Original Article

Frequency of Susceptibility to Cefiderocol in Carbapenem-Resistant Gram-Negative Bacteria by Kirby-Bauer Disc Diffusion Method; A Study in Tertiary Care Hospital of Southern Punjab

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Abstract

Objective: The study was planned to assess the frequency of susceptibility to Cefiderocol in carbapenem resistant gram-negative bacteria by Kirby-Bauer disc diffusion method.

Methodology: This was a cross-sectional study performed at the Department of Microbiology, Combined Military Hospital, from 1st July to 31st December 2024. A total of 101 specimens submitted to the department and growing carbapenem-resistant gram-negative rods were included consecutively; duplicate specimens were excluded. Specimens, including urine, blood, pus, respiratory samples, and body fluids, were processed by conventional methods. Organisms were recognized by colony morphology, microscopic features, biochemical tests, and API-10S. The Kirby-Bauer disc diffusion approach was performed on Cation-Adjusted Mueller-Hinton agar plates per CLSI guidelines, and Cefiderocol disk diffusion was applied on commercial preparation of CAMH-agar plates and manually produced ID-CAMH-agar plates. Data were analyzed using SPSS version 23 with descriptive statistics and Fischer's exact test to assess Cefiderocol sensitivity across demographic strata.

Results: Mean age of patients was 42.6 ± 14.3 years, with 55.4% males and 70.3% indoor cases. Pus (28.7%), urine (26.7%), and blood (23.8%) were the most common specimens. Enterobacterales (66.3%) and Pseudomonas aeruginosa (21.8%) were frequently isolated. Cefiderocol sensitivity was observed in 77.2% of isolates, with resistance in 17.8%.

Conclusion: Cefiderocol demonstrates high response against carbapenem-resistant gram-negative microorganisms, supporting its clinical utility in managing severe and complex infections effectively. These findings highlight Cefiderocol as a promising therapeutic option.

Keywords: Acinetobacter baumannii, Anti-Bacterial Agents, Bacterial Drug Resistance, Carbapenem-Resistant Enterobacteriaceae, Cefiderocol, Gram-Negative Bacteria, Microbial Sensitivity Tests, Pseudomonas aeruginosa.

Introduction

One of the biggest threats to public health is the growth of microorganisms that are resistant to drugs. Since carbapenem-resistant gram-negative bacteria (CR-GNB) have a multi-resistant phenotype against practically all routinely used classes of antibiotics, they are regarded as superbugs in healthcare settings.[1] The World Health Organization (WHO) has since identified them as highly important microorganisms for which the discovery of new medications is desperately needed.[2] A number of antibacterial agents, e.g., new β-lactam/β-lactamase inhibitor combos like imipenem-relebactam and ceftazidime-avibactam, have been authorized for clinical utility.[3]

A new siderophore cephalosporin, Cefiderocol facilitates cell entrance and attains high concentrations in the periplasmic region by means of bacterial iron transfer.[4] Following its attachment to penicillin-binding proteins, the cephalosporin moiety prevents the formation of peptidoglycans, which eventually results in the death of bacterial cells.[5] Enterobacterales, Pseudomonas aeruginosa, and A. baumannii complex are among the many carbapenem-resistant gram-negative bacteria that it effectively combats.[6]

When doing cefiderocol antimicrobial susceptibility testing (AST), specific consideration must be given to iron concentrations of media because cefiderocol enters bacteria by using active iron transport, while iron vehicles are increased in iron-depleted environments, as happens in vivo.[7] Because normal cation-adjusted Mueller-Hinton broth (CAMHB) does not yield repeatable minimum inhibitory concentrations (MICs) that appropriately represent predicted in vivo activity, iron-depleted CAMHB is necessary to get accurate cefiderocol MICs as assessed by broth microdilution (BMD). Microbiology labs may encounter difficulties because of the challenging nature of both preparations.[8] As an alternative, AST techniques such as the disc diffusion (DD) and Sensititre lyophilised BMD panel have been developed that make it easier to acquire cefiderocol results. Iron depletion is also not necessary for in vitro testing using Disc Diffusion method on Mueller-Hinton agar (MHA) since the iron is adequately bound within the agar.[9]

