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TO EVALUATE THE IN-VITRO ACTION OF LEVONADIFLOXACIN AND CEFEPIME-ENMETAZOBACTAM ON CLINICAL ISOLATES OF GRAM-NEGATIVE NON-FERMENTATIVE BACTERIA.

Microbiology

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ABSTRACT

The increase in hospital-acquired infections due to Gram-negative non-fermentative bacilli (GNNFB) is a challenge, particularly due to their intrinsic resistance to many antibiotics. We assessed the in-vitro antimicrobial activity of Levonadifloxacin and cefepime enmetazobactam against 64 clinical isolates of GNNFB. 58.3% (n=43) of Pseudomonas aeruginosa were susceptible to levonadifloxacin, while all isolates of Chryseobacterium (n=1), S. maltophilia (n=8), Burkholderia (n=2) and Elizabethkingia (n=1) were susceptible to levonadifloxacin. 63.9 % of Pseudomonas aeruginosa (n=43) and all isolates of Chryseobacterium (n=1), S. maltophilia (n=8), Burkholderia (n=2) and Elizabethkingia (n=1) were susceptible to cefepime enmetazobactam. None of the isolates of Acinetobacter spp. (n=9) were susceptible to either antimicrobials. Our findings highlight the potential clinical role of these agents as valuable options in treatment of infections by resistant Gram-negative non-fermenters.

KEYWORDS

Antimicrobial Susceptibility Test, Cefepime-Enmetazobactam, Levonadifloxacin, Non-Fermenters

1. INTRODUCTION

In the past few years, Gram-negative non-fermentative bacilli (GNNFB) have become important causes of healthcare-associated infections, especially in intensive care units and in people with weak immune systems. Common organisms in this group such as Pseudomonas aeruginosa, Acinetobacter baumannii, and Stenotrophomonas maltophilia are very difficult to treat because they are naturally resistant to many antibiotics, can form biofilms, and easily acquire new resistance genes like those for carbapenemases and efflux pumps. [1] Because of these factors, infections caused by these bacteria are linked with high illness rates, longer hospital stays, and increased chances of death, making them a serious global health concern. The rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) GNNFB has made many regular antibiotics less effective, creating an urgent need for new treatment options. [2]

Among the newer antimicrobial agents, Levonadifloxacin and Cefepime–Enmetazobactam have shown promising results. Levonadifloxacin is a new benzoquinolizine fluoroquinolone that works against a wide range of bacteria, including both Gram-positive and Gram-negative types that are resistant to quinolones. It acts by blocking DNA gyrase and topoisomerase IV, which are essential for bacterial DNA replication. It also has strong intracellular activity, good lung tissue levels, and can overcome efflux-related resistance. [3] Cefepime–Enmetazobactam is a combination of cefepime, a fourth-generation cephalosporin, and Enmetazobactam, an extended-spectrum β-lactamase (ESBL) inhibitor. This combination improves cefepime's activity by helping it act against bacteria that produce β-lactamases and are resistant to other β-lactam antibiotics. [4]

The aim of the present study was to determine the in-vitro activity of Levonadifloxacin and Cefepime–Enmetazobactam against clinical isolates of Gram-negative non-fermentative bacteria.

2. Methodology

The study was conducted at Max Super Speciality Hospital, Dwarka, to evaluate antimicrobial resistance by assessing the efficacy of levonadifloxacin and cefepime–enmetazobactam against Gram-negative non-fermentative bacteria. The study population included 64 clinical isolates of Pseudomonas aeruginosa, Acinetobacter baumannii, Burkholderia cepacia, and other GNNFB, which were confirmed using standard identification techniques and/or VITEK. Duplicate isolates from the same patient, as well as isolates exhibiting mixed growth of multiple organisms, was excluded from the study.

The method of measurement of outcome of interest was carried out through lawn culture and antimicrobial susceptibility testing (AST). For lawn culture, each strain was subcultured on suitable agar to ensure purity, and a bacterial suspension was prepared and standardized to a 0.5 McFarland turbidity standard. AST was performed using the Disk Diffusion (Kirby-Bauer) method. In this procedure, Mueller-Hinton agar (MHA) plates were uniformly inoculated with the standardized inoculum, and antibiotic discs of levonadifloxacin and cefepime-enmetazobactam were placed on the plates. The MHA plates were then incubated aerobically overnight (16-18 hours) at 35±2°C. [5] Following incubation, the diameters of inhibition zones were measured in millimeters and compared with CLSI/EUCAST breakpoints to classify the isolates as sensitive, intermediate, or resistant. The standard disc concentration recommended by CLSI for levonadifloxacin is 10 µg, while the susceptibility testing disc for cefepime-enmetazobactam contains 30 µg of cefepime and 20 µg of enmetazobactam. [6,7,8]

For statistical analysis, all categorical data was entered into Microsoft Excel and analyzed using SPSS version 25. The results were expressed as percentages to report susceptibility and resistance patterns.

3. RESULTS

In this study, the in-vitro activity of levonadifloxacin and cefepime–enmetazobactam was evaluated against clinical isolates of Gram-negative non-fermenters, including Pseudomonas (P.) aeruginosa, Acinetobacter spp., Elizabethkingia spp., Stenotrophomonas (S.) maltophilia, Burkholderia (B.)cepacia and Chryseobacterium (C.)indologenes. The majority of isolates were recovered from respiratory samples, followed by blood, urine, and pus samples.

