BPR MCS | Bulletin of Pioneering Researches of Medical and Clinical Science

Bulletin of Pioneering Researches of Medical and Clinical Science

Available online: https://bprmcs.com
2025 | Volume 4 | Issue 1 | Page: 117-125

Cefiderocol: A Potent Agent against Multidrug-Resistant Infections Facing Diagnostic Challenges in Routine Susceptibility Testing

Roberto Lozano[1], Laia Briones-Buixassa[2], Marta Llorente-Alonso[3*]

[1] Critical Care Department, Hospital Universitario La Paz, IdiPAZ, 28046 Madrid, Spain.
[2] Microbiology Department, Hospital Universitario La Paz, 28046 Madrid, Spain.
[3]Internal Medicine Department, Infectious Diseases Unit, Hospital Universitario La Paz, CIBERINFEC, IdiPAZ, 28046 Madrid, Spain.

Abstract

Cefiderocol is a novel cephalosporin–siderophore conjugate antibiotic with significant potential in combating infections caused by multidrug-resistant (MDR) Gram-negative bacilli. Its antibacterial activity remains largely unaffected by most β-lactamases, including metallo-β-lactamases, and—due to its siderophore-mediated uptake—it demonstrates reduced susceptibility to resistance mechanisms such as porin loss or active efflux, compared to other β-lactam agents. This study aimed to evaluate the *in vitro* susceptibility of carbapenemase-producing Gram-negative bacilli isolated from hospitalized patients to cefiderocol. A total of 102 clinical isolates of carbapenemase-producing *Enterobacterales* and non-fermenting Gram-negative bacteria were collected from hospitals in Łódź, Poland. Antimicrobial susceptibility to cefiderocol was determined using minimum inhibitory concentration (MIC) test strips and disc diffusion methods. The findings were inconclusive, as the presence of a technical uncertainty zone made the interpretation of results challenging. The high cost of cefiderocol therapy and the difficulties associated with interpreting susceptibility results currently limit its clinical application. Further research is needed to establish standardized, reliable, and widely accessible methods for determining cefiderocol susceptibility.

Keywords: Cefiderocol, Multidrug resistance, *Enterobacterales*, Pseudomonas spp., *Acinetobacter* spp.

Corresponding author: Marta Llorente-Alonso
E-mail: Marta.lalonso@outlook.com

Received: 18 March 2025
Revised: 14 June 2025
Accepted: 16 June 2025

How to Cite This Article: Lozano R, Briones-Buixassa L, Llorente-Alonso M. Cefiderocol: A Potent Agent against Multidrug-Resistant Infections Facing Diagnostic Challenges in Routine Susceptibility Testing. Bull Pioneer Res Med Clin Sci. 2025;4(1):117-25. https://doi.org/10.51847/lxcS7wl61g

Introduction

Cefiderocol is a cephalosporin antibiotic uniquely linked to a siderophore moiety, enabling it to exploit bacterial iron transport systems for cell entry. Unlike conventional β-lactams, which rely primarily on passive diffusion through porin channels, cefiderocol actively chelates extracellular ferric iron and is transported into the periplasmic space of Gram-negative bacteria. Once inside, it binds to penicillin-binding proteins (PBPs), disrupting peptidoglycan synthesis and leading to bacterial cell death. Resistance to cefiderocol can arise through various mechanisms, including PBP mutations, β-lactamases capable of hydrolyzing the drug, alterations in iron acquisition pathways, mutations in siderophore transport proteins, and overproduction of native siderophores. Laboratory studies show that cefiderocol retains activity

against most β-lactamase-producing pathogens, including those harboring metallo-β-lactamases, and is less affected by porin loss or efflux pump activity than other β-lactams [1, 2].

Cefiderocol is recommended for infections caused by aerobic Gram-negative bacteria in adults with limited treatment options, under the supervision of an infectious disease specialist. Its activity against Gram-positive and anaerobic bacteria is negligible due to intrinsic resistance [3]. Marketed as Fetroja® or Fetcroja®, it is approved in the EU and USA for complicated urinary tract infections caused by *Enterobacterales*, infections due to *Pseudomonas aeruginosa* when therapeutic options are limited, and for hospital-acquired or ventilator-associated pneumonia caused by *Enterobacterales*, *P. aeruginosa*, or *Acinetobacter baumannii* complex. The safety and efficacy of cefiderocol in pediatric populations remain unestablished [3].

