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RESEARCH

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Commercially available tests for determining cefiderocol susceptibility display variable performance in the *Achromobacter* genus

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Abstract

Background Cefiderocol is a siderophore-conjugated cephalosporin increasingly used in the management of *Achromobacter* infections. Testing for cefiderocol susceptibility is challenging with distinct recommendations depending on the pathogens.

Objectives We evaluated the performance of commercial tests for testing cefiderocol susceptibility in the *Achromobacter* genus and reviewed the literature.

Methods Diffusion (disks, MIC gradient test strips [MTS], Liofilchem) and broth microdilution (BMD) methods (ComASP™, Liofilchem; UMIC®, Bruker) were compared with the BMD reference method according to the EUCAST guidelines on 143 *Achromobacter* strains from 14 species with $MIC_{50/90}$ of ≤ 0.015/0.5 mg/L. A literature search was conducted regardless of method or species.

Results None of the methods tested fulfilled an acceptable essential agreement (EA). MTS displayed the lowest EA (30.8%) after UMIC® (49%) and ComASP™ (76.9%). All methods achieved an acceptable bias, with MICs either underestimated using MTS (-1.3%) and ComASP™ (-14.2%) or overestimated with UMIC® (+ 9.1%). Inhibition zone diameters ranged from 6 to 38 mm ($IZD_{50/90}$=33/30 mm). UMIC® and ComASP™ failed to categorize one or the two cefiderocol-resistant strains of this study as resistant unlike the diffusion-based methods. The literature review highlighted distinct performance of the available methods according to pathogens and testing conditions.

Conclusions The use of MTS is discouraged for *Achromobacter* spp. Disk diffusion can be used to screen for susceptible strains by setting a threshold diameter of 30 mm. UMIC® and ComASP™ should not be used as the sole method but have to be systematically associated with disk diffusion to detect the yet rarely described cefiderocol-resistant *Achromobacter* sp. strains.

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Highlights

- Performance of commercial methods are highly diverse and species-dependent.
- The use of MTS is discouraged due to low essential agreement.
- UMIC® and ComASP™ failed to detect one or the two cefiderocol-resistant strains.
- UMIC® or ComASP™ should not be used as the sole method for *Achromobacter* cefiderocol susceptibility testing.
- A threshold diameter of 30 mm is proposed for susceptible strain screening.

Keywords Cefiderocol, *Achromobacter*, Susceptibility testing, Microdilution, Disk, Diffusion

Introduction

Achromobacter spp. are recognized as opportunistic pathogens, predominantly infecting immunocompromised individuals and patients with cystic fibrosis (CF). These microorganisms are often considered as difficult-to-treat pathogens due to their intrinsic antimicrobial resistance and potential acquisition of additional resistances [1, 2]. Cefiderocol is a broad-spectrum siderophore cephalosporin increasingly used in the management of *Achromobacter* infections [2–5]. Considering studies which determined minimal inhibitory concentrations (MICs) using the broth microdilution (BMD) reference method according to the EUCAST (European committee on antimicrobial susceptibility testing) guidelines, cefiderocol was shown to have excellent in vitro activity against *Achromobacter* spp. In total, according to the EUCAST 2023 non-species-related breakpoint (2 mg/L), only 2.1% (13/606) of strains were resistant to cefiderocol [6–10] with MIC_{50} values ranging from ≤0.015 mg/L [6] to 0.5 mg/L [7], and MIC_{90} values ranging from 0.125 mg/L [8] to 1 mg/L [7].

However, the BMD reference method, which is based on iron-depleted-cation-adjusted Mueller-Hinton broth (ID-CAMHB), is complex, time-consuming, and therefore difficult to apply routinely in a clinical microbiology laboratory for in vitro susceptibility testing of cefiderocol [11]. Consequently, more widely accessible antimicrobial susceptibility testing (AST) methods have been developed for routine cefiderocol susceptibility tests, such as the disk diffusion method, cefiderocol-impregnated strips, both tested on regular Mueller-Hinton-agar (r-MHA), and several microdilution panels. To date, the recommendations for use of these different methods vary depending on the antibiogram committee or regulatory agency and the bacterial species under consideration, and so do the breakpoints applied for categorization (Table 1). In the absence of specific recommendations for *Achromobacter* spp., the most accurate method to be used for cefiderocol susceptibility testing of *Achromobacter* spp. strains can therefore not be inferred from the current recommendations published for other opportunistic pathogens and thus warranted a specific evaluation.

In this study, commercialized BMD methods, *i.e.*, the compact antimicrobial susceptibility panel (ComASP™) microdilution assay (Liofilchem, Roseto degli Abruzzi, Teramo, Italy) and UMIC® (Bruker Daltonics GmbH & Co. KG, Bremen, Germany), MIC gradient test strips (MTS) and disks (Liofilchem) were compared with the previous results we obtained with the BMD reference method on a collection of *Achromobacter* spp. clinical isolates accurately identified by *nrdA* gene-based analysis [6]. Our goal was to identify the most accurate commercial method for testing *Achromobacter* spp. Specifically, for disk diffusion, we aimed to determine an inhibition zone diameter (IZD) threshold that might be helpful for distinguishing susceptible *Achromobacter* strains from strains with abnormal susceptibility that would require further testing. Finally, we conducted a literature review to summarize the data on methods that displayed the best performance for cefiderocol susceptibility testing according to bacterial species and compared the results with those of the evaluation conducted on *Achromobacter* spp. herein.

