Cefiderocol: Systematic Review of Mechanisms of Resistance, Heteroresistance and In Vivo Emergence of Resistance

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Abstract: Cefiderocol appears promising, as it can overcome most β-lactam resistance mechanisms (including β-lactamases, porin mutations, and efflux pumps). Resistance is uncommon according to large multinational cohorts, including against isolates resistant to carbapenems, ceftazidime/avibactam, ceftolozane/tazobactam, and colistin. However, alarming proportions of resistance have been reported in some recent cohorts (up to 50%). A systematic review was conducted in PubMed and Scopus from inception to May 2022 to review mechanisms of resistance, prevalence of heteroresistance, and in vivo emergence of resistance to cefiderocol during treatment. A variety of mechanisms, typically acting in concert, have been reported to confer resistance to cefiderocol: β-lactamases (especially NDM, KPC and AmpC variants conferring resistance to ceftazidime/avibactam, OXA-427, and PER- and SHV-type ESBLs), porin mutations, and mutations affecting siderophore receptors, efflux pumps, and target (PBP-3) modifications. Coexpression of multiple β-lactamases, often in combination with permeability defects, appears to be the main mechanism of resistance. Heteroresistance is highly prevalent (especially in *A. baumannii*), but its clinical impact is unclear, considering that in vivo emergence of resistance appears to be low in clinical studies. Nevertheless, cases of in vivo emerging cefiderocol resistance are increasingly being reported. Continued surveillance of cefiderocol's activity is important as this agent is introduced in clinical practice.

Keywords: cefiderocol; resistance; heteroresistance

1. Introduction

Extensively drug-resistant (XDR) and pandrug-resistant Gram-negative bacteria are increasingly being reported worldwide [1]. Attributable mortality appears to be high [2], and treatment options very limited [3], with synergistic combinations of in vitro inactive (alone) antimicrobials often being a last resort [3–5]. Cefiderocol appears promising, as it can overcome most of the mechanisms of β-lactam resistance (including β-lactamases, porin mutations and efflux pumps) [3].

Cefiderocol is a novel siderophore cephalosporin [6,7]. It has a structure similar to that of cefepime (pyrrolidinium group on the C-3 side chain, which improves stability against β-lactamases) and ceftazidime (same C-7 side chain conferring improved stability against β-lactamases and improved transport across the outer membrane) [6,7]. The major difference is the addition of a chlorocatechol group on the end of the C-3 side chain, which further enhances β-lactamase stability and confers siderophore activity [6,7]. Siderophores are natural iron-chelating molecules used by bacteria to facilitate iron transport. Notably, the natural siderophores enterobactin (*E. coli*) and pyoverdine (*P. aeruginosa*) contain similar catechol groups as an iron-chelating moiety. By utilizing natural iron transportation systems

(often referred to as a "Trojan horse" strategy), cefiderocol is actively transported across the outer membrane of Gram-negative bacteria, therefore overcoming resistance mediated by porin loss or efflux pumps [6]. This, in combination with improved β-lactamase stability, makes cefiderocol an ideal candidate for treatment of infections by XDR/PDR Gram-negative bacteria.

Based on several prior studies, including large multinational cohorts [8–13], cefiderocol appears to be active against the majority of Enterobacterales, *A. baumannii*, *P. aeruginosa*, and *S. maltophilia*, including isolates that are resistant to carbapenems, ceftazidime/avibactam, ceftolozane/tazobactam, meropenem/vaborbactam, imipenem/relabactam, and polymyxins. Nevertheless, cefiderocol resistance has been reported to be high in selected cohorts [14–19]. The prevalence of heteroresistance may also be high [20], and cases of resistance emerging during treatment are increasingly being reported [21–26]. The aim of this manuscript was to systematically review mechanisms of resistance to cefiderocol, prevalence of heteroresistance and reports of in vivo emerging resistance.

2. Results

The study flow chart is depicted in Figure 1. A total of n = 52 relevant studies were reviewed [12,14–17,19–64]. All studies were published after 2018; n = 17 (33%) in 2022, n = 18 (35%) in 2021, n = 12 (23%) in 2020, n = 1 (2%) in 2019, and n = 4 (8%) in 2018. Evaluation of mechanisms of resistance was based on: n = 13 group 1 studies [14,17,27,29,30,37,45,52–54,61,63,64], n = 4 group 2 studies [12,15,19,62], n = 11 group 3 studies [14,16,21,24,25,32,42–44,55,59], n = 7 group 4 studies [28,31,40,46,57,58,60], and n = 13 group 5 studies [29,31,41,47–51,54–57,64] (see Section 4.2 for grouping of studies). Data about the prevalence of heteroresistance were available in only two studies [20,33]. Data about resistance emerging in vivo (during/after cefiderocol treatment) were available in 12 studies [21–26,32,34–36,38,39].
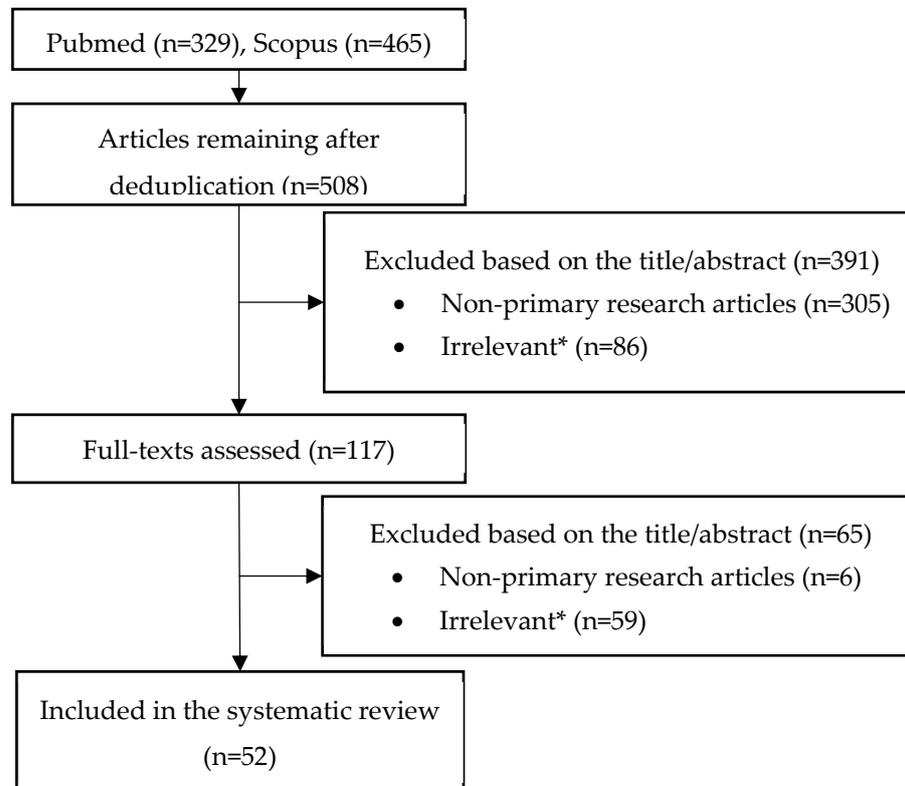