Performing antimicrobial susceptibility testing (AST) for cefiderocol is challenging, primarily because its antibacterial activity is influenced by iron availability in the culture medium, and current interpretive standards differ among CLSI, FDA, and EUCAST guidelines [4–6]. Cefiderocol exhibits potent activity against a wide range of multidrug-resistant Gram-negative pathogens, including both Enterobacterales and non-fermenting bacteria [7]. By leveraging iron transport systems, it bypasses common resistance mechanisms and selectively inhibits PBP3, preventing proper cross-linking of peptidoglycan and inducing bacterial death [8]. Consequently, careful consideration of iron concentration is crucial during *in vitro* susceptibility testing [9].

In Poland, data on cefiderocol susceptibility among carbapenemase-producing Enterobacterales (CPE) are limited. This study therefore aimed to evaluate the *in vitro* susceptibility of clinical carbapenemase-producing Gram-negative bacilli, including both Enterobacterales and non-fermenters, isolated from hospitalized patients [10].

Materials and Methods

A total of 102 carbapenemase-producing isolates—including KPC, metallo-β-lactamase (MBL), and OXA-48 producers—were analyzed. Specimens were obtained from diverse clinical sources, including blood, bronchial alveolar lavage (BAL), other lower respiratory tract samples, urine, surgical swabs, nasal swabs, wound swabs, pressure ulcer swabs, and rectal swabs collected for CPE screening.

Isolates were preserved in Viabank™ beads (Medical Wire and Equipment, Corsham, UK) at −80 °C for up to six months. Prior to testing, bacteria were cultured on Columbia Agar supplemented with 5% sheep blood (Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C for 18–24 h. Susceptibility to cefiderocol was determined using both 30 µg disk diffusion (DD) and minimum inhibitory concentration (MIC) test strips (MTS) (Liofilchem, Italy) with standardized inocula on Mueller-Hinton Agar (Thermo Fisher Scientific) following EUCAST guidelines [6]. DD zones were read visually at full inhibition from a distance of ~30 cm, ignoring colonies within the inhibition zone [11, 12]. Reference strains *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were included as quality controls [13].

Carbapenemase production was assessed using the carbapenem inactivation method (CIM) [14] and confirmed phenotypically according to EUCAST 2024 [6] and the Polish National Reference Centre for Microbial Susceptibility (KORLD) [15]. The presence of carbapenemase genes—including KPC, OXA-48, NDM, and VIM—was confirmed via PCR at KORLD. Detection of the GES gene was performed at the Department of Microbiology and Laboratory Medical Immunology, Medical University of Łódź, with positive control strains previously validated at KORLD. Genomic DNA was extracted using the Genomic Mini AX Bacteria Spin kit (A&A Biotechnology, Gdansk, Poland) and amplified using HS PCR Kit 1 (A&A Biotechnology). PCR products were analyzed by electrophoresis on 2% agarose gels using the GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific) as a molecular weight marker.

Descriptive statistical analyses were conducted using Microsoft Excel 2019 (Microsoft Corporation, Redmond, USA).

Table 1. Cefiderocol clinical breakpoint for Enterobacterales and non-fermenting bacilli (following the 2024 EUCAST guidelines [5])

Organism	MIC Breakpoint (mg/L)	Disk Diffusion Zone (mm)	Area of Technical Uncertainty (ATU)
	Susceptible (S) ≤	Resistant (R) >	Susceptible (S) ≥
Enterobacterales	2	2	23
Pseudomonas aeruginosa	2	2	22
Stenotrophomonas maltophilia	–	–	20[a]
Acinetobacter spp.	–	–	17[b]

Abbreviations: S – susceptible, R – resistant, ATU – area of technical uncertainty, MIC – minimum inhibitory concentration, PK–PD - pharmacokinetic–pharmacodynamic.

aZone diameters of ≥20 mm for the cefiderocol 30 µg disk correspond to MIC values below the PK-PD breakpoint of S ≤ 2 mg/L.

bZone diameters of ≥17 mm for the cefiderocol 30 µg disk correspond to MIC values below the PK-PD breakpoint of S ≤ 2 mg/L.

Ethical considerations

This study was conducted in accordance with the principles of the 1964 Helsinki Declaration and its subsequent revisions. Approval from a formal Bioethics Committee was not required, as the study utilized only

anonymized records, making identification of individual patients impossible. All bacterial isolates had been previously archived in our laboratory culture collection and were labeled with consecutive code numbers. Available clinical information was limited to patient sex, age, and the type of biological specimen from which each isolate was obtained.

Results

Among the 102 Gram-negative isolates tested, minimum inhibitory concentration (MIC) values determined by the MTS method indicated susceptibility to cefiderocol for all but one strain. The exception was a CIM-positive *Pseudomonas aeruginosa* isolate, which exhibited resistance (MIC = 4 mg/L). Detailed results are provided in **Table 2**.