Materials and methods

Bacterial isolates

Achromobacter spp. collection beyond study

A collection of 143 clinically-documented strains of *Achromobacter* spp., representing a subgroup of the 230 strains previously described by Jean-Pierre et al., was selected for this study [6] (Supplementary Table 1). The collection included 67 strains from 67 CF patients and 76 strains from 76 non-CF (NCF) patients. These strains were isolated between 2010 and 2023 during routine microbiological analysis of samples from patients attending CF centres (CRCM, Centre de Ressource et de Compétence de la Mucoviscidose) of the University Hospitals of Paris, Montpellier (France) and Aarhus (Denmark), and at the University Hospitals of Montpellier and Nîmes, and Hospital of Alès-Cévennes (France) for NCF patients. Most strains originated from the respiratory tract, 100% of CF strains (67/67) and 39.5% of NCF strains (30/76), followed by blood cultures (18.4% of NCF strains, 14/76) and ear-nose-throat samples (15.8% of NCF strains, 12/76) and various other sources including skin wounds and pus, biopsies, the digestive tract,

Table 1 Clinical breakpoints for cefiderocol published by antibiogram committees and/or regulatory agencies in 2024

Microorganism and non-species related PK/PD breakpoints	CA-SFM					EUCAST				CLSI				FDA			
	MIC, mg/L		IZD, mm[a]			MIC, mg/L		IZD, mm[a]			MIC, mg/L		IZD, mm[a]		MIC, mg/L		IZD, mm[a]	
	≤ S	> R	≥ S	< R	ATU	≤ S	> R	≥ S	< R	ATU	≤ S	> R	≥ S	< R	≤ S	> R	≥ S	< R
Enterobacterales	2	2	-	-	-	2	2	23	23	21–23	4	16	16	8	4	16	16	8
Pseudomonas aeruginosa	2	2	27	-	< 27	2	2	22	22	20–21	4	16	18	12	1	4	22	12
Acinetobacter baumannii complex	IE	IE	_[b]	_[b]	-	IE	IE	_[d]	_[d]	-	4	16	15	_[f]	1	4	19	11
Stenotrophomonas maltophilia	IE	IE	_[c]	_[c]	-	IE	IE	_[e]	_[e]	-	1	-	15[g]	-	-	-	-	-
PK/PD	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Abbreviations ATU: area of technical uncertainty; CA-SFM: Antibiogram Committee of the French Society of Microbiology; CLSI: Clinical & Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; FDA: Food and Drug Administration; IE: insufficient evidence; IZD: inhibition zone diameter; MIC: minimal inhibitory concentration; PK/PD: pharmacokinetics and pharmacodynamics; R: resistant; S: susceptible

[a] 30 µg disk content

[b] IZD of ≥ 22 mm for the cefiderocol 30 µg disk correspond to MIC values below the PK/PD breakpoint of S ≤ 2 mg/L

[c] IZD of ≥ 20 mm for the cefiderocol 30 µg disk correspond to MIC values below the PK/PD breakpoint of S ≤ 2 mg/L

[d] IZD of ≥ 17 mm for the cefiderocol 30 µg disk correspond to MIC values below the PK/PD breakpoint of S ≤ 2 mg/L

[e] IZD of ≥ 20 mm for the cefiderocol 30 µg disk correspond to MIC values below the PK/PD breakpoint of S ≤ 2 mg/L

[f] Disk diffusion diameters ≤ 14 mm should not be interpreted or reported because IZD ≤ 14 mm occur with resistant, intermediate and susceptible isolates. For isolates with IZD ≤ 14 mm, do not report cefiderocol without performing an MIC test

[g] Breakpoints are based on PK/PD properties, and limited clinical data

implantable devices, and eyes (26.3% of NCF strains, 20/76). These strains had been identified in a previous study by *nrdA* gene sequencing (765 bp), analysis, and phylogeny [12]. Strains beyond study were genetically diverse, displaying 50 alleles of the *nrdA* gene and were assigned to 14 species of the *Achromobacter* genus. *Achromobacter xylosoxidans* and 13 other species represented 65% (93/143) and 35% (50/143) of strains, respectively. Among the 143 strains, most isolates were susceptible to meropenem (MEM) (87.4%, 125/143), piperacillin-tazobactam (TZP) (86.7%, 124/143) and trimethoprim-sulfamethoxazole (SXT) (81.1%, 116/143), applying the IZD breakpoints of *A. xylosoxidans* to all *Achromobacter* species [13]. Reference cefiderocol MIC values were determined by the National Reference Centre for Antibiotic Resistance (Besançon, France) using an ID-CAMHB as described by Devoos et al. [14] and ranged from ≤0.015 to 16 mg/L ($MIC_{50/90}$ of 0.03/0.5 mg/L overall and 0.125/2 mg/L against MEM non-susceptible strains). In total, 141 strains (98.6%) were susceptible (MIC ≤ 2 mg/L) and two strains were resistant to cefiderocol (MIC > 2 mg/L) according to the EUCAST 2023 non-species-related breakpoint (Supplementary Table 1) [15]. All strains were stored frozen at -80 °C in glycerol Trypticase-soy broth.

Quality control strain

Quality control using *Pseudomonas aeruginosa* strain CIP 76110 (= ATCC 27853) was included in each series of BMD, ComASP™, and UMIC®, and five series of MTS and disk diffusion to ensure the validity of methods, checking that the results were within the specified range: 0.06 to 0.5 mg/L (for MIC determination) and 23 to 29 mm (for IZD determination) according to EUCAST recommendations [13].

Commercial AST of cefiderocol of *Achromobacter* spp.

All tests were performed from overnight cultures on Difco™ r-MHA plates (Becton Dickinson) at 35–37 °C for 18–24 h (unless additional incubation was required to achieve sufficient growth). A bacterial suspension adjusted to 0.5 McFarland in 0.9% sodium chloride solution was used for the four methods in this study. All tests were performed according to both the manufacturer's recommendations and the EUCAST guidelines, and results were compared with those of the BMD reference method. Clinical breakpoints used for cefiderocol results interpretation were EUCAST 2023 non-species-related pharmacokinetic/pharmacodynamic (PK/PD) values (susceptible strain: MIC ≤ 2 mg/L; resistant strain: MIC > 2 mg/L) [15]. At that point, no IZD breakpoints had been defined for *Achromobacter* spp. and cefiderocol. Both IZD and MIC values were determined separately by

two operators, and by a third operator if there was any disagreement.