Figure 1. Flow chart. * not satisfying the eligibility criteria described in Methods.

2.1. Role of β-Lactamases

Various β-lactamases have been correlated to cefiderocol resistance, and we discuss the most notable below. A detailed summary of the available data is presented in Table A1.

2.1.1. NDM Metallo-β-Lactamases (MBL)

The role of NDM-type β-lactamases has been supported by several findings, including multifold increases in cefiderocol MIC by introduction of NDM in isogenic mutants [29,54,64] and much higher prevalence (42–59% in some cohorts [12,19,62]) of cefiderocol nonsusceptibility in NDM-producing clinical isolates. Additionally, in vivo emergence of cefiderocol resistance in *E. coli* associated with increased copy numbers of bla_{NDM} genes has been reported [25]. Moreover, expression of NDM appears to facilitate the emergence of cefiderocol resistance by additional mechanisms (such as mutations in siderophore receptors) [40,64].

2.1.2. KPC Variants

The role of KPC variants, particularly those conferring resistance to ceftazidime/avibactam, has been supported by both multifold increases in MIC by introduction of selected KPC variants in isogenic mutants [41,56,64] and clinical data [12,42,43,56]. Notably, cefiderocol resistance (MIC > 2 mg/L) was considerably higher (83% vs. 7%) in ceftazidime/avibactam-resistant (n = 40) than in ceftazidime/avibactam-susceptible (n = 60) KPC-producing Enterobacterales (mostly *K. pneumoniae*) in one cohort [12]. In addition, in vivo emerging resistance to cefiderocol in *K. pneumoniae* attributable to KPC variants has been reported [12,42,56].

There is evidence that binding of cefiderocol by KPC-3 variants KPC-41 and KPC-50 (rather than hydrolysis) is responsible for reduced susceptibility to cefiderocol [56]. Therefore, lack of hydrolysis does not exclude a contribution of β-lactamase to cefiderocol resistance.

2.1.3. Role of OXA-Type β-Lactamases

Among OXA-type β-lactamases, only OXA-427 has been correlated to cefiderocol resistance [15]. OXA-427 is a novel type of carbapenem-hydrolysing class D β-lactamase, sharing only 22–29% amino acid identity with OXA-48-like enzymes and conferring resistance to extended-spectrum cephalosporins (mostly ceftazidime) [65]. Uniform cefiderocol nonsusceptibility on disk diffusion was reported among n = 26 OXA-427-producing Enterobacterales isolates in Belgium [15]. OXA-427 was been recently confirmed to have hydrolytic activity against cefiderocol [56], although introduction of OXA-427 in *E. coli* resulted in only twofold increase in cefiderocol MIC [56]. Various OXA-type β-lactamases (especially OXA-23) have also been commonly reported in cefiderocol-resistant *A. baumannii* clinical isolates [17,29,30,63]. However, cefiderocol appears to be stable against OXA-23 (as well as OXA-48 and OXA-40) [66]. Furthermore, introduction of OXA-1, OXA-48, OXA-23, OXA-24, OXA-40, OXA-58, and OXA-232 in *E. coli/K. pneumoniae/A. baumannii/ P. aeruginosa* have not been shown to affect cefiderocol MIC [29,32,54,56,64,66].

2.1.4. Role of PER-Type, SHV-Type, and BEL-Type ESBLs

Both PER-type [29,54,56] and SHV-type EBSLs [29,54,56] have been associated with increased cefiderocol MIC following their introduction to isogenic mutants. Both types of ESBLs have been detected in cefiderocol-resistant clinical isolates. Specifically, PER-type ESBLs have been detected predominantly in cefiderocol-resistant *A. baumannii* [27,29,54], but also in *P. aeruginosa* [19]. SVH-type β-lactamases have been correlated to cefiderocol resistance in *K. pneumoniae* and *A. baumannii* [29]. BEL-type β-lactamases also have the potential to contribute to cefiderocol resistance based on isogenic mutant experiments [54,56].