Of the 58 metallo-β-lactamase (MBL)-positive isolates, six (including three NDM-positive strains) were resistant according to disk diffusion (DD), while 16 (including nine NDM-positive) showed inhibition zones within the area of technical uncertainty (ATU). Importantly, all MBL-positive isolates were susceptible when assessed by MTS. Among six OXA-48-positive isolates, two were resistant by DD and four fell within the ATU; however, all were susceptible according to MTS results. For the 11 KPC-positive isolates, four were DD-susceptible, and seven exhibited ATU results, yet all were susceptible using MTS. Similarly, of 35 GES-positive isolates, 25 were DD-susceptible and 10 showed ATU measurements, with full susceptibility confirmed via MTS. **Table 3** summarizes growth inhibition zone ranges, MIC ranges, and MIC50 and MIC90 values. **Figures 1 and 2** illustrate the *in vitro* activity of cefiderocol against the studied species.

All *Acinetobacter baumannii* isolates and approximately 87% of *P. aeruginosa* strains were classified as susceptible by DD. Resistance by DD was observed in three *Escherichia coli*, three *Klebsiella pneumoniae*, and one *P. aeruginosa* isolate. ATU results were predominantly associated with Enterobacterales isolates. **Figure 3** presents a visual summary of DD-based susceptibility outcomes.

Table 2. Cefiderocol susceptibility testing results for studied bacterial isolates using MIC test strips and DD methods.

Isolate No.	Organism	Carbapenemase Detected		MTS Method (MIC, mg/L)	DD Method (Zone, mm)
		CIM	MBL		OXA-48
1	Aeromonas sobria	+			
2	Escherichia coli	+			
3	Escherichia coli				
4	Escherichia coli				
5	Escherichia coli			+	
6	Escherichia coli			+	
7	Escherichia coli			+	
8	Escherichia coli			+	
9	Klebsiella pneumoniae			+	
10	Klebsiella pneumoniae			+	+
11	Klebsiella pneumoniae			+	
12	Klebsiella pneumoniae			+	
13	Klebsiella pneumoniae			+	
14	Klebsiella pneumoniae	+			
15	Klebsiella pneumoniae			+	
16	Klebsiella pneumoniae			+	
17	Klebsiella pneumoniae			+	
18	Klebsiella pneumoniae			+	
19	Klebsiella pneumoniae			+	
20	Klebsiella pneumoniae			+	+
21	Klebsiella pneumoniae			+	
22	Klebsiella pneumoniae	+			
23	Klebsiella pneumoniae			+	
24	Klebsiella pneumoniae				
25	Klebsiella pneumoniae			+	
26	Klebsiella pneumoniae			+	
27	Klebsiella pneumoniae			+	+
28	Klebsiella pneumoniae			+	
29	Klebsiella pneumoniae			+	
30	Klebsiella pneumoniae			+	
31	Klebsiella pneumoniae			+	
32	Klebsiella pneumoniae			+	
33	Klebsiella pneumoniae				
34	Klebsiella pneumoniae				
35	Klebsiella pneumoniae				
36	Klebsiella pneumoniae			+	
37	Klebsiella pneumoniae			+	+
38	Klebsiella pneumoniae			+	

39	Klebsiella pneumoniae			+	+
40	Klebsiella pneumoniae			+	+
41	Klebsiella pneumoniae			+	
42	Klebsiella pneumoniae				
43	Klebsiella pneumoniae				
44	Klebsiella pneumoniae			+	
45	Klebsiella pneumoniae			+	
46	Klebsiella pneumoniae			+	
47	Klebsiella pneumoniae			+	
48	Klebsiella pneumoniae			+	
49	Klebsiella pneumoniae			+	
50	Klebsiella pneumoniae			+	
51	Klebsiella pneumoniae			+	
52	Klebsiella pneumoniae			+	
53	Klebsiella pneumoniae			+	
54	Klebsiella pneumoniae				
55	Klebsiella pneumoniae			+	
56	Klebsiella pneumoniae			+	
57	Klebsiella pneumoniae			+	
58	Klebsiella pneumoniae			+	
59	Klebsiella variicola			+	
60	Pseudomonas aeruginosa	+			
61	Pseudomonas aeruginosa			+	
62	Pseudomonas aeruginosa			+	
63	Pseudomonas aeruginosa			+	
64	Pseudomonas aeruginosa			+	
65	Pseudomonas aeruginosa	+			
66	Pseudomonas aeruginosa	+			
67	Pseudomonas aeruginosa	+			
68	Pseudomonas aeruginosa			+	
69	Pseudomonas aeruginosa	+			
70	Pseudomonas aeruginosa			+	
71	Pseudomonas aeruginosa			+	
72	Pseudomonas aeruginosa			+	
73	Pseudomonas aeruginosa			+	
74	Pseudomonas aeruginosa			+	
75	Pseudomonas alcaligenes			+	
76	Pseudomonas putida	+			
77	Acinetobacter baumannii	+			
78	Acinetobacter baumannii	+			
79	Acinetobacter baumannii	+			
80	Acinetobacter baumannii	+			
81	Acinetobacter baumannii	+			
82	Acinetobacter baumannii	+			
83	Acinetobacter baumannii				
84	Acinetobacter baumannii				
85	Acinetobacter baumannii				
86	Acinetobacter baumannii	+			
87	Acinetobacter baumannii				
88	Acinetobacter baumannii				
89	Acinetobacter baumannii				
90	Acinetobacter baumannii				
91	Acinetobacter baumannii				
92	Acinetobacter baumannii			+	
93	Acinetobacter baumannii	+			
94	Acinetobacter baumannii	+			
95	Acinetobacter baumannii	+			
96	Acinetobacter baumannii	+			
97	Acinetobacter baumannii	+			
98	Acinetobacter baumannii	+			
99	Acinetobacter baumannii	+			
100	Acinetobacter baumannii	+			
101	Acinetobacter baumannii	+			
102	Acinetobacter baumannii				
QC1	ATCC 25922 (E. coli)				
QC2	ATCC 27853 (P. aeruginosa)				