Commercial diffusion methods: disk diffusion and MTS

Cefiderocol disk diffusion assays were performed with disks loaded at 30 µg (Liofilchem). Cefiderocol MTS assays (Liofilchem) generate a range of concentrations from 0.016 to 256 mg/L and are only validated and approved for *P. aeruginosa*. Both disk and MTS assays were tested on unsupplemented MH II agar plates (Liofilchem). Colonies within the inhibition zones were considered after excluding any possible contamination, according to the EUCAST recommendations [13].

Commercial BMD methods: ComASP™ and UMIC®

The ComASP™ microdilution assay (Liofilchem) is a two-test panel using an iron-depleted MH broth supplied in the kit, generating a range of concentrations from 0.008 to 128 mg/L. The UMIC® assay (Bruker Daltonics GmbH & Co. KG, Bremen, Germany) is a unit test that uses iron-depleted MH broth, which is not supplied in the kit, generating a range of concentrations from 0.03 to 32 mg/L. Both tests were used according to the manufacturer's instructions. Briefly, for ComASP™, the bacterial suspension was diluted to 1:20 and 0.4 mL of this suspension was used to inoculate ID-CAMHB (3.6 mL). Next, 100 µL were dispensed into each well followed by 16–20 h of incubation at 35–37 °C under aerobic conditions. For UMIC®, a volume of 25 µL of the bacterial suspension was used to inoculate ID-CAMHB (5 mL) and 100 µL were dispensed into each well of the strip followed by 18–24 h of incubation at 35–37 °C under aerobic conditions. For both commercial BMD methods, the MIC of cefiderocol was then read as the first well in which the growth reduction corresponded to a dot of < 1 mm or was replaced by the presence of light haze/faint turbidity according to the recommendations [11]. The positive control should show strong growth in the form of a dot of > 2 mm or heavy turbidity. If considerable growth was not observed in the growth control well, the panels were incubated for a further incubation period.

Literature review

A literature search was conducted using the PubMed database, with the last search date being June 18th, 2024. The search was performed using a combination of Mesh terms: "disk or disc" and/or "MTS" and/or "ComASP" and/or "UMIC" and/or "Sensititre" and "cefiderocol", and followed by manual selection of studies that had made a comparative analysis of the performance of different methods for cefiderocol susceptibility testing. Of the 49 studies that were identified and screened, 33 were excluded because they did not involve a comparison of the performance of a commercial test with a BMD method, and 16 were included in the review. The results were aggregated according to species or bacterial group and were then used to compare the results of our evaluation on *Achromobacter* spp.

Data analysis and interpretation

Essential agreement (EA), inferior and superior bias were calculated according to ISO 20776-2:2021 guidelines, with the BMD method and EUCAST 2023 PK/PD breakpoints as references [15, 16]. Percentages ≥ 90% for EA, and a difference for bias ± 30% were considered as acceptable [16]. These criteria were also those applied to evaluate the performance of studies retrieved from our literature review. The ISO 20776-2:2007 guidelines require 10% of resistant strains for the whole study before categorical agreement (CA), major errors (ME) and very major errors (VME) can be calculated [17]. The small number of resistant strains included in this study ($n=2$) and, more largely, the small number of cefiderocol-resistant *Achromobacter* strains reported in the literature thus avoided calculating CA, ME and VME in this genus. The generally acceptable criteria considered for evaluating the performance of the studies retrieved from our literature review were: CA > 90%, ME rate < 3% of the susceptible isolates tested, and a lower rate of VME < 1.5% of the resistant isolates according to the FDA.

Statistical analysis of discrepancies in MICs between commercial tests and the BMD reference method were performed using the Fisher test with GraphPad Prism (GraphPad Software, La Jolla, CA). A two-tailed *p-value* < 0.05 was chosen as being statistically significant.

Results

Commercial BMD methods: ComASP™ and UMIC®

The MIC values of cefiderocol for the quality control strain *P. aeruginosa* CIP 76110 with both BMD tests ($range_{ComASP™}$: 0.064–0.25 mg/L, $n=23$; $range_{UMIC®}$: 0.125-0.5 mg/L, $n=5$) were within the acceptable range although ComASP™ and UMIC® tended to result in MIC respectively lower and higher than the target values (Supplementary Fig. 1).

Compared with the BMD reference method, neither the ComASP™ nor the UMIC® methods achieved an acceptable 90% EA rate (EA = 76.9%, $n=110/143$, 95% CI [69.4–83.1] for ComASP™ and EA = 49%, $n=70/143$, 95% CI [40.9–57.1] for UMIC®) (Fig. 1; Table 2). EAs reached the 90% rate for both techniques only when a difference from the reference method of ± three 2-fold dilutions was considered (modified $EA_{ComASP™}$ = 97.2%, $n=139/143$, 95% CI [93–98.9] and modified $EA_{UMIC®}$ = 92.3%, $n=132/143$, 95% CI [86.7–95.6]). Although the ComASP™ test tended to provide lower MIC values, as illustrated by a negative bias (-14.2%), the UMIC® tended to provide higher MIC values (bias = + 9.1%), compared to the BMD reference