2.1.5. Role of AmpC Variants

In vivo emerging cefiderocol cross-resistance attributable to *ampC* mutations was reported in two patients, with *Enterobacter cloacae* infections being treated with ceftazidime/ avibactam [43]. The role of these AmpC variants in cefiderocol resistance has also been

confirmed by introducing them in isogenic *E. coli* mutants, resulting in a 32-fold increase in cefiderocol MIC by A292_L293del AmpC variant [43] and a 4-fold increase by A294_P295del AmpC [43]. In vivo emerging cefiderocol cross-resistance potentially attributable to *ampC* mutation was reported in three patients with *P. aeruginosa* infections being treated with ceftolozane/tazobactam [16,44].

2.1.6. Reversal of Cefiderocol Susceptibility by β-Lactamase Inhibitors

The role of β-lactamases in cefiderocol resistance has been further supported by potentiation of cefiderocol activity by dipicolinic acid (an MBL inhibitor) against MBL-producing (mainly NDM-producing) isolates and by avibactam against isolates producing serine β-lactamases (e.g., SHV- or PER-type ESBLs) [19,29,30,40,47,63,64]. In isolates coexpressing both MBL and serine β-lactamases, potentiation of cefiderocol activity is greatest in the presence of both dipicolinic acid and avibactam [29].

2.2. Permeability Defects/Increased Efflux

Mechanisms affecting siderophore receptors are summarized in Table A2, while mechanisms involving porins and efflux pumps are summarized in Table A3.

2.2.1. Mutations Affecting Siderophore Receptors

Several studies based on isogenic mutants have proven the role of siderophore receptor mutations in cefiderocol resistance. PiuA and PiuD (an ortholog of PiuA) appear to be most important in *P. aeruginosa* [47,50]. Mutations affecting *pirA* alone appear to be less important in *P. aeruginosa* but may further raise cefiderocol MIC in combination with mutations affecting either *piuA* or *piuD* [50]. One study, however, did not find a correlation between siderophore receptors (including PiuA/PiuD and PirA) and cefiderocol MIC in 10 *P. aeruginosa* isolates with cefiderocol MIC ranging from 0.03 to 8 mg/L [45]. In Enterobacterales, mutations in siderophore receptors CirA and/or Fiu appear to be most important [40,47,57,64], especially in the presence of NDM MBLs [40,57,64]. Differences in the degree of resistance caused by mutations in specific siderophore receptors may be due to the relative contribution of each iron acquisition system in a given strain [57]. Mutations affecting iron transport have also been detected in in vitro derived (after serial passaging) cefiderocol-resistant *S. maltophilia* [58,60]. Mutations affecting the aforementioned genes have also been detected in clinical cefiderocol-resistant isolates, including *P. aeruginosa* (*piuD* and *pirR*) [44], *A. baumannii* (*pirA* and *piuA*) [52], and *K. pneumoniae* (*cirA*) [24].

2.2.2. Porin Mutations

Porin mutations can raise cefiderocol MIC based on isogenic *K. pneumoniae* (OmpK35, OmpK36) and *P. aeruginosa* (OprD) mutants [47]. Mutations in porins have also been detected in cefiderocol-resistant clinical *K. pneumoniae* (OmpK35, OmpK36, OmpK37) and *Enterobacter* spp. (OmpC, OmpF) isolates, in combination with expression of various β-lactamases [37,53,55]. Finally, a high percentage of cefiderocol resistance (38.5% with MIC > 2 mg/L) was reported in ESBL-Enterobacterales with porin mutations [19].

2.2.3. Overexpression of Efflux Pumps

Efflux pumps may contribute to cefiderocol resistance based on transformed isogenic mutants or in vitro derived cefiderocol-resistant mutants in *K. pneumoniae* (sugE and chrA) [37], *P. aeruginosa* (MexAB–OprM efflux pump) [47], and *S. maltophilia* (smeDEF efflux pump) [60]. Furthermore, overexpression of AxyABM efflux pump in *Achromobacter xylosoxidans* was associated with a threefold higher cefiderocol MIC [51].

2.3. Target Modification and Other Genes Potentially Involved in Cefiderocol Resistance

Target modification (mutations in PBP-3) has been noted in few cefiderocol-resistant *E. coli* [31,59] and *A. baumannii* [30,52]. However, the role of this mechanism in cefiderocol resistance is uncertain, and based on isogenic *E. coli* mutants, it only minimally raised

(2-fold) cefiderocol MIC [31]. Mutations in other genes potentially involved in cefiderocol resistance are summarized in Table A4. Most of these mutations have been detected only in vitro and not in clinical isolates.

2.4. Combination of Mechanisms Contribute to Cefiderocol Resistance

Considering the results of studies of isogenic strains, each of the mechanisms described above appears to be insufficient to raise, on its own, cefiderocol MICs above current PK/PD breakpoints [29,31,40,46,47,50,51,54,55]. Indeed, the majority of strains harbouring various mechanisms of resistance, including various β-lactamases, remain susceptible to cefiderocol [8–12,47]. This suggests that a combination of different mechanisms is necessary, including coexpression of different β-lactamases, mutations affecting siderophore–drug receptors expression/function, mutations affecting porin expression/function, overexpression of efflux pumps, and target (PBP-3) modification [37,59,64]. This is further supported by the fact that various studies of clinical isolates have not correlated cefiderocol resistance with specific mechanisms but detected multiple resistance mechanisms comprising predominantly coexpression of various β-lactamases in combination with permeability defects in cefiderocol-resistant isolates [17,37,45,53,63].