Notes:

- CIM: Carbapenem Inactivation Method
- MBL: Metallo-β-lactamase

- OXA-48: OXA-48-type carbapenemase
- KPC: Klebsiella pneumoniae carbapenemase
- GES: Guiana Extended-Spectrum β-lactamase
- MTS Method: Minimum Inhibitory Concentration (MIC) in mg/L
- DD Method: Disk Diffusion method, zone diameter in mm
- "+": Indicates detection of the specified carbapenemase
- Blank cells: No carbapenemase detected for that type
- QC1 and QC2: Quality control strains (ATCC 25922 for E. coli and ATCC 27853 for P. aeruginosa)

Abbreviations: *Aero.* – Aeromonas; *Esch.* – Escherichia; *Kleb.* – Klebsiella; *Pseud.* – Pseudomonas; *Acin.* – Acinetobacter; MIC – minimum inhibitory concentration; MTS – MIC test strips; DD – disk diffusion; CIM – carbapenem inactivation method; MBL – metallo-β-lactamase; OXA-48 – oxacillinase-48; KPC – Klebsiella *pneumoniae* carbapenemase; GES – Guiana Extended-Spectrum.

Table 3. Antimicrobial *in vitro* activity of cefiderocol against carbapenemase-producing species depending on the method used

	DD method		MTS method			
Resistance mechanism	**Growth inhibition zone range [mm]**	**Susceptible**	**MIC_{50} [mg/L]**	**MIC_{90} [mg/L]**	**MIC range [mg/L]**	***Susceptible***
CIM	10–28	94%	0.25	0.38	0.016–4	97%
MBL	10–28	62%	0.125	1	≤0.016–2	100%
OXA-48	10–22	0%	0.5	1	0.023–1	100%
KPC	19–23	36%	0.064	0.19	≤0.016-0.25	100%
GES	19–28	71%	0.125	0.25	0.016-1.5	100%
NDM	10–27	56%	0.125	1	0.047-1.5	100%

Abbreviations: DD – disk diffusion; MIC – minimum inhibitory concentration; MIC_{50} – MIC required to inhibit the growth of 50% of bacteria; MIC_{90} – MIC required to inhibit the growth of 90% of bacteria; CIM – carbapenem inactivation method; MBL – metallo-β-lactamase; OXA-48 – oxacillinase-48; KPC – *Klebsiella pneumoniae* carbapenemase; GES – Guiana extended-spectrum; NDM – New Delhi metallo-β-lactamase.

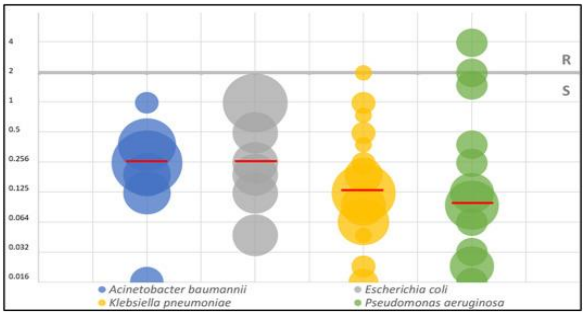

Figure 1. Antimicrobial *in vitro* activity of cefiderocol against carbapenemase-producing bacteria depending on species – MIC test strip method. The size of the bubble depends on the percentage of strains with a given MIC value, the grey line indicates the breakpoint between susceptible-resistant, the red lines indicate the average MIC values.

Abbreviations: R – resistant; S – susceptible.