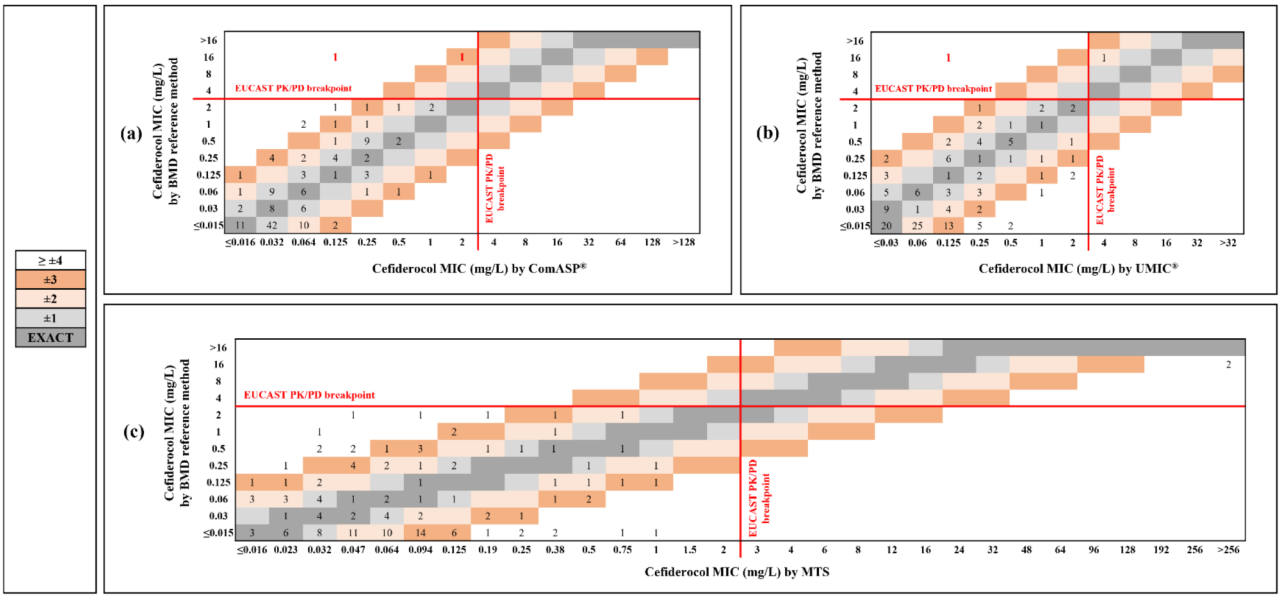

Fig. 1 Correlation between cefiderocol MICs determined by the reference BMD method and by commercial methods **(a)** ComASP™, **(b)** UMIC® and **(c)** MTS. MTS assays were performed on unsupplemented MH II agar plates (Liofilchem). The number of strains with MIC determined by the commercial method corresponding to the BMD method, ± one 2-fold dilution, ± two 2-fold dilution, ± three 2-fold dilution and ≥ ± four 2-fold dilution compared to the reference MIC, are highlighted in dark grey, light grey, light orange, dark orange and white areas, respectively. BMD: broth microdilution; MIC: minimal inhibitory concentration.

Table 2 Performance of ComASP™, UMIC®, Liofilchem MTS and disk tested on unsupplemented MH II agar plates (Liofilchem) for the determination of susceptibility to cefiderocol of 143 *Achromobacter* spp. clinical isolates according to EUCAST PK/PD breakpoint in comparison with BMD reference method

Method	MIC (mg/L) or IZD (mm) range	MIC_{50} (mg/L) or IZD_{50} (mm)	MIC_{90} (mg/L) or IZD_{90} (mm)	EA	Bias high	Bias low	Difference of biais	Number of resistant strains detected (*n* = 2)
BMD	≤ 0.015 to 16	0.03	0.5	-	-	-	-	-
ComASP™*	≤ 0.016 to 2	0.032	0.25	76.9 (110)	+46.1 (66/143)	-60.3 (47/78)	-14.2	0
UMIC®*	≤ 0.03 to 4	0.125	0.5	49 (70)	+47.6 (68/143)	-38.5 (30/78)	+9.1	1
MTS*	≤ 0.016 to > 256	0.064	0.38	30.8 (44)	+53.8 (77/143)	-55.1 (43/78)	-1.3	2
Disk diffusion**	39 to 6	33	30	-	-	-	-	2

BMD: broth microdilution; EA: essential agreement; IZD: inhibition zone diameter; MIC: minimal inhibitory concentration; -: not applicable

As there are no specific indications for this genus: *MIC were interpreted according to the EUCAST 2023 PK/PD breakpoints, **IZD were interpreted according to *P. aeruginosa*-specific breakpoints. In accordance with ISO 20776-2:2021, the acceptable rates are indicated in bold (EA ≥ 90%, and difference of bias low and high ± 30%)

method, as also observed for the quality control strain (Fig. 1; Table 2, Supplementary Fig. 1, Supplementary Table 2).

Of the two strains 2579 and 2463, classified as resistant to cefiderocol with MIC values of 16 mg/L using the BMD reference method, strain 2579 was wrongly categorized as susceptible with both ComASP™ and UMIC® with MIC values of 0.125 mg/L, far from the reference value, whereas strain 2463 was wrongly categorized as susceptible with ComASP™ alone, with an MIC of 2 mg/L, close to the EUCAST 2023 PK/PD breakpoint [15] (Supplementary Table 1).

Commercial diffusion methods: disk diffusion and MTS

Disk diffusion

IZDs for the *P. aeruginosa* quality control strain (range: 26–27 mm, *n* = 5) were all within the acceptable range (23–29 mm) and met the acceptability criteria (± 1 mm from the target value) defined by the EUCAST in its warning published in August 2022 [18].

IZD varied greatly for a same MIC: for strains with a reference MIC ≤ 0.015 mg/L (*n* = 65), IZD ranged from 27 mm to 38 mm overall ($IZD_{50/90}$ = 34/31 mm) and from 27 mm to 37 mm ($IZD_{50/90}$ = 30/27 mm) for strains with a reference MIC of 2 mg/L (*n* = 5), suggesting that the

disk diffusion method did not correlate very well with the BMD reference method (Supplementary Fig. 2). However, the only two strains resistant to cefiderocol with the BMD reference method (strains 2579 and 2463) both demonstrated no inhibition zone in the disk diffusion assay (6 mm), suggesting that the combination between the Liofilchem disk with Liofilchem r-MHA could detect cefiderocol-resistant *Achromobacter* strains. Unlike strain 2463, which showed homogeneous resistance (no visible inhibition zone), strain 2579 showed heterogeneous growth (double halo of growth with an outer IZD of 31 mm and no inner inhibition zone) (Supplementary Fig. 3). Strains with cefiderocol MICs below the PK/PD critical concentration value (≤2 mg/L) [15] had IZDs systematically ≥27 mm (range: 27 to 39 mm with no microcolonies in the IZD) around a disk loaded with 30 µg cefiderocol.