3.1 Levonadifloxacin Susceptibility

Levonadifloxacin exhibited notable activity against multidrug-resistant (MDR) isolates, especially Pseudomonas aeruginosa with 58.3% (n=43) of Pseudomonas aeruginosa were susceptible to levonadifloxacin, while all isolates of Chryseobacterium (n=1), S. maltophilia (n=8), Burkholderia (n=2) and Elizabethkingia (n=1) were susceptible to levonadifloxacin. None of the isolates of Acinetobacter spp. were susceptible to this antibiotic (Table 1). Among fluoroquinolone-resistant non-fermenters, Pseudomonas aeruginosa (n=3) and Chryseobacterium indologenes (n=1) were Levonadifloxacin sensitive.

3.2 Cefepime-Enmetazobactam Susceptibility

Among isolates of Pseudomonas aeruginosa, 63.9% (n=43) were susceptible to cefepime enmetazobactam, while all isolates of Chryseobacterium (n=1), S. maltophilia (n=8), Burkholderia (n=2) and Elizabethkingia (n=1) were susceptible to cefepime

enmetazobactam. None of the isolates of Acinetobacter spp. were susceptible to this antibiotic. (Table 1). Among Piperacillin/Tazobactam-resistant non-fermenters, Pseudomonas aeruginosa (n=4), Chryseobacterium indologenes (n=1) and Elizabethkingia spp.(n=1) were cefepime enmetazobactam sensitive. When compared to other tested antibiotics, all non-fermenters, except Pseudomonas aeruginosa and Acinetobacter spp., showed good activity against levonadifloxacin and cefepime enmetazobactam.

Table 1. Susceptibility of Gram-negative Non-fermenters to Levonadifloxacin and Cefepime–Enmetazobactam

Organism (n)	Levonadifloxacin Susceptibility n (%)	Cefepime-Enmetazobactam Susceptibility n (%)
Pseudomonas aeruginosa (43)	25 (53.8%)	27 (63.9%)
Acinetobacter spp. (9)	0 (0%)	0 (0%)
Chryseobacterium spp. (1)	1 (100%)	1 (100%)
S. maltophilia (8)	8 (100%)	8 (100%)
Burkholderia spp. (2)	2 (100%)	2 (100%)
Elizabethkingia spp. (1)	1 (100%)	1 (100%)

4. DISCUSSION

This study evaluated the in-vitro activity of levonadifloxacin and cefepime–enmetazobactam against clinical isolates of Gram-negative non-fermenters, particularly multidrug-resistant (MDR) strains such as Pseudomonas aeruginosa, Acinetobacter spp., Elizabethkingia spp., Stenotrophomonas maltophilia, and Chryseobacterium indologenes. These pathogens are well-known for their intrinsic resistance mechanisms and pose significant clinical challenges, especially in hospital-acquired infections.

Levonadifloxacin demonstrated promising activity, especially against Pseudomonas aeruginosa, with 58.3% of isolates being susceptible. This is consistent with a recent study by Chakraborty et al., which reported that levonadifloxacin had potent in-vitro activity against Stenotrophomonas maltophilia and Pseudomonas aeruginosa, with lower minimum inhibitory concentrations (MICs) compared to older fluoroquinolones, supporting its use against resistant non-fermenters. [9] Interestingly, levonadifloxacin remained active against fluoroquinolone-resistant Pseudomonas aeruginosa and Chryseobacterium indologenes, suggesting it may be efficient where older fluoroquinolones fail. This aligns with findings from a broader in vitro study of levonadifloxacin against ICU bacterial isolates, which confirmed its significant activity against resistant strains of Pseudomonas aeruginosa. [10] None of the Acinetobacter spp. isolates were susceptible to levonadifloxacin, which is in contradiction to the earlier studies reported. [9,10,11] The reason for the findings remain unexplored though, the possibility of efflux pumps cannot be ruled out and needs to be tested.

Cefepime–enmetazobactam exhibited broader-spectrum activity, showing 63.9% susceptibility in Pseudomonas aeruginosa and full susceptibility in Elizabethkingia spp., Stenotrophomonas maltophilia, and Chryseobacterium indologenes. These results are supported by a recent study by Falagas et al., which demonstrated strong in-vitro activity of cefepime–enmetazobactam against beta-lactamase-producing non-fermenters. [12] Furthermore, the ability of cefepime–enmetazobactam to inhibit Piperacillin/Tazobactam-resistant isolates underscores its potential as an effective carbapenem-sparing agent, as also reported by Bakthavatchalam YD et al., who found this combination highly effective against a broad spectrum of Gram-negative pathogens including Pseudomonas aeruginosa. [13]

In conclusion, our findings reinforce the potential clinical role of levonadifloxacin and cefepime–enmetazobactam as valuable options in the treatment of infections caused by resistant Gram-negative non-fermenters, particularly Pseudomonas aeruginosa, Stenotrophomonas maltophilia, and Elizabethkingia spp.. These agents demonstrated better in vitro efficacy compared to many older antibiotics, offering hope in settings of multidrug resistance. Nevertheless, the lack of activity against Acinetobacter spp. suggests that alternative strategies such as combination therapy or development of novel agents remain essential.

A key limitation of our study is the relatively small number of isolates for other GNNFB, apart from P. aeruginosa, which restricts the statistical power of the conclusions. Additionally, in the absence of specific disc-diffusion breakpoints for Burkholderia cepacia, we applied Pseudomonas-based criteria, which may not accurately reflect the true susceptibility.

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