Bianco G et al (2010) assessed 286 hard-to-treat Gram-negative pathogens and in vitro response of cefiderocol by use of disc diffusion method and a ComASP® cefiderocol microdilution panel. BMD in iron-depleted MHB served as the standard procedure. Cefiderocol was found to be susceptible in 78%, 88%, 85%, and 100% of isolates of Enterobacterales, Pseudomonas aeruginosa, A. baumannii, and S. maltophilia, respectively, using disc diffusion. General categorical agreement and essential agreement were 94% and 84%, respectively, according to the ComASP® cefiderocol panel.[10]

The rationale of this study is to assess the prevalence of cefiderocol susceptibility in Carbapenem resistant Gram-negative bacteria in samples obtained from a tertiary care hospital. While global studies suggest increased in-vitro response of cefiderocol against these organisms, regional variations in susceptibility exist. The study will help in rationalizing cefiderocol use to preserve its efficacy and delay resistance development. The results will contribute to antimicrobial resistance surveillance data, helping shape treatment guidelines and infection control strategies.

Methodology

This cross-sectional study was performed at the microbiology department of Combined Military Hospital over a period of six months from 1st July to 31st December 2024 after approval from the institutional ethics review committee (ERC no. 89/2024 dated 30-06-2024). All the specimen submitted to microbiology department and growing gram-negative rods resistant to carbapenems were consecutively enrolled in the study. Carbapenem resistance was assessed by Kirby-bauer disc diffusion method with meropenem 10µg disc. Zone for resistance is less than or equal to 19mm. Duplicate specimen from the same patients were excluded. Age, gender and patient location was recorded. The specimens included urine, blood, pus, respiratory specimens and body fluids and processed by conventional methods as per hospital protocol. A sample size of 101 was calculated by use of WHO sample size calculator, keeping confidence level 95%, margin of error 8% and taking 78.6% of Enterobacterales as susceptible to Cefiderocol by disc diffusion.[10]

These specimens were inoculated on culture plates and incubated at 35+/-2°C for 18 to 24 hours. Identification of isolates was carried out by colony morphology, microscopic characteristics, various biochemical tests and API-10S as per standard protocol. The disc diffusion method was performed on CAMH agar plates (BioMérieux) based on CLSI guidelines. Also, Cefiderocol disk diffusion was done on CAMH-agar plates from another company (Liofilchem) and on locally produced ID-CAMH-agar plates. Twenty grams of agar was added to 1 L of CAMHB to prepare ID-CAMH-agar plates. After aseptic measures, 25 mL media was put in empty petri dishes. Cefiderocol (30µg) disks was obtained from Liofilchem. After eighteen hours incubation at temperature of 35 °C, the inhibition zone of Cefiderocol was visually examined and noted. Sensitivity to Cefiderocol was labelled if inhibition zone (mm) for P. Aeruginosa was ≥18, A. Baumannii ≥15, S. maltophilia ≥15 and Enterobacterales ≥16.

The data was analysed through SPSS version 23. Descriptive statistics e.g., mean ± SD for numerical data and frequency and percentages for categorical data were measured. Cefiderocol sensitivity across the strata of demographic characteristics was determined

through Fischer's exact test at 5% significance level.

Results

The mean age of the patients was 42.6 ± 14.3 years and 55.4% (n=56) were males. The samples were from indoor patients in 70.3% (n=71) cases. The most common specimens submitted were pus in 28.7% (n=29) followed by urine in 26.7% (n=27) and blood in 23.8% (n=24) [Table 1]. The most common pathogen isolated were Enterobacterales 66.3% (n=67) followed by Pseudomonas aeruginosa 21.8% (n=22) [Table 2].

In 77.2% (n=78) of the carbapenem-resistant gram-negative cases, the isolates were sensitive to Cefiderocol and resistant only in 17.8% (n=18) [Fig. 1]. There was no difference in Cefiderocol sensitivity across different strata of patient variables [Table 3].

Discussion

In the present study, the most common carbapenem resistant pathogen isolated were enterobacterales (66.3%) followed by Pseudomonas aeruginosa (21.8%). In 77.2% of the cases, the isolates were sensitive to Cefiderocol and resistant only in 17.8%. In hospital settings, multidrug-resistant (MDR) GNB, such as MDR SM (Stenotrophomonas maltophilia), CR-AB (carbapenem-resistant Acinetobacter baumannii), CR-PA (carbapenem-resistant Pseudomonas aeruginosa), and carbapenem-resistant Enterobacteriaceae, are regarded as superbugs. They are linked to resistance to almost all the antibiotic classes that are often employed in clinical settings.