MTS

The MIC values for cefiderocol against the quality control strain *P. aeruginosa* (range: 0.19–0.5 mg/L, $n=5$) were within the acceptable range (Supplementary Fig. 1).

Compared with both the ComASP™ and UMIC® assays, MTS was the commercial method that gave the lowest percentage of EA (30.8%, $n=44/143$, 95% CI [23.8–38.8]) (Fig. 1; Table 2). Even when considering isolates with a difference from the reference method of up to ±three 2-fold dilutions, the modified EA (87.4%, $n=125/143$, 95% CI [81–91.9]) did not reach the 90% rate required to consider the method as acceptable, confirming MTS a poorly accurate technique for determining reliable cefiderocol MICs. However, unlike ComASP™ and UMIC®, this method successfully classified both cefiderocol-resistant strains with MICs>256 mg/L (Fig. 1; Table 2).

Analysis of discordant results between commercial tests according to strain and species

According to the BMD reference method, the two cefiderocol-resistant strains (strains 2579 and 2463) were both detected as resistant by the diffusion-based methods whereas they were erroneously categorized as susceptible by either one method (UMIC®, strain 2463) or both (ComASP™ and UMIC®, strain 2579) commercial BMD methods. Thus, the use of commercial BMD methods as the sole method for cefiderocol susceptibility testing has to be discouraged for *Achromobacter* spp. to avoid reporting false-susceptible results.

Distribution of the cefiderocol MICs or IZDs according to *Achromobacter* species is shown in Fig. 2. Analysis according to *Achromobacter* species showed that discrepancies in MICs (at least±two 2-fold dilutions) observed between both MTS or UMIC® commercial tests and the BMD reference method were dependent on the species under study, unlike with ComASP™ ($p\text{-}value_{MTS} < 0.001$, $p\text{-}value_{UMIC®} = 0.002$, $p\text{-}value_{ComASP™} = 0.3$) according to the Fisher test. ComASP™ was the commercial technique with the highest EA for *A. xylosoxidans* (EA=78.5%, $n=73/93$, 95% CI [69.1–85.6]) compared to both UMIC® (EA=41.9%, $n=39/93$, 95% CI [32.4–52.1]) and MTS (EA=23.7%, $n=22/93$, 95% CI [16.2–33.2]), which provided more discordant than concordant MIC results for that species (the most common species recovered from clinical samples worldwide). Note that *A. insolitus* was the only species represented by more than four strains in this study ($n=5$), with 100% EA whatever the commercial technique used.

Performance of methods in the literature

We found 16 comparative studies including one to four methods for cefiderocol susceptibility testing among disk diffusion, MIC strips, Sensititre EUMDROXF microplates, ComASP™ and UMIC®, mostly compared to the BMD reference method [10, 14, 19–32]. Eleven of these studies included a unique bacterial group [*Acinetobacter baumannii* complex ($n=6$), Enterobacterales ($n=3$), and *P. aeruginosa* ($n=2$)] whereas the five remaining studies included three to six bacterial groups, either multidrug-resistant (MDR), carbapenemase-producing or not, among Enterobacterales, *P. aeruginosa*, *A. baumannii* complex, *Stenotrophomonas maltophilia*, *Burkholderia* spp. and *Achromobacter* spp. (Supplementary Table 2). Depending on the method under evaluation, data were available for 38 to 342 Enterobacterales, for 3 to 150 *P. aeruginosa*, for 7 to 468 *A. baumannii* complex, for 10 to 30 *S. maltophilia*, for 30 *Burkholderia* spp. and 27 *Achromobacter* spp. strains. Performance of the different methods are presented in Supplementary Table 2 according to bacterial species or group.

Studies evaluating disk diffusion showed conflicting performance, which depended not only on the bacterial species under study but also on the combination disk/r-MHA selected [10, 14, 19–23, 26–32]. For some studies, the difference in performance also depended on the breakpoints used for categorization [27, 30, 31] (Supplementary Table 2). For Enterobacterales, CAs ranging from 77 to 100% were observed in the eight studies retrieved, and half of them showed insufficient performance with the disk diffusion method (CA<90%) to assess cefiderocol susceptibility. Only the results obtained with the Mast disks tested on r-MHA showed CA rates that were consistently ≥90% for Enterobacterales [10, 21]. For *P. aeruginosa* (six studies) and *A. baumannii* complex (ten studies), none of the disks tested provided CA rates consistently>90% for all studies. By contrast, studies evaluating the performance of cefiderocol disks on *S. maltophilia* (three studies, 11–30 strains) as well as the only study on a small number of *Burkholderia* spp. and *A.*