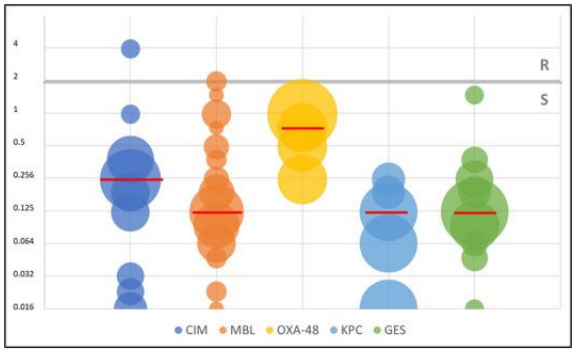

Figure 2. Antimicrobial *in vitro* activity of cefiderocol against carbapenemase-producing bacteria depending on resistance mechanism – MIC test strip method. The size of the bubble depends on the percentage of strains with a given MIC value, the grey line indicates the breakpoint between susceptible-resistant, the red lines indicate the average MIC values.

Abbreviations: KPC – Klebsiella pneumoniae carbapenemase; OXA-48 – oxacillinase-48; GES – Guiana extended-spectrum; MBL – metallo-β-lactamase; CIM – carbapenem inactivation method; R – resistant; S – susceptible.

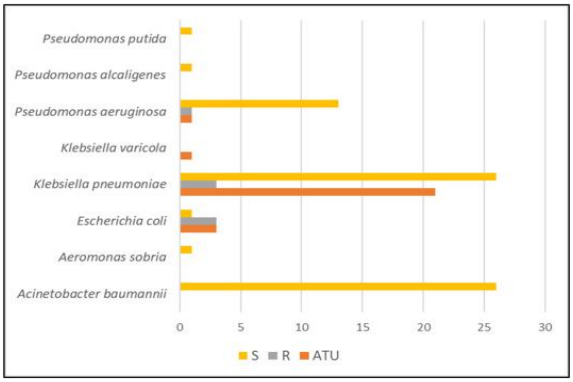

Figure 3. Susceptibility interpretations of antimicrobial *in vitro* activity of cefiderocol against carbapenemase-producing bacteria depending on resistance mechanism – disk diffusion method

Abbreviations: R – resistant; S – susceptible; ATU – area of technical uncertainty

Discussion

In 2017, the World Health Organization highlighted the urgent need for new antimicrobials targeting multidrug-resistant Gram-negative bacteria, particularly

carbapenemase-producing Enterobacterales (CPE) [16]. Since then, several broad-spectrum agents have been approved by the European Medicines Agency (EMA), including novel carbapenem–β-lactamase inhibitor combinations—relebactam/imipenem and vaborbactam/meropenem—designed to combat certain carbapenemase types. Cefiderocol, a siderophore-conjugated cephalosporin, represents a distinct class with a unique mechanism of bacterial entry, enabling it to evade carbapenemase-mediated hydrolysis [17, 18].

Antimicrobial susceptibility testing (AST) of cefiderocol remains challenging due to its dependence on iron-depleted conditions for optimal activity and the differing interpretive criteria established by CLSI and EUCAST [1, 4, 6, 19]. Matuschek *et al.* [20] demonstrated that disk diffusion (DD) is generally reliable for cefiderocol, with quality control data indicating reproducibility. However, results falling within the area of technical uncertainty (ATU) require careful consideration, as they may be retested, assessed with alternative methods, or reported as "unreliable." Currently, EUCAST defines ATU zones only for Enterobacterales (21–23 mm) and P. aeruginosa (20–21 mm) [6]. Failure to retest ATU isolates risks misclassifying susceptible strains (MIC ≤2 mg/L) as resistant, which could limit therapeutic options, particularly for patients intolerant to polymyxin-based regimens [1].

Cefiderocol overcomes common β-lactam resistance mechanisms, including porin loss, efflux pump overexpression, and β-lactamase production. Nonetheless, high MICs (128 to >256 mg/L) have been reported in *A. baumannii*, *Enterobacter cloacae*, *Proteus mirabilis*, *Providencia rettgeri*, and *Morganella morganii* [21, 22]. Surveillance programs such as SIDERO-WT have noted elevated cefiderocol MICs (>4 mg/L) in a minority of isolates, particularly *A. baumannii* and certain Enterobacterales [23, 24].

Bianco *et al.* [25, 26] evaluated broth microdilution and DD methods for cefiderocol and found high concordance in detecting resistant isolates (~95–96%), but a substantial proportion of Enterobacterales (37.8%) and *P. aeruginosa* (40%) fell within ATU, supporting the combined use of both methods in routine laboratories. Reports of cefiderocol resistance are emerging; Wang *et al.* [27] identified 30 resistant isolates among 1,158 carbapenemase-producing strains, most notably NDM-positive *E. coli*. Similarly, Nurjadi *et al.* [28] and Isler *et al.* [29] highlighted the role of NDM in facilitating cefiderocol resistance, underscoring the importance of resistance monitoring.

Resistance in *A. baumannii* has been linked to ESBLs such as PER and VEB, whereas in *E. cloacae* and *K. pneumoniae*, NDM is implicated [9]. Importantly, the mere presence of these enzymes does not guarantee resistance, as some isolates remain susceptible. Mutations affecting iron transport systems (e.g., *pvdS*, *fecI*) have been associated with cefiderocol resistance in *P. aeruginosa*, while elevated MICs have also been reported for *Stenotrophomonas maltophilia* [30]. In our study, one carbapenemase-producing *P. aeruginosa* isolate demonstrated both quantitative (MIC = 4 mg/L) and qualitative (DD inhibition zone = 10 mm) resistance.

Our findings also highlight limitations of the MTS strips, which underestimated MICs for Enterobacterales, while DD correctly categorized 81.7% of isolates in a study of 827 carbapenem-resistant strains [31]. Mutational resistance mechanisms include alterations in siderophore synthesis and regulation, iron uptake, two-component systems, and PBPs [7]. Structural changes in AmpC β-lactamase (e.g., R2 loop deletion in *E. cloacae*) can reduce susceptibility to both cefiderocol and ceftazidime-avibactam [32], suggesting that further molecular studies on our isolates could elucidate resistance mechanisms.

In line with Zalas-Więcek *et al.* [10], our study confirms that MTS testing identifies nearly all MDR isolates as susceptible, while DD often classifies a substantial proportion as resistant or within ATU. For *E. coli*, MTS showed full susceptibility (MIC <0.047–1 mg/L), whereas DD categorized ~43% as resistant, 43% as ATU, and only ~14% as susceptible. A similar trend was observed for *K. pneumoniae*, where MTS confirmed universal susceptibility (MIC <0.016–2 mg/L), while DD indicated 6% resistance, 42% ATU, and 52% susceptibility. These findings underscore the importance of method selection and careful interpretation when evaluating cefiderocol activity in MDR Gram-negative pathogens.

Discrepancies in susceptibility testing

Interpretation of cefiderocol susceptibility across different methods—broth microdilution, MTS, and disk diffusion (DD)—is currently under review by EUCAST [33]. In August 2022, it was reported that commercially available MTS strips do not reliably ensure accuracy or reproducibility, complicating the interpretation of results for isolates within the area of technical uncertainty (ATU). Given these limitations and the critical need to test multidrug-resistant bacteria, EUCAST recommends using microdilution and DD methods until confirmatory MIC results can be obtained.

Laboratories are advised to begin testing cefiderocol using the DD method. With rising β-lactam resistance and limited therapeutic options, properly performed DD assays—using high-quality materials and adhering to quality control guidelines—can provide reliable predictions of susceptibility, even for isolates in the ATU. Template zone diameter distributions for relevant species allow laboratories to calibrate internal results [34]. When alternative interpretive methods are unavailable, EUCAST advises that ATU results can be interpreted using the

standard breakpoint diameter cutoffs [6]. As with all AST methods, outcomes are dependent on the quality of reagents and procedural rigor. The use of MTS strips is not recommended, as they tend to underestimate MIC values; DD should be preferred for initial screening [35].

Study limitations

This study included 102 isolates, a relatively small sample size. However, the strains were collected from multiple hospital centers in a large urban area in central Poland, and carbapenem-resistant isolates are uncommon in routine clinical practice. Some species were represented by only a few isolates, so antimicrobial susceptibility analysis focused on the four most frequently identified species.

To avoid duplication and ensure isolate uniqueness, only the first isolate from each patient with a distinct resistance profile was included. Clonality of the isolates was not verified due to logistical constraints. Additionally, the MIC test strips used were designed specifically for *P. aeruginosa*, limiting their reliability for other species. While DD provides useful screening data, confirmatory microdilution testing remains necessary for most CPE strains [31]. Importantly, the reference standard for antimicrobial susceptibility testing—the microdilution method—was not employed in this study.

Conclusions

This study demonstrates that carbapenemase-producing Gram-negative bacilli remain largely susceptible to cefiderocol *in vitro*. The data confirm its potential as a potent treatment option against a broad range of multidrug-resistant pathogens, although susceptibility outcomes varied depending on the testing method. Discrepancies between DD and MTS methods underscore the need for standardized susceptibility testing protocols for cefiderocol.