Furthermore, a higher baseline cefiderocol MIC due to the presence of one or more of the above mechanisms of resistance appears to facilitate the emergence of cefiderocol resistance. The closer the MIC is to the breakpoints, the easier it is for additional mutations to raise the MIC above PK/PD breakpoints [40,64]. For example, NDM-5 production has been shown to facilitate emergence of cefiderocol resistance (by additional mutations affecting CirA) in *E. cloacae* [40] and *K. pneumoniae* [64]. Notably, the emergence of resistance was not possible in vitro in *E. cloacae* isolates with alternative β-lactamases (not affecting cefiderocol) and was prevented in the presence of dipicolinic acid [40].

Finally, combinations of different mechanisms of resistance may be synergistic. For example, introduction of NDM-5 alone or CirA deficiency alone in *K. pneumoniae* resulted in eightfold (0.5→4 mg/L) and twofold (0.5→1 mg/L) higher cefiderocol MIC. However, introduction of both NDM-5 and CirA deficiency resulted in a cefiderocol MIC > 256 mg/L [64].

2.5. Heteroresistance (In Vitro Emergence of Resistant Subpopulations)

Heteroresistance was systematically assessed in only two studies [20,33], both conducted by the same group. Heteroresistance was defined as presence of resistant (×2–4 the relevant CLSI breakpoints) subpopulations at a frequency of ≥ 1 in 10^6 [20,33]. The prevalence of heteroresistance was much higher than that of nonsusceptibility, at 59% (64/108) in carbapenem-resistant *A. baumannii* (vs. 8.3% nonsusceptible), 48% (14/29) in *S. maltophilia* (vs. 0% nonsusceptible), 30% (27/89) in carbapenem-resistant *K. pneumoniae* (vs. 5.6% nonsusceptible), and 9% (6/69) in carbapenem-resistant *P. aeruginosa* (vs. 0% nonsusceptible). Furthermore, prevalence of heteroresistance was higher in carbapenem-resistant than in carbapenem-susceptible isolates and much lower in isolates susceptible to both carbapenems and extended spectrum cephalosporins [33], an observation that further supports the potential role of β-lactamases in facilitating emergence of cefiderocol resistance.

The frequency of cefiderocol-resistant subpopulations depends on a variety of factors, including methodological factors (such as bacterial density used considering potential inoculum effect [40,41]) as well as strain-specific factors [20,31,33,46,67]. With regard to the latter, and considering that cefiderocol resistance is a result of various different mechanisms acting in concert, a high baseline MIC appears to facilitate the in vitro and in vivo emergence of resistant subpopulations (i.e., the frequency of resistant subpopulations is higher) [40,57]. Similarly to what has been previously described for colistin heteroresistance [68,69], the frequency of resistant subpopulations increased after exposure to cefiderocol and decreased after removal from the drug [20].

2.6. In Vivo Emergence of Resistance or Reduced Cefiderocol Susceptibility

In vivo emergence of resistance was systematically addressed in two randomized controlled trials (CREDIBLE-CR [23] and APEKS-NP [22]) [21]. In these trials, a more than fourfold cefiderocol MIC increase during or after treatment was found in 7% (19 of 265) of the patients [21]. However, for most patients, the post-treatment cefiderocol MICs were relatively low. Specifically, post-treatment MICs were higher than susceptibility breakpoints in six (1.6%), three (1.1%), and four (1.5%) cases considering FDA, CLSI, and EUCAST breakpoints, respectively.

In vivo emerging resistance has also been reported in small observational case series [26,34–36]. In one study, 17 patients were treated with cefiderocol for difficult-to-treat *P. aeruginosa* infection, and relapses occurred in 3 of the patients, but repeat MIC testing was available in only 1 patient (fourfold increase from 0.25 to 1 mg/L) [26]. In another small series of 10 patients (*A. baumannii* n = 8, *K. pneumoniae* n = 2) who received cefiderocol as salvage therapy, microbiological failure was reported in 2 of the patients, but repeat MIC testing was available in only 1 (16-fold increase from 0.25 to 4 mg/L) [35]. In 13 patients with *A. baumannii* infections, microbiological failure was observed in about half (n = 7) of the patients but was associated with suboptimal attainment of PK/PD targets rather than emergence of resistance [36]. Finally, in 47 patients with carbapenem-resistant *A. baumannii* infections, microbiological failure occurred in 8 patients, in 4 of whom emergence of cefiderocol resistance was documented (MICs ranging from 4 to >32 mg/L) [34].

In addition, several case reports have described in vivo emergence of cefiderocol resistance: (1) on day 21 of treatment of a patient with hepatic abscesses by NDM-1 producing carbapenem-resistant *E. cloacae* (MIC 4→256 mg/L associated with *cirA* mutations) [24]; (2) on day 19 of treatment of a patient with intraabdominal abscesses by NDM-5-producing *E. coli* (MIC 2→>32 mg/L, associated with increased copy numbers of *bla*$_{NDM}$ genes) [25]; (3) after 32 days of a 6-week course of cefiderocol in a patient with pancreatic abscess by *P. aeruginosa* (a cefiderocol resistant *C. koseri* was also detected later in the same patient) [38]; (4) after a 3-week course of cefiderocol therapy in a patient with empyema by XDR *P. aeruginosa* (MIC 0.25→1 mg/L) [39]. In three of these four reports, emergence of resistance was associated with persistent or recurrent infection [24,25,38]. Notably, these reports involved patients with infections characterized by high bacterial load, insufficient source control, and/or prolonged treatment durations, which provide ideal conditions for in vivo emergence of resistance. Indeed, a higher bacterial density increases the chance of emergence of resistant subpopulations [40]. Furthermore, an inoculum effect has been described by which higher cefiderocol MIC is observed in/associated with higher bacterial densities [41].

In vivo emergence of cross-resistance to cefiderocol in ceftazidime/avibactam-treated patients has also been described [14,42]. Bianco et al. [14] described in vivo emergence of cross-resistance between cefiderocol and ceftazidime/avibactam after ceftazidime/ avibactam treatment in 16 patients with KPC-producing *K. pneumoniae* isolates. Similarly, Tiseo et al. [42] reported emergence of cross-resistance to cefiderocol during treatment with ceftazidime/avibactam resulting from emergence of a KPC-3 variant (KPC-31). In addition, emergence of cross-resistance to cefepime, ceftazidime/avibactam, and cefiderocol in *Enterobacter hormaechei* strains resulting from emergence of A292_L293del AmpC variant was described in two cefepime-treated patients [43].

Finally, the potential of in vivo emergence of cross-resistance between ceftolozane/ tazobactam and cefiderocol in *P. aeruginosa* has been reported [16,44]. Analysing 16 pairs (before/after ceftolozane/tazobactam treatment) of *P. aeruginosa* isolates Simner et al. detected ≥4-fold increases in cefiderocol MICs in 4 of the 16 isolates, although the MIC was above CLSI susceptibility breakpoint in only 1 case [16]. In a case report, emergence of cross-resistance to cefiderocol was reported in a ceftolozane/tazobactam-treated patient [44]. Potential contributing mechanisms involved an amino acid substitution in *AmpC* [16,44], overexpression of the MexAB–OprM efflux pump [16], and mutations in siderophore re-

ceptors PiuD and PirA [44], although the relative contribution of each of these mechanisms to cefiderocol resistance was unclear from these studies [16,44].

3. Discussion

3.1. Overview of Mechanisms of Cefiderocol Resistance

Resistance to cefiderocol is associated with a combination of mechanisms, each contributing to reduced cefiderocol susceptibility; β-lactamases, mutations affecting expression/function of siderophore receptors (most commonly involved genes: *cirA* and *fiu* in Enterobacterales, *piuA* and *piuD* in *P. aeruginosa, pirA* and *piuA* in *A. baumannii*) and to a lesser extent mutations affecting expression/function of porins and/or efflux pumps, or target (PBP-3) modification. Each of these mechanisms alone are usually insufficient to raise cefiderocol MIC above PK/PD breakpoints. Therefore, cefiderocol resistance is typically the result of various combinations of the aforementioned mechanisms of resistance. A combination of β-lactamases and reduced permeability (due to mutations affecting porins or siderophore receptors) appears to be the most common mechanism resulting in cefiderocol nonsusceptibility.

3.2. Role of β-Lactamases in Cefiderocol Resistance

One of the main advantages of cefiderocol is that it remains active (MIC below susceptibility breakpoints) against the majority of isolates producing various β-lactamases, including isolates that are resistant to carbapenems, ceftazidime/avibactam, ceftolozane/tazobactam, meropenem/vaborbactam, and imipenem/relebactam [8–13]. However, cefiderocol is not completely stable against various β-lactamases. Reduced susceptibility has been reported in the presence of specific β-lactamases, including KPC variants conferring resistance to ceftazidime/avibactam, AmpC variants conferring resistance to ceftazidime/avibactam and/or ceftolozane tazobactam, selected SHV- and PER-type ESBLs, selected NDM, and OXA-427. Of interest is that cefiderocol resistance mediated by serine β-lactamases can be reversed by avibactam [19,29,47], which could be a useful combination in clinical practice to overcome cefiderocol resistance. Similarly, MBL inhibitors (when they become available) could prove useful in combination with cefiderocol against NDM-producing isolates.

Generally, β-lactamases alone are not sufficient to raise cefiderocol MIC above susceptibility breakpoints, and nonsusceptibility is usually the result of coexpression of multiple β-lactamases and/or overexpression of β-lactamases, possibly in combination with other mechanisms of resistance described above (especially mutations associated with reduced permeability). Additionally, β-lactamases can facilitate the emergence of resistance by additional mechanisms [33,40]. Notably, prevalence of cefiderocol resistance was very high is some cohorts of isolates producing specific β-lactamases (OXA-427 [15], KPC variants conferring resistance to ceftazidime/avibactam [14], NDM [12,19,62]). This suggests that emerging β-lactamases could become a major contributor to cefiderocol resistance in the future.

3.3. Cross-Resistance between Other Antibiotics and Cefiderocol

Cefiderocol appears to be active against the vast majority of Gram-negative bacteria that are nonsusceptible to ceftazidime/avibactam or ceftolozane/tazobactam [8]. However, cross-resistance among ceftazidime/avibactam, ceftolozane/tazobactam, and cefiderocol has been reported, associated with KPC variants in *K. pneumoniae* [14,41,55] or AmpC variants in *Enterobacter* spp. [43] and *P. aeruginosa* [16]. Notably, in a recent study, cefiderocol resistance was very high (83%) in ceftazidime/avibactam-resistant KPC-producing Enterobacterales (predominantly *K. pneumoniae*) [14]. Furthermore, metallo-β-lactamases, which are known to confer resistance to ceftazidime/avibactam and ceftolozane/tazobactam, have also been associated with decreased susceptibility to cefiderocol (especially NDM metallo-β-lactamases) [12,25,29,40,54]. Therefore, widespread clinical use of ceftazidime/avibactam and ceftolozane/tazobactam may contribute to the emergence and spread of cefiderocol resistance, even in the absence of exposure to cefiderocol.

On the other hand, cross-resistance to cefiderocol and ceftazidime/avibactam conferred by KPC variants may be associated with reversal of susceptibility to carbapenems [14,42] and may be amenable to treatment with meropenem/vaborbactam [42]. Furthermore, *P. aeruginosa* AmpC variants conferring resistance to cefiderocol, ceftazidime/avibactam, and ceftolozane/tazobactam may remain susceptible to imipenem/relebactam [16].

Finally, potential for cross-resistance between colistin and cefiderocol has been proposed [37], although cefiderocol appears to retain activity against the majority of colistin-resistant Enterobacterales [12,61] and colistin-resistant *A. baumannii* [63].

3.4. Importance of Heteroresistance and In Vivo Emergence of Resistance

The clinical impact of heteroresistance is that resistant subpopulations can emerge during treatment, resulting in treatment failure and the spread of resistant strains [68–70]. The high prevalence of heteroresistance to cefiderocol has been proposed as an explanation for the suboptimal efficacy of cefiderocol against carbapenem-resistant bacteria, especially *A. baumannii* [20,33]. However, there are no clinical data to support this hypothesis, and emergence in vivo of resistance to cefiderocol during treatment appears to be rare (<2%) in clinical studies [21,71].

The potential impact of cefiderocol heteroresistance in the longer term and as cefiderocol is increasingly being used in clinical practice is yet unclear. Yearly data (2014–2019) from the SIDERO-WT studies are not informative, considering that cefiderocol was approved only in 2020 in North America and Europe and remains unavailable in some countries. Based on experience with polymyxins (with a similarly high prevalence of heteroresistance [68] and similarly low reported frequency of in vivo emerging resistance [69]), emergence of resistance in cefiderocol is likely in the future, especially in *A. baumannii*.

Notably, the definition of and methodologies for detecting heteroresistance are not well-defined [68,70,72,73]. Furthermore, traditional susceptibility testing methods, which are based on a few individual colonies (rather than the bacterial population), may miss resistant subpopulations even if they are present at a relatively high frequency [73]. Additionally, heteroresistance can be unstable and very dynamic (resistant subpopulations rapidly increasing/decreasing in the presence/absence of antibiotic pressure), which may also result in failure to detect resistance (reversal to the susceptible phenotype has been documented after only 12 h of growth in blood culture flasks) [74]. Finally, appropriately designed clinical studies are needed to assess the impact of heteroresistance on clinical outcomes and to uncover characteristics of heteroresistance (in combination with patient characteristics) that predict treatment failure [70]. Such studies are necessary to define appropriate methods to detect resistance (and clinically relevant heteroresistance).

In theory, the following factors (often acting in concert) can facilitate clinically relevant in vivo emergence of resistance [69,70,73]: (1) infections associated with higher bacterial burden or poor source control, (2) higher baseline MIC (closer to breakpoints of resistance), (3) higher frequency of resistant subpopulations and especially of resistant subpopulations with preserved fitness and virulence, (4) immunosuppression, (5) monotherapy, and (6) failure to achieve appropriate PK/PD targets at the site of infection.

3.5. Limitations

Mutations identified by in vitro selection of mutants following exposure to cefiderocol may not be relevant to in vivo observed mutations, as has been described previously for colistin [68,69]. Mutations observed in vitro, especially those affecting iron transport [58,60], may be associated with fitness cost [40,58] and can be unstable or reversible in the absence of continued cefiderocol exposure [58,60]. This is further supported by the fact that resistance emerging in vivo in animal models [58,67] or clinical studies [21–23,75] is much less frequent than heteroresistance [20] and much less frequent than resistance emerging in vitro after exposure to cefiderocol. Furthermore, mutations identified in cefiderocol-resistant clinical isolates do not prove causality. Additionally, mutations identified in single-centre cohorts may not be generalizable to other sites and may overestimate the role

of certain mechanisms of resistance by considering only isolates that represent one or few related clones. Nevertheless, comparisons of in vivo emerging cefiderocol resistant strains with their parental strains are very useful for identifying clinically relevant mechanisms of resistance [21–23,75]. Furthermore, on many occasions, the role of various resistance mechanisms has been confirmed in isogenic mutants.

Finally, it has recently been suggested that in vitro susceptibility testing of cefiderocol against *A. baumannii* may overestimate its activity compared with conditions in vivo [76]. Specifically, cefiderocol MIC was 2- to 16-fold higher in the presence of human serum, human serum albumin, or human pleural fluid in the culture medium [76]. Under these conditions, higher expression of β-lactam resistance-associated genes (β-lactamases and PBPs) was observed, combined with downregulation of iron uptake-related genes, which could explain the higher cefiderocol MIC [76], and may explain lower efficacy of cefiderocol against carbapenem-resistant *A. baumannii* infections [23].

4. Materials and Methods

4.1. Search Strategy and Sources

PubMed and Scopus were searched from inception to 6 May 2022, using the keywords cefiderocol OR s-649266. After deduplication, screening for eligibility of retrieved articles was conducted by the first author using the Rayyan online app for collaborative systematic reviews [77]. Eligibility of included articles was validated by a second author.

4.2. Eligibility Criteria

The following types of studies were eligible for review: (1) cohorts of cefiderocol-resistant clinical isolates evaluating for the presence of specific resistance mechanisms in these isolates (e.g., by PCR or whole genome sequencing to identify mutations in genes potentially contributing to cefiderocol nonsusceptibility); (2) cohorts of clinical isolates reporting high proportions (>20%) of cefiderocol resistance and correlating such resistance with specific mechanisms; (3) studies trying to identify resistance mechanisms by comparing in vivo emerging cefiderocol-resistant clinical isolates with their parental strains; (4) studies trying to identify resistance mechanisms by comparing in vitro derived (by subculturing in the presence of cefiderocol) cefiderocol-resistant isolates with their parental strains; (5) studies evaluating the impact of specific resistance mechanisms on cefiderocol minimum inhibitory concentration (MIC) by introducing the relevant resistance determinants in vitro (comparison of the cefiderocol MIC of the derived with that of the isogenic parental strain); (6) studies examining the prevalence of heteroresistance; (7) studies evaluating for the emergence of resistance in vivo during or after cefiderocol treatment (even if no evaluation for the mechanism of resistance was conducted).

The following types of studies were excluded: (1) nonoriginal articles (e.g., reviews, commentaries, editorials); (2) irrelevant original articles (not satisfying the eligibility criteria described in the paragraph above); (3) potentially relevant articles written in languages other than English.

4.3. Data Items

The following data were extracted from each eligible article: mechanisms of resistance, methods for confirming contribution of each mechanism to cefiderocol resistance, proportion of heteroresistance and the definition thereof used, and frequency of emergent resistance during treatment in clinical studies (case reports of emergent resistance were also recorded).

4.4. Assessment of the Evidence for the Reported Mechanisms of Resistance

Studies were grouped (groups 1 to 6) as described above in Section 4.2. The evidence for a resistance mechanism was considered strongest if both of the following were true: (1) the resistance mechanism was detected in cefiderocol-resistant clinical isolates (group 1, 2, or 3 studies) and (2) resistance determinants detected in clinical isolates were confirmed

to be able to raise cefiderocol MIC in vitro (group 5 studies). Detection of resistance mechanisms in in vitro derived cefiderocol-resistant isolates (group 4 studies) in the absence of detection of the same mechanisms in vivo suggests that these mechanisms may not be clinically relevant (e.g., due to fitness cost in vivo [69]). Similarly, confirmation in vitro that specific resistance determinants can raise cefiderocol MIC (group 5 studies) does not necessarily mean that these mechanisms are relevant/common in vivo. Finally, detection of potential resistance determinants in clinical isolates does not, alone, confirm that the detected mechanism truly contributes to cefiderocol nonsusceptibility. More than one mutation in potentially relevant resistance genes may be present simultaneously, and the relative (if any) contribution to cefiderocol nonsusceptibility of each mutation would be unclear in group 1–2 studies (and to a lesser extent in group 3 studies).

4.5. Synthesis of Results

A qualitative synthesis of the data was conducted. The various potential resistance mechanisms were recorded, and the relevant evidence supporting each reported mechanism was assessed as described above.

5. Conclusions

Although cefiderocol appears to retain activity against most XDR Gram-negative bacteria, resistance is increasingly being reported and is high in some cohorts. Various mechanisms of resistance have been identified, including β-lactamases, mutations affecting siderophore receptors, mutations affecting porins and efflux pumps, and mutations in PBP-3 (the target of cefiderocol). However, on many occasions, the mechanism of resistance remains unclear or appears to result from a combination of mechanisms. Especially worrisome are the emergence of various β-lactamases able to cause multifold increases in cefiderocol MIC and the high prevalence of cefiderocol resistance in the presence of selected β-lactamases (mainly NDM-1, KPC-variants conferring resistance to ceftazidime/avibactam, and OXA-427). Heteroresistance is also highly prevalent, mainly in *A. baumannii*, but its clinical impact is yet unclear, and emergence of resistance during treatment is uncommon based on available data. Continued surveillance of cefiderocol activity is important as this agent is being introduced in clinical practice.

Appendix A

Table A1. Role of β-lactamases in cefiderocol resistance.

β-Lactamase	Organism(s)	Findings
NDM	*Enterobacterales, A. baumannii*	**Based on isogenic mutants:** ○ Up to 64-fold increases in cefiderocol MIC have been reported by introduction of NDM MBLs in isogenic *E. coli* mutants: 8- to 64-fold increase by NDM-1 (0.5→4 mg/L [64]) (≤0.125→1 mg/L [56]) (0.03→1 mg/L [54]) (0.06→4 mg/L [29]) [29,54], 8- to 32-fold by NDM-5 (≤0.125→1 mg/L [56]) (0.125→2 mg/L [64]) (0.03→1 mg/L [54]), ≥8-fold (≤0.125→1 mg/L) by NDM-7 [56], and 16- to 64-fold by NDM-9 (≤0.125→1 mg/L [56]) (0.03→2 mg/L [54]). ○ In a *K. pneumoniae* mutant, an 8-fold increase in MIC (0.5→4 mg/L) was demonstrated by NDM-5 [64]. ○ In *A. baumannii* mutants, a ≥16-fold increase in MIC (≤0.125→2) was demonstrated by NDM-1 and NDM-9, as was a 4-fold increase (≤0.125→0.5 mg/L) by NDM-5 [54]. ○ In *P. aeruginosa*: 8-fold higher (0.5→4 mg/L) MIC by NDM-1, NDM-7, and NDM-9 and 4-fold higher (0.5→2 mg/L) by NDM-5 [56]. **Based on clinical isolates:** ○ In SIDERO-WT-2014 42% (5/12) of NDM-positive Enterobacterales were nonsusceptible to cefiderocol (MIC > 4 mg/L) [12]. ○ In a multinational European collection, 49% (18/37) of NDM-positive Enterobacterales were nonsusceptible (MIC > 2 mg/L) to cefiderocol [62]. ○ In a cohort from the United Kingdom, 59% and 28% of n = 61 NDM-positive Enterobacterales had an MIC > 2 mg/dL and >4 mg/dL, respectively [19]. Among n = 11 NDM-positive *P. aeruginosa*, 54% had MIC > 2 mg/dL, and 27% had MIC > 4 mg/dL) [19]. Among n = 20 NDM-positive *A. baumannii*, 50% had MIC > 2 mg/dL, and 20% had MIC > 4 mg/dL) [19]. ○ In the case of in vivo emerging cefiderocol resistance (intraabdominal abscesses by NDM-5 producing *E. coli* being treated with cefiderocol), a >16-fold increase in MIC (2→>32 mg/L) was observed associated with increased copy numbers of *blaNDM* genes) [25]. ○ NDM-1 was detected in a cefiderocol-resistant *K. pneumoniae* (in combination with SHV-12 and DHA-1) [64]. ○ NDM-5 was detected in in a cefiderocol-resistant *K. pneumoniae* (in combination with CirA deficiency) [64]. ○ A combination of NDM-1 and PER-1 was detected in a pandrug-resistant *Providencia rettgeri* clinical isolate [78]. ○ A combination of NDM-1 and TMB-1 was found in a cefiderocol-resistant (MIC = 32 mg/L) *A. baumannii* [79].
KPC-variants (conferring resistance to ceftazidime/avibactam)	*K. pneumoniae, E. coli* *	**Based on isogenic mutants:** ○ Introduction of KPC-variants in *E. coli* mutants resulted in multifold increases in cefiderocol MIC: ≥8-fold (≤0.125→1) by KPC-3 [55], ≥32-fold (≤0.125→4) by KPC-41 [55], ≥8-fold (≤0.125→1) by KPC-50 [55], 64-fold (0.06→4 mg/L) by KPC-31 [41], 32-fold (0.06→2 mg/L) by KPC-33 [41], 16-fold (0.06→1 mg/L) by KPC-39 [41], 8-fold (0.06→0.5 mg/L) by KPC-44 and KPC-29 [41], and 4-fold (0.06→0.25 mg/L) by KPC-25 [41]. ○ Introduction of KPC-2 in *K. pneumoniae* resulted in 4-fold higher cefiderocol MIC (0.5→2 mg/L) [64]. **Based on clinical isolates:** ○ Cefiderocol resistance (MIC > 2 mg/L) was considerably higher (82.5% vs. 6.7%) in ceftazidime/avibactam-resistant (n = 40) than in ceftazidime/avibactam-susceptible (n = 60) KPC-producing Enterobacterales in one cohort [14]. ○ Among 17 paired (before and after ceftazidime/avibactam treatment) KPC-producing *K. pneumoniae* isolates, 2- to 512-fold higher cefiderocol MICs were noted (0.25→4, 0.25→8, 16→32, 1→4, 1→16, 0.12→64, 0.25→64, 0.25→32, 0.12→4, 1→32, 1→16, 0.25→0.5, 2→32, 1→16, 0.5→>64, 0.25→4, 0.12→4 mg/L) [14]. ○ Emergence of cross-resistance between ceftazidime/avibactam and cefiderocol was reported in two clinical associates associated with mutations in KPC (KPC-41 and KPC-50). MIC was 2- to 4-fold higher (2→ 4–8 mg/L) in the KPC-41 mutant and 8-fold higher in the KPC-50 mutant (2→16 mg/L). In both strains, truncation of OmpK35 was also detected [55]. ○ Emergence of cross-resistance between ceftazidime/avibactam and cefiderocol was reported in another case associated with KPC-31, resulting in a 4-fold higher cefiderocol MIC (4→16 mg/L) [42].

Table A1. *Cont.*

β-Lactamase	Organism(s)	Findings
PER-type ESBL	*A. baumannii*, *P. aeruginosa*, *E. coli* *	- Based on isogenic mutants: ○ Introduction of PER-1 in isogenic *E. coli* mutants was reported to result in 8- to 16-fold higher cefiderocol MICs (0.063–0.125→1 mg/L [29]). ○ In another study [56], introduction of PER-1, PER-6, and PER-7 in isogenic *E. coli* resulted in ≥32-fold (≤0.125→4 mg/L) higher MIC, while PER-2 resulted in ≥8-fold (≤0.125→1 mg/L) [56]. In *P. aeruginosa*: 32-fold higher (0.5→16 mg/L) MIC by PER-1, 16-fold higher (0.5→8 mg/L) by PER-6 and -7, and 2-fold higher (0.5→1 mg/L) by PER-2. ○ Similarly, a 64-fold increase in MIC (0.03→2 mg/L) was demonstrated by introduction of PER-1 in isogenic *A. baumannii* mutants [54]. - Based on clinical isolates: ○ PER ESBLs were detected in 25 of 28 cefiderocol-nonsusceptible *A. baumannii* in one study (all PER-1) [29], in all 8 nonsusceptible *A. baumannii* isolates in another study (either PER-1 or PER-7) [54], in 5 of 24 cefiderocol nonsusceptible *A. baumannii* in another study [27], but only in 1 of 21 cefiderocol-resistant *A. baumannii* isolates in another single-centre cohort [30]. ○ In a cohort from the United Kingdom, 33% (5 of 15) and 27% (4 of 15) of PER-producing *P. aeruginosa* isolates had a cefiderocol MIC >2 mg/dL and >4 mg/L, respectively [19].
SHV-type ESBL	*K. pneumoniae*, *A. baumannii*, *E. coli* *	- Based on isogenic mutