacter baumannii* (carbapenem-resistant) and *Enterobacterales* (third-generation cephalosporin and carbapenem-resistant) as critical priority pathogens, and *Pseudomonas aeruginosa* (carbapenem-resistant) as a high priority pathogen (2). This escalating trend in antimicrobial resistance underscores the imperative need for novel, sensitive antibiotics, albeit the development of such agents is fraught with challenges, making the conservation of existing antibiotics' efficacy crucial in the battle against antibiotic resistance.

Cefoperazone, a third-generation cephalosporin, exhibits broad-spectrum antimicrobial activity against frequently encountered Gram-positive cocci, Gram-negative bacilli, and anaerobes (3). With a prolonged elimination half-life of approximately two hours, cefoperazone facilitates bi-daily dosing and was extensively utilized during the 1980s for treating infections in both immunocompetent and neutropenic patients (4-7). Due to its susceptibility to β-lactamases, cefoperazone was amalgamated with the β-lactamase inhibitor sulbactam in an equimolar ratio (1 g CFP and 1 g SUL) (8). The burgeoning resistance of bacteria to antimicrobial agents has emerged as a pivotal public health quandary globally. Widely employed antimicrobials such as beta-lactams, cephalosporins, aminoglycosides, and quinolones are increasingly facing resistance from both Gram-positive and Gram-negative bacilli. The cefoperazone-sulbactam combination (CFP/SUL) is posited as a potential alternative, owing to its expansive antimicrobial spectrum and efficacy across various geographic locales in treating diverse infections, including nosocomial pneumonia, intra-abdominal infections, gynecological infections, sepsis, and infections in febrile neutropenic patients (9). Regrettably, there is a dearth of data regarding the susceptibility of CFP/SUL against clinically significant pathogens prevalent in this region of India. Against this backdrop, we undertook this study to elucidate the susceptibility patterns of cefoperazone-sulbactam vis-à-vis Gram-negative bacteria isolated from blood and juxtapose its efficacy with other commonly utilized antimicrobials.

## MATERIALS AND METHODS

This retrospective, hospital-based investigation was executed within the Department of Microbiology at a tertiary care institution in North India. Gram-negative organisms analyzed in this study were procured from blood samples collected from both outpatient and inpatient departments over a span of one year, from January 2023 to December 2023. All blood cultures were processed utilizing the BacT/Alert 3D system. The identification and antimicrobial susceptibility testing (AST) for cefoperazone/sulbactam and other comparator agents were conducted using the VITEK 2 ID and AST Compact System, respectively. The comparator antibiotics included Piperacillin/tazobactam, Ampicillin/sulbactam, Amikacin, Gentamicin, Tobramycin, Cefepime, Ceftazidime, Cefotaxime, Ciprofloxacin, Levofloxacin, Meropenem, Imipenem, Tigecycline, Colistin and Cotrimoxazole. Susceptibility profiles for each antibiotic were established according to the criteria delineated by the Clinical and Laboratory Standards Institute (CLSI) (10). The organisms scrutinized in this study included *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter* species, and *Pseudomonas aeruginosa*. Concurrent quality control (QC) testing was conducted to ensure the accuracy of test conditions and procedures, employing QC strains such as *Escherichia coli* ATCC 25922 and 35218, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, and *Acinetobacter* ATCC 19606. Data was collected and analysed in a Microsoft Excel sheet with various charts and Tables. The statistical tests of hypothesis were not applicable.

Ethical clearance for the execution of this study was granted by the institute's ethical committee under the approval number: SIMS 131/IEC-SKIMS/2024-16 dared 04/01/2024.

## RESULTS

Over the course of one annum, we amassed a total of 700 Gram-negative bacterial isolates from blood specimens. The most frequently isolated pathogen was *Escherichia coli*, accounting for 250 isolates, followed closely by *Klebsiella pneumoniae* with 240 isolates. *Pseudomonas aeruginosa* constituted 130 isolates, while *Acinetobacter* species comprised the remaining 80 isolates (Fig. 1). The susceptibility pattern

of isolated organisms to cefoperzone-sulbactam and other comparator antibiotics is outlined in the Table 1. *Escherichia coli* exhibited a remarkable 97% susceptibility to the cefoperazone/sulbactam combination at an MIC <16 mg/l (MIC50/90, 0.5/16 mg/l). Comparatively, a mere 39% of isolates were sensitive to ampicillin/sulbactam, with an average susceptibility pattern to other antibiotics (Fig. 2). In contrast, 80% of *Klebsiella pneumoniae* isolates (MIC50/90, 0.5/32 mg/l), 83% of *Pseudomonas aeruginosa* (MIC50/90, 8/32 mg/l), and 46% of *Acinetobacter* species (MIC50/90, 8/64 mg/l) demonstrated sensitivity to cefoperazone/sulbactam. Among comparator antibiotics, amikacin exhibited robust sensitivity profiles: 95% for *Escherichia coli*, 95% for *Klebsiella pneumoniae*, 93% for *Pseudomonas aeruginosa*, but only 31% for *Acinetobacter*. Meropenem showed commendable activity against 95% of *Escherichia coli*, 82% of *Klebsiella pneumoniae*, and 81% of *Pseudomonas aeruginosa*. Tigecycline and colistin emerged as the most efficacious among comparator antibiotics, with sensitivity percentages ranging from 97-100% (Table 1). *Acinetobacter* displayed the lowest sensitivity to all comparator antibiotics, with 100% of isolates sensitive to colistin, followed by 46% to cefoperazone/sulbactam. From Table 1 and Fig. 2, it is evident that cefoperazone/sulbactam shows substantial efficacy against all four isolates, second only to colistin and tigecycline.

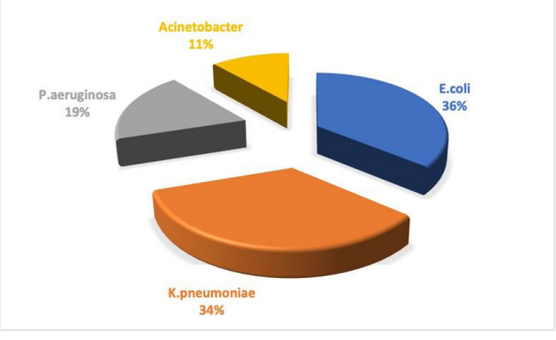


**Fig. 1.** Frequency Distribution of isolated organisms.

## DISCUSSION

The escalating global threat of antimicrobial resistance (AMR) poses a significant challenge. One of the primary mechanisms of resistance is the production of beta-lactamase enzymes. Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* species are the most frequently isolated multidrug-resistant Gram-negative organisms (MDRGN), severely limiting the therapeutic arsenal (3). To restore and expand the spectrum of beta-lactam antibiotics, beta-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam have been combined with amoxicillin, ampicillin, and piperacillin, respectively (11).

**Table 1.** % age sensitivity of the isolates to Cefoperazone/sulbactam and the comparator agents.

| | *E. coli* | *K. pneumoniae* | *P. aeruginosa* | *Acinetobacter* |
|---|---|---|---|---|
| Cefoperazone/sulbactam | 97 | 80 | 83 | 46 |
| Piperacillin/tazobactam | 94 | 79 | 83 | 20 |
| Ampicillin/sulbactam | 39 | 59 | - | 19 |
| Amikacin | 95 | 95 | 93 | 31 |
| Gentamicin | 84 | 80 | 92 | 30 |
| Tobramycin | 85 | 81 | - | 34 |
| Cefepime | 80 | 70 | 87 | 15 |
| Ceftazidime | 82 | 73 | 85 | 21 |
| Cefotaxime | 75 | 74 | - | - |
| Ciprofloxacin | 72 | 67 | 82 | 20 |
| Levofloxacin | 75 | 75 | 80 | 23 |
| Meropenem | 95 | 82 | 81 | 25 |
| Imipenem | 90 | 79 | 80 | 24 |
| Tigecycline | 97 | 98 | - | - |
| Colistin | 100 | 100 | 100 | 100 |
| Cotimoxazole | 59 | 45 | - | - |

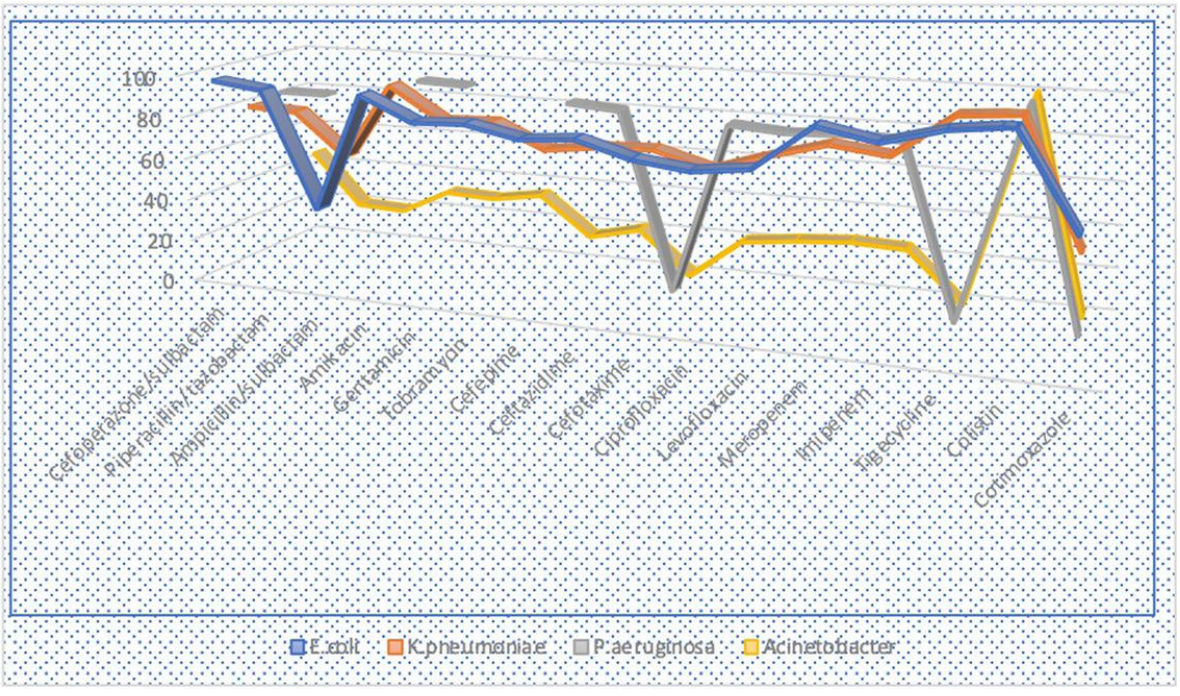


**Fig. 2.** Susceptibility pattern for the tested antibiotics.

This study elucidates that cefoperazone-sulbactam exhibits superior *in vitro* activity against Gram-negative isolates compared to other antibiotics, corroborated by previous studies showing that sulbactam significantly enhances cefoperazone's antimicrobial activity and mitigates the bacterial inoculum effect (4, 11-14). Colistin exhibited the highest activity against all four isolates, consistent with findings in the literature (8, 9, 15, 16). Although carbapenems were previously among the few effective options for MDRGN, resistance, particularly in hospital-acquired *Pseudomonas aeruginosa* and *Acinetobacter*, is rising (17-19). In this study, cefoperazone-sulbactam demonstrated notable activity against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex, indicating its potential as an alternative treatment.

The addition sulbactam fully potentiates cefoperazone against *Pseudomonas* species and *Enterobacteriaceae*, even those harboring plasmid-mediated and extended-spectrum enzymes (20). Antimicrobial susceptibility rates vary widely among geographic regions and are generally the lowest in Eastern Europe. Cefoperazone-sulbactam continues to show strong *in vitro* activity against *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolates from the Asia-Pacific region. Its potency and broad-spectrum activity sustain its role in treating infections caused by Gram-negative organisms, remaining among the most active compounds *in vitro* against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Acinetobacter* species at established breakpoints (9).

The strains included in this study were procured from the blood samples of both the OPD and IPD patients which can be a reason for their high sensitivity to certain drugs. This was the limitations of our study.

## CONCLUSION

In summary, Cefoperazone/sulbactam exhibits promising in vitro activity against Gram-negative organisms isolated from blood samples, suggesting its potential efficacy in treating infections caused by these pathogens. However, vigilance against antimicrobial resistance remains imperative, necessitating ongoing surveillance and judicious antibiotic use to preserve their effectiveness.

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