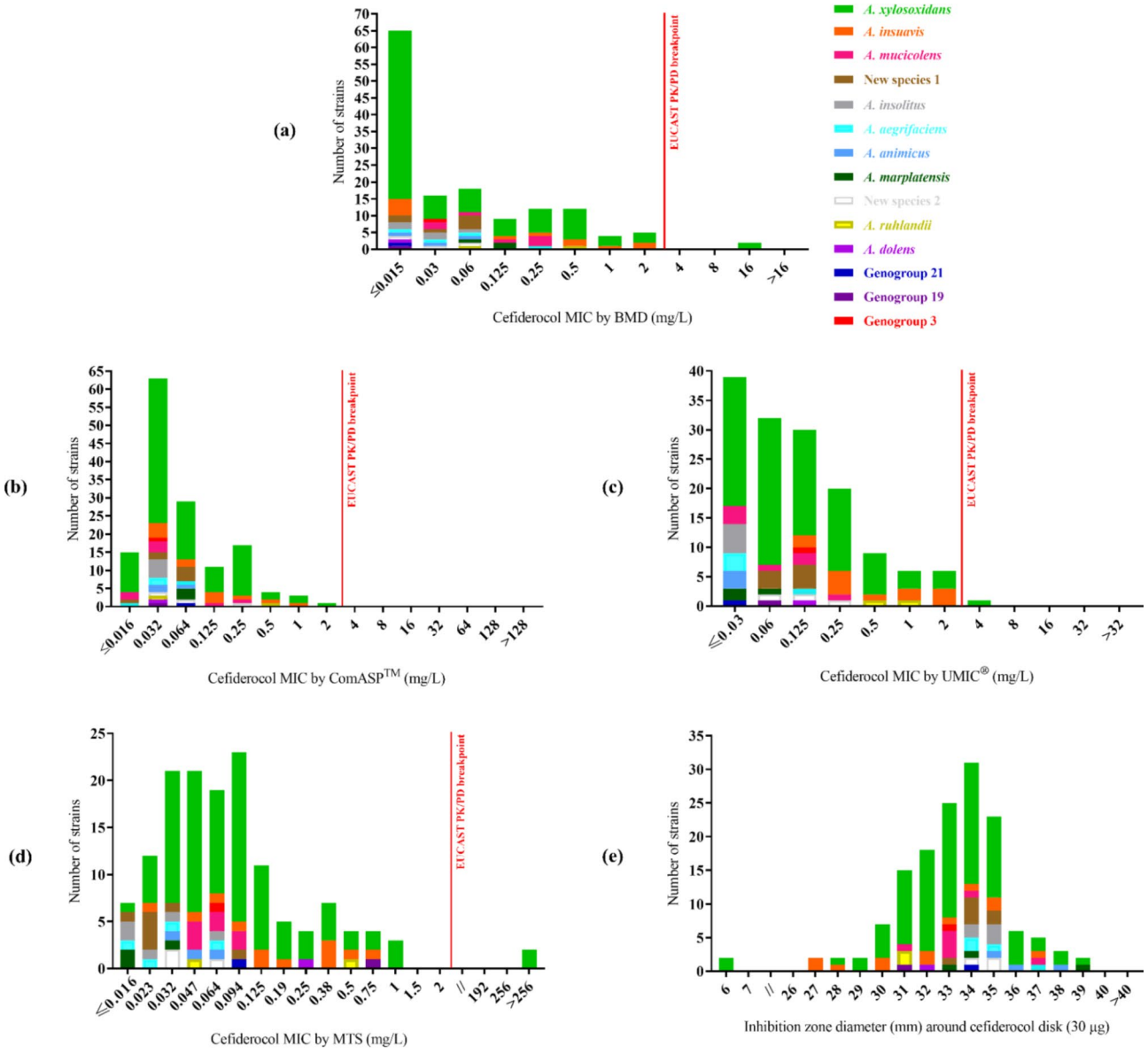

Fig. 2 Distribution of the cefiderocol MICs (mg/L) or IZDs (mm) determined by several methods for 143 *Achromobacter* strains according to species. **(a)** BMD reference method, **(b)** ComASP™, **(c)** UMIC®, **(d)** Liofilchem MTS, **(e)** Liofilchem disks. Disk and MTS assays were performed on unsupplemented MH II agar plates (Liofilchem). BMD: broth microdilution; IZD: inhibition zone diameter; MIC: minimal inhibitory concentration

xylosoxidans strains (30 and 27 strains, respectively) suggested that the disk method performed well (CA = 100%), irrespective of the disk manufacturer (Liofilchem, Oxoid - Thermo Fisher Scientific or Mast Diagnostic) [10, 20, 21].

Regarding the MTS gradient strip that was formulated for *P. aeruginosa* only, the four studies which had evaluated this method on bacteria other than *P. aeruginosa* found very poor performance with EA ranging from 6% [23] to 75% [30], high biases of up to -48.4% when calculated [30], and unacceptable VME rates confirming that MTS should be avoided for these bacteria (Supplementary Table 2). The use of MTS was also discouraged for *P. aeruginosa* showing insufficient performance (EA = 69.3% and bias = -30.4%) [14].

The Sensititre EUMDROXF microplate was evaluated in two studies comparing it with the BMD reference method. The EA and CA rates were higher for Enterobacterales than for *P. aeruginosa*, for which the method overestimated MIC values. However, both studies showed unacceptably high rates of VME [14, 23] (Supplementary Table 2).

ComASP™ has already been evaluated for Enterobacterales, *P. aeruginosa* and *A. baumannii* complex strains [20, 24, 29, 30, 32] either as first-line antimicrobial susceptibility method or as second-line on isolates that

presented an IZD within area of technical uncertainty (ATU) using the disk diffusion method. All species considered, performance of the test was not sufficient for use in clinical diagnostics according to the ISO 20776-2:2021 validation criteria (Supplementary Table 2). Indeed, ComASP™ never achieved 90% EA, ranging from a minimum of 29.6% for carbapenem-resistant *Acinetobacter* spp. ($n=27$) [32] to a maximum of 86.8% for carbapenemase-producing Enterobacterales ($n=38$) [20]. In all studies including more than 10 strains, unacceptable VME rates were also observed with this method despite the fact that CA values compared to the BMD reference method ranged from 74.1% for *Acinetobacter* spp. ($n=27$) [32] to 95.9% for the same bacterial genus ($n=97$) [29].

Finally, UMIC® appeared to perform better on *P. aeruginosa* than on Enterobacterales, with acceptable EA, CA and VME rates in the two studies reviewed, despite an unacceptable bias value in one of those two studies [10, 25] (Supplementary Table 2). Contrasting evaluation results have been published for other pathogens. The values of biases varied significantly from one study to another for Enterobacterales and *A. baumannii* complex (Supplementary Table 2) [10, 24, 25, 29]. The two studies on *S. maltophilia* or *Burkholderia* spp. found unacceptable EA and bias rates with an overestimation of the MIC when calculated, but CA rates were ≥ to 90% [10, 25].