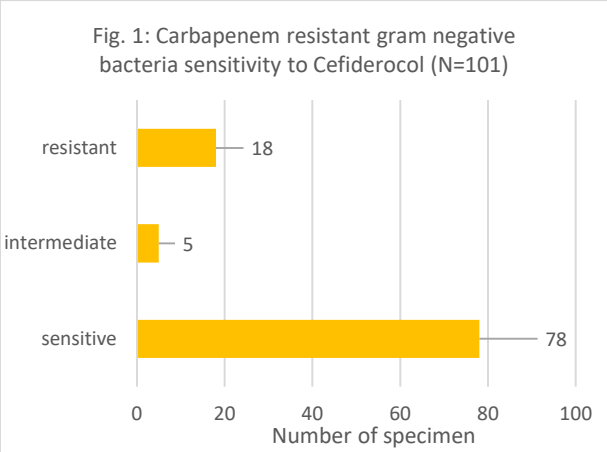
Table 1. Characteristics of patients providing carbapenem-resistant gram-negative isolates (N=101)

Age (years)	42.6 ± 14.3
Age(years)	1-60 years
Gender	
Male	56 (55.4%)
Female	45 (44.6%)
Patient location	
Indoor	71 (70.3%)
Outdoor	30 (29.7%)
Specimen type	
Urine	27 (26.7%)
Blood	24 (23.8%)
Pus	29 (28.7%)
Respiratory	11 (10.9%)
Body Fluids	10 (9.9%)

There are currently few alternatives for treating systemic infections produced by MDR GNB. There is hope for fighting against these high-profile pathogens since Cefiderocol, a new siderophore cephalosporin, has shown strong in vitro efficacy against Gram-negative, carbapenem-resistant bacteria. But there may already be resistance to a new antibiotic. Therefore, information for justified use of antimicrobial drugs and the creation of sensible antibiotic stewardship programs can be obtained from both local and worldwide antimicrobial resistance surveillance.

The efficacy of imipenem/relebactam and ceftazidime/avibactam (CAZ/AVI) against Klebsiella pneumoniae carbapenemase–producing Enterobacterales and ceftolozane/tazobactam against non-metallo-β-lactamase-producing CR-PA isolates was highlighted by Piotrowski PM et al, who also found a notable frequency of CR Gram-negative bacteria.[11] In a study by Bianco G et al, 78.6%, 88%, 85%, and 100% of the Enterobacterales, Pseudomonas aeruginosa, A. baumannii, and S. maltophilia pathogens tested showed cefiderocol susceptibility.[10] These findings are consistent with our results.

KPC (Klebsiella Pneumoniae Carbapenemase) and NDM (New Delhi metallo-β-lactamase) are the two primary carbapenemases in CR-KP in China.[12] According



Carbapenem resistant gram negative bacteria sensitivity to Cefiderocol (N=101)

Table 2. Pathogen isolated from patients providing carbapenem-resistant gram-negative isolates (N=101)

Pathogens Isolated	
Pseudomonas aeruginosa	22 (21.8%)
Acinetobacter baumannii	10 (9.9%)
Enterobacterales	67 (66.3%)
Stenotrophomonas maltophilia	2 (2.0%)

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Table 3. Effect of demographic characteristics on Cefiderocol sensitivity in carbapenem-resistant gram-negative isolates (N=101)

Demographic characteristics		Cefiderocol sensitivity			p-value*
		Sensitive	Intermediate	Resistant	
Gender	Male	45 (80.4)	3 (5.4)	8 (14.3)	0.646
	Female	33 (73.3)	2 (4.4)	10 (22.2)	
Patient location	Indoor	55 (77.5)	2 (2.8)	14 (19.7)	0.246
	Outdoor	23 (76.7)	3 (10)	4 (13.3)	
Specimen type	Urine	20 (74.1)	2 (7.4)	5 (18.5)	0.923
	Blood	17 (70.8)	1 (4.2)	6 (25)	
	Pus	23 (79.3)	1 (3.4)	5 (17.2)	
	Respiratory	10 (90.9)	0 (0.0)	1 (9.1)	
	Body fluid	8 (80)	1 (10)	1 (10)	
Pathogen Isolated	P. aeruginosa	18 (81.8)	0 (0.0)	4 (18.2)	0.896
	A. baumannii	8 (80)	0 (0.0)	2 (20)	
	Enterobacterales	50 (74.6)	5 (7.5)	12 (17.9)	
	S. maltophilia	2 (100)	0 (0.0)	0 (0.0)	

*Fischer's exact test

to a study, cefiderocol was less effective than other isolates at inhibiting the production of NDM.[13] According to reports, treating KPC-producing CR-KP with ceftazidime/avibactam may cause the landscape of carbapenemase to change from KPC to MBLs (Metallo-β-Lactamase).[14] The selection of isolates that produce NDM may also result from Cefiderocol's widespread use in the future.

Prior susceptibility has shown Cefiderocol's effectiveness against the CR-AB. Cefiderocol's MIC values ranged from ≤0.03 to >64 mg/L, with MIC50 and MIC90 levels of 0.25 and 1 mg/L, respectively, when tested against 101 CR-AB isolates in the ARGONAUT-I trial.[15] Falagas et al, from Greece, studied 107 CR-AB positive samples from 18 hospitals in their investigation; these isolates had cefiderocol MIC50 and MIC90 values of 0.06 and 0.5 mg/L, respectively.[16]

There were 452 patients in all, 303 treated with cefiderocol and 149 treated with imipenem/cilastatin, in a study by Zhanel GG et al. The microbiological intent-to-treat (MITT) population showed aggregate of clinical and pathological response rates of 54.6% (65/119) for imipenem/cilastatin and 72.6% (183/252) for cefiderocol.[17] In the investigation by Hackel et al., 368 MDR AB isolates were studied between 2014 and 2016 from labs in 52 different countries. They discovered that cefiderocol had MIC50 and MIC90 levels of 0.25 and 8 mg/L, respectively.[18]

Our study's cefiderocol susceptibility rate against CR-AB was surprisingly lower than those found in the previous research. Cefiderocol resistance in CR-AB has been linked to PER β-lactamase, according to reports.[19] Cefiderocol had strong in vitro response against these two microorganisms, in the research by Wang Q et al, inhibiting 100% of all carbapenem resistant PA isolates and 98% of all MDR SM pathogens at a dosage of 4 mg/L.12 These findings are comparable to those of our investigation, which showed that 100% of CR-SM and 81.8% of CR-PA were cefiderocol-sensitive. In other research, the MIC distribution for SM was shown to be comparable.[20]

However, compared to other centres, the MIC value for CR-PA (MIC90 = 4 mg/L) was typically greater. Cefiderocol's MIC readings from 0.03 to 1 mg/L, while its MIC50 and MIC90 levels were 0.2 and 0.5 mg/L, respectively, against 27 CR-PA isolates in the ARGONAUT-I research.[15] The MIC50 and MIC90 levels of cefiderocol were 0.1 and 1 mg/L, respectively, according to a research by Kazmierczak et al that enrolled 353 isolates of meropenem non susceptible PA obtained from North America and Europe as part of the SIDERO-WT-2014 monitoring project.[21] A multicentre evaluation compared cefiderocol disk diffusion (DD) with broth microdilution (BMD) on carbapenem-resistant Enterobacterales and non-fermenters. Categorical agreement ranged from 75–90%, with notably lower performance for Acinetobacter baumannii (high very-major error rate). This highlights limitations of DD when iron-depleted media are not used.[22]

There are various limitations to our investigation. First,

testing was done on a small sample size that was gathered from a particular area. Data regarding further species percentage from the Enterobacterales group was not collected. Also, we did not conduct a thorough analysis of the isolates' molecular epidemiology. Therefore, additional validation is needed to determine whether our findings are generalisable to other centres and locations where the prevalence and genotypic characteristics of distinct β-lactamase genes may differ.

Conclusion

To sum up, cefiderocol demonstrated strong antibacterial efficacy against CR-Gram-negative bacteria. The type of pathogen did not appear to have a substantial impact on this activity. Cefiderocol may therefore be a good option for treating patients whose infections are due to gram negative bacteria resistant to carbapenem.

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Authors' Contribution Statement

MZ contributed to the conception, design, acquisition, analysis, interpretation of data, drafting of the manuscript, critical review of the manuscript, and final approval of the version to be published. IUK contributed to the conception, design, acquisition, interpretation of data, drafting of the manuscript, and final approval of the version to be published. FF contributed to the conception, design, acquisition, drafting of the manuscript, critical review of the manuscript, and final approval of the version to be published. All authors are accountable for their work and ensure the accuracy and integrity of the study.

Conflict of Interest

Authors declared no conflict on interest

Grant Suppport and Financial Disclosure

None

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.