Continuous surveillance of antimicrobial susceptibility is essential to preserve cefiderocol's clinical effectiveness, as resistance may emerge over time. Overall, these findings provide important insights into the utility of cefiderocol for treating infections caused by carbapenem-resistant *Enterobacterales* and non-fermenters. While cefiderocol represents a promising therapeutic alternative amid rising antibiotic resistance, careful implementation of susceptibility testing and further research are needed to optimize its clinical use.

Acknowledgments: None.

Conflict of interest: None.

Financial support: None.

Ethics statement: None.

References

1. Simner PJ, Patel R. Cefiderocol antimicrobial susceptibility testing considerations: the achilles' heel of the trojan horse? J Clin Microbiol 2020;59:e00951.
2. Sato T, Yamawaki K. Cefiderocol: discovery, chemistry, and in vivo profiles of a novel siderophore cephalosporin. Clin Infect Dis 2019;69:S538–43.
3. European Medicines Agency. Fetcroja 2022. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/fetcroja. [Accessed 12 January 2024].
4. Clinical and Laboratory Standards Institute. In: M100: performance standards for antimicrobial susceptibility testing. 34rd ed. 2024 Available from: https://em100.edaptivedocs.net/Login.aspx?_ga¼2.110071623.370038418.1716067494-667 485923.1716067494. [Accessed 18 May 2024].
5. U.S. Food and Drug Administration. Cefiderocol injection FDA identified breakpoints. Available from: https://www.fda.gov/drugs/development-resources/ cefiderocol-injection. [Accessed 18 May 2024].
6. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, ver. 14.0. 2024. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf. [Accessed 18 May 2024].
7. Zhanel GG, Golden AR, Zelenitsky S, Wiebe K, Lawrence CK, Adam HJ, et al. Cefiderocol: a siderophore cephalosporin with activity against carbapenem- resistant and multidrug-resistant gram-negative bacilli. Drugs 2019;79:271–89.
8. Page MGP. The role of iron and siderophores in infection, and the development of siderophore antibiotics. Clin Infect Dis 2019;69:S529–37.
9. Yamano Y. In vitro activity of cefiderocol against a broad range of clinically important gram-negative bacteria. Clin Infect Dis 2019;69:S544–51.
10. Zalas-Więcek P, Płachta K, Gospodarek-Komkowska E. Cefiderocol against multi- drug and extensively drug-resistant Escherichia coli: an in vitro study in Poland. Pathogens 2022;11:1508.
11. European Committee on Antimicrobial Susceptibility Testing. Antimicrobial susceptibility testing – EUCAST disk diffusion method, ver. 12.0. 2024. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2024_manuals/

Manual_v_12.0_EUCAST_Disk_Test_2024.pdf. [Accessed 12 April 2024].
12. European Committee on Antimicrobial Susceptibility Testing. Reading guide – EUCAST disk diffusion method for antimicrobial susceptibility testing, ver. 10.0. 2023. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2024_manuals/Manual_v_12.0_EUCAST_Disk_T est_2024.pdf. [Accessed 12 April 2024].
13. European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST, ver. 14.0. 2024. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/QC/v_14.0_EUCAST_QC_tables_routine_and_extended_QC.pdf. [Accessed 12 January 2024].
14. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PLoS One 2015 Mar 23;10(3):e0123690. https://doi.org/10.1371/ journal.pone.0123690.
15. Krajowy O´srodek Referencyjny ds. Lekowraz_liwo´sci Drobnoustrojo´w. Wykrywanie karbapenemaz–zalecenia KORLD. 2017. Available online: https://korld.nil.gov.pl/pdf/Wykrywanie_karbapenemaz-zalecenia_2017_strona.pdf. [Accessed 12 January 2024].
16. Wu JY, Srinivas P, Pogue JM. Cefiderocol: a novel agent for the management of multidrug-resistant gram-negative organisms. Infect Dis Ther 2020;9:17–40.
17. Jung E, Gademann K. Clinically approved antibiotics from 2010 to 2022. Chimia 2023;77(4):230–4.
18. Athans V, Neuner EA, Hassouna H, Richter SS, Keller G, Castanheira M, et al. Meropenem-vaborbactam as salvage therapy for ceftazidime-avibactam-resistant Klebsiella pneumoniae bacteremia and abscess in a liver transplant recipient. Antimicrob Agents Chemother 2018;63:e01551.
19. Morris CP, Bergman Y, Tekle T, Fissel JA, Tamma PD, Simner PJ. Cefiderocol antimicrobial susceptibility testing against multidrug-resistant gram-negative bacilli: a comparison of disk diffusion to broth microdilution. J Clin Microbiol 2020; 59:e01649.
20. Matuschek E, Longshaw C, Takemura M, Yamano Y, Kahlmeter G. Cefiderocol: EUCAST criteria for disc diffusion and broth microdilution for antimicrobial susceptibility testing. J Antimicrob Chemother 2022;77:1662–9.
21. Karlowsky JA, Hackel MA, Tsuji M, Yamano Y, Echols R, Sahm DF. In vitro activity of cefiderocol, a siderophore cephalosporin, against gram-negative bacilli isolated by clinical laboratories in north America and europe in 2015-2016: sidero-WT- 2015. Int J Antimicrob Agents 2019;53:456–66.
22. U.S. Food and Drug Administration. Briefing document on cefiderocol injection indication. Available from: https://www.fda.gov/media/131703/download. [Accessed 12 January 2024].
23. Naas T, Lina G, Santerre Henriksen A, Longshaw C, Jehl F. *In vitro* activity of cefiderocol and comparators against isolates of Gram-negative pathogens from a range of infection sources: SIDERO-WT-2014-2018 studies in France, vol. 3. JAC Antimicrob Resist; 2021. p. dlab081 [published correction appears in JAC Antimicrob Resist. 2021; 3: dlab102].
24. Stracquadanio S, Torti E, Longshaw C, Henriksen AS, Stefani S. *In vitro* activity of cefiderocol and comparators against isolates of Gram-negative pathogens from a range of infection sources: SIDERO-WT-2014-2018 studies in Italy. J Glob Antimicrob Resist 2021;25:390–8.
25. Bianco G, Boattini M, Comini S, Gaibani P, Cavallo R, Costa C. Performance evaluation of Bruker UMIC® microdilution panel and disc diffusion to determine cefiderocol susceptibility in Enterobacterales, *Acinetobacter baumannii, Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Achromobacter xylosoxidans* and *Burkolderia species*. Eur J Clin Microbiol Infect Dis 2024;43(3):559–66.
26. Bianco G, Boattini M, Comini S, Banche G, Cavallo R, Costa C. Disc diffusion and ComASP® cefiderocol microdilution panel to overcome the challenge of cefiderocol susceptibility testing in clinical laboratory routine. Antibiotics (Basel) 2023;12(3): 604.
27. Wang Q, Jin L, Sun S, Yin Y, Wang R, Chen F, et al. Occurrence of high levels of cefiderocol resistance in carbapenem-resistant *Escherichia coli* before its approval in China: a report from China CRE-network. Microbiol Spectr 2022;10(3):e0267021.
28. Nurjadi D, Kocer K, Chanthalangsy Q, Klein S, Heeg K, Boutin S. New Delhi metallo- beta-lactamase facilitates the emergence of cefiderocol resistance in *Enterobacter cloacae*. Antimicrob Agents Chemother 2022;66(2):e0201121.
29. Isler B, Vatansever C, O€ zer B, Çınar G, Aslan AT, Falconer C, et al. Higher rates of cefiderocol

resistance among NDM producing *Klebsiella* bloodstream isolates applying EUCAST over CLSI breakpoints. Inf Disp 2023;55(9):607–13.

30. Ito A, Nishikawa T, Ishii R, Kuroiwa M, Ishioka Y, Kurihara N, Sakikawa I, Ota T, Rokushima M, Tsuji M, Sato T, Yamano Y. 696. Mechanism of Cefiderocol high MIC mutants obtained in non-clinical FoR studies. Open Forum Infect Dis 2018 Nov 26; 5(Suppl 1):S251. https://doi.org/10.1093/ofid/ofy210.703.
31. Bonnin RA, Emeraud C, Jousset AB, Naas T, Dortet L. Comparison of disk diffusion, MIC test strip and broth microdilution methods for cefiderocol susceptibility testing on carbapenem-resistant enterobacterales. Clin Microbiol Infect 2022;28(8): 1156.e1–5.
32. Kawai A, McElheny CL, Iovleva A, Kline EG, Sluis-Cremer N, Shields RK, et al. Structural basis of reduced susceptibility to ceftazidime-avibactam and cefiderocol in Enterobacter cloacae due to AmpC R2 loop deletion. Antimicrob Agents Chemother 2020;64:e00198.
33. European Committee on Antimicrobial Susceptibility Testing. Warnings concerning antimicrobial susceptibility testing products or procedures. Available from: https://www.eucast.org/ast-of-bacteria/warnings. [Accessed 12 January 2024].
34. European Committee on Antimicrobial Susceptibility Testing. Template zone diameter distributions for relevant species against which to calibrate in-house results. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2022_manuals/Cefiderocol_disk_diffusion_training.pdf. [Accessed 12 January 2024].
35. Jeannot K, Gaillot S, Triponney P, Portets S, Pourchet V, Fournier D, et al. Performance of the disc diffusion method, MTS gradient tests and two commercially available microdilution tests for the determination of cefiderocol susceptibility in acinetobacter spp. Microorganisms 2023;11(8):1971.