Discussion

Cefiderocol susceptibility testing remains challenging due to technical specificities and the distinct performance of available testing methods depending both on the pathogen and the testing conditions. The reference BMD method for in vitro susceptibility testing of cefiderocol involves a complex and time-consuming preparation of an ID-CAMHB, making this technique inappropriate for routine testing in a clinical microbiology laboratory [11]. Consequently, various commercial systems have been developed for routine cefiderocol susceptibility testing. Agar diffusion methods, using either the disk method or cefiderocol-impregnated strips, which do not require iron-depleted media, and commercial microdilution techniques mimicking the BMD reference method, have become available. However, the performance of these different methods has been found to vary greatly depending on the pathogen tested and, for diffusion assays, according to the testing conditions, as highlighted in our literature review.

Diffusion assays and the value of disk diffusion for cefiderocol susceptibility testing in the *Achromobacter* genus

Generally speaking, the comparative studies reviewed all agree that determining MICs *via* the MTS assay cannot currently be recommended for cefiderocol susceptibility testing due to its severe underestimation of the cefiderocol MICs generating unacceptable rates of VME in Enterobacterales and *A. baumannii* complex isolates, bacteria for which the MTS is not validated, but also in *P. aeruginosa* strains, for which the MTS is approved. Our study evaluating MTS on *Achromobacter* spp. strains highlighted, for the first time, that although MTS succeeded in correctly categorizing the two cefiderocol-resistant *Achromobacter* strains, it proved to be inaccurate for cefiderocol MIC determination with a low EA of 30.8% and unacceptable values for high and low biases, +53.8 to -55.1%, respectively. MTS is thus a poor accurate technique for determining reliable cefiderocol MICs and its use must be discouraged, whatever the species under consideration [14, 22, 23, 29, 30, 32]. Some studies consider disk diffusion as a convenient, alternative testing method to BMD for determining cefiderocol susceptibility, but found variable performance according to the bacterial species being studied and the disk/r-MHA combination selected. Altogether, these studies lead to distinct recommendations for both the use of the disk diffusion assay and result interpretation depending on the committee on antimicrobial susceptibility testing under consideration [10, 14, 19–23, 26–32]. Although disk diffusion might be useful for screening, many strains should be re-tested by alternative methods providing MIC results to assess definitive categorization [14, 23].

To date, our study is the only one to have tested the performance of cefiderocol disks on such a wide panel of 143 *Achromobacter* spp. strains assigned to 14 species by *nrdA* gene sequencing and encompassing various sources of isolation. Indeed, the only data available so far on *Achromobacter* spp. are those recently published by Bianco et al. for 27 *A. xylosoxidans* strains, of which six cefiderocol-resistant strains were obtained after in vitro induction from three originally susceptible strains [10]. On that collection tested with disks from three manufacturers (Oxoid, Liofilchem, Mast Group) on the same r-MHA (non-communicated brand), the authors reported a 100% CA rate compared to the BMD reference method whatever the test conditions when using the EUCAST 2023 breakpoints for *P. aeruginosa* (Supplementary Table 2). With a larger, more taxonomically diverse collection of *Achromobacter* strains, including a few cefiderocol-resistant strains, the present study confirmed that the disk diffusion method performs well for *Achromobacter* spp. as it led to the detection of the two resistant strains in the study with the combination of Liofilchem disks and Liofilchem r-MHA. The phenomenon of heterogeneous growth and resistance observed with strain 2579 (found resistant with the BMD reference method) leads us to recommend considering even weak bacterial growth up to contact with the disk: the inner edge of the IZD should be measured in the event of heterogeneous growth [33,

34]. Heteroresistance to cefiderocol, previously reported in other MDR Gram-negative pathogens with debated correlation between heteroresistance and clinical outcomes, is thus also observed in the *Achromobacter* genus and its contribution to cefiderocol treatment failure remains to be evaluated.

Another input of our study was to report the FDC IZD distribution for the collection beyond study, showing that the population susceptible to cefiderocol consistently displayed an IZD≥27 mm. However, this result alone cannot be considered as a breakpoint, as our study did not fulfil the EUCAST procedure for establishing zone diameter breakpoints requiring that both several disk and agar medium suppliers be tested in several laboratories [35]. Indeed, although Matuschek et al. showed that the susceptibility results of quality control strains were largely unaffected by the disk and media manufacturers [36], other studies showed large variations in IZDs with clinical strains depending on the disk and r-MHA combinations, notably linked to variable iron concentrations from one batch of r-MHA to another, which may influence cefiderocol susceptibility results [14, 37]. It would thus be interesting to test other combinations of cefiderocol disk and MHA from other manufacturers on our collection of *Achromobacter* spp. strains to go further with a breakpoint proposal. It should be noted that data from the study by Bianco et al. still suggest that the disk manufacturer has a low impact on IZD with a maximum of +/- 3 mm observed between manufacturers (no variation, variations of +/- 1 mm, +/- 2 mm, +/- 3 mm observed for 7.5%, 59.2%, 29.6% and 3.7% of the 27 *A. xylosoxidans* strains tested) [10]. Thus, to generalise a cefiderocol susceptibility threshold for *Achromobacter* when using disk/r-MHA combinations from manufacturers other than Liofilchem, it seems more appropriate to propose a susceptibility threshold of 30 mm (*i.e.* 3 mm beyond the susceptible strain with the lowest IZD measured at 27 mm) above which strains would be susceptible to cefiderocol and below which a determination of the MIC with a BMD method will be required to confirm or exclude cefiderocol resistance. This proposition is congruent with the data published by Bianco et al. showing that the six cefiderocol-resistant induced strains that display IZDs ranging from 6 to 26 mm regardless of the disk brand used [10].

Commercial BMD methods and warnings on the non-detection of cefiderocol-resistant *A. xylosoxidans* strains

Sensititre EUMDROXF microplates (Thermo Fisher Scientific, Cleveland, OH, USA) corresponding to cefiderocol pre-coated wells with a specific iron chelator which allowed the use of a standard CAMHB (Thermo Fisher Scientific) without iron depletion, were not evaluated in our study as they were withdrawn from the market in February 2022 [14]. An excess of free iron in the microplate wells once inoculated with non-ID-CAMHB was suspected to be the cause of MIC overestimation. The issue has not yet been resolved, and this method thus remains to be evaluated on *Achromobacter* spp. strains.

To date, ComASP™ has been evaluated in Enterobacterales, *P. aeruginosa*, *A. baumannii* complex, but never in the *Achromobacter* spp. genus (Supplementary Table 2) [20, 24, 29, 30, 32]. Despite the differences observed among pathogens, performance of the ComASP™ test was insufficient for use in clinical diagnostics according to the ISO 20776-2:2021 validation criteria (Supplementary Table 2). However, in evaluating the performance of the ComASP™ for 50 carbapenemase-producing GNB isolates that presented an IZD within ATU by using the disk diffusion method, Bianco et al. concluded that ComASP™ achieved good performance compared to the BMD reference method with a CA of 94% and both the VME (2.0%) and ME (4.0%) observed for MIC values were nearly close to the resistance breakpoint (2 mg/L) [20]. Thus, the combination of disk diffusion with ComASP™ was shown to be a valid strategy to resolve the susceptibility interpretation of isolates in the ATU and overcome the challenge of routine cefiderocol susceptibility testing in microbiology laboratories.

One limitation of our work is the small number of cefiderocol-resistant isolates (n=2) which made it difficult to assess the performance of commercial tests against these rarely isolated strains [2–6, 9, 38, 39]. However, the performance of ComASP™ in *Achromobacter* spp. evaluated here for the time was similar to that published for other bacterial genera. Indeed, this technique failed to detect the two strains resistant to cefiderocol, mainly because the MICs were underestimated (EA=76.9%, bias = -14.2%). In contrast to the diffusion methods, ComASP™ wrongly categorized the only two *Achromobacter* resistant strains in our study as susceptible to cefiderocol, discouraging its use for routine cefiderocol susceptibility testing. Compared with ComASP™, the literature review data showed that UMIC® appears to provide better performance, achieving the acceptable criteria defined by the ISO for both Enterobacterales (EA=91.7%, bias = -10%, n=60 [24] and EA=91.7%, bias = -25%, n=180 [25]) and *P. aeruginosa* (EA=93.9%, bias=+12.2%, n=49 [25]). The UMIC® also appears to perform better than the ComASP™ in *A. baumannii*, with EA ranging from 76% [30] to 89.7% [10] with UMIC®, and 29.6% [32] to 85.7% [20] with ComASP™. All species considered, ComASP™ test tended to provide lower MIC values while the UMIC® tended to provide higher MIC values compared to the BMD reference method that was also observed for the quality control strain *P. aeruginosa* and *Achromobacter* spp. isolates in our study as illustrated by a bias = -14.2% for ComASP™ and a bias=+9.1% for

UMIC®. However, poorer overall performance of UMIC® were noticed in our study (EA = 49%) compared with the only study published by Bianco et al. to date (EA = 92.6%, CA = 96.2%, VME = 16.7%) [10]. Taken together that UMIC® successfully classified one of the two *Achromobacter* resistant strains, unlike ComASP™, but showed poorer EA (49%) compared to ComASP™ (76.9%) makes them two equivalent techniques that would be interesting to evaluate on a larger number of cefiderocol-resistant *Achromobacter* strains.

Conclusion

As cefiderocol is currently unavailable in all commercial automated AST panels [40], commercial tests may be suitable alternative methods for routine testing of *Achromobacter* susceptibility to cefiderocol in microbiology laboratories. To the best of our knowledge, this is the first study to evaluate the performance of disk diffusion, MTS and two BMD commercial tests (ComASP™ and UMIC®) to assess susceptibility to cefiderocol of an accurately identified collection of *Achromobacter* spp. clinical strains, compared to the standard BMD method. This is also the first literature review to summarize the performance of commercially available AST methods for cefiderocol, for all bacterial species.

Although the use of MTS should be discouraged for *Achromobacter* spp. due to the low EA compared with the standard BMD method, the disk diffusion method can be used as a susceptibility screening tool with a cut-off diameter of 30 mm: strains with an IZD ≥ 30 mm could be categorized as susceptible to cefiderocol without further testing. However, for strains with an IZD < 30 mm, it would be necessary to determine the MIC of cefiderocol by a BMD method. Commercialized BMD methods, either ComASP™ or UMIC®, should not be used as the sole method for cefiderocol susceptibility testing but have to be systematically associated with a disk diffusion test to detect the yet rarely described cefiderocol-resistant *Achromobacter* sp. strains that could be overlooked by ComASP™ or UMIC®. In case of persisting interpretation difficulties, strains have to be sent to a reference laboratory performing the BMD reference method, as ComASP™ or UMIC® do not meet the acceptable EA as defined by ISO 20776-2:2021, despite showing an acceptable bias < ± 30%.

Abbreviations

AST	Antimicrobial susceptibility testing
ATU	Area of technical uncertainty
BMD	Broth microdilution
CF	Cystic fibrosis
ComASP™	Compact antimicrobial susceptibility panel
EUCAST	European committee on antimicrobial susceptibility testing
GNB	Gram-negative bacilli
ID-CAMHB	Iron-depleted-cation-adjusted Mueller-Hinton broth
IZD	Inhibition zone diameter
r-MHA	Regular Mueller-Hinton-agar
MDR	Multidrug resistant
MEM	Meropenem
MIC	Minimal inhibitory concentration
MTS	MIC gradient test strips
NCF	Non-cystic fibrosis
PK/PD	Pharmacokinetics and pharmacodynamics
TZP	Piperacillin-tazobactam
SXT	Trimethoprim-sulfamethoxazole

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

HM and AP conceived and designed the study. RC, HM collected the CF clinical isolates. AP, JPL, HM collected the non-CF clinical isolates. VJP, PS, KJ and HM collected the microbiological data, analyzed and interpreted the data. VJP and HM drafted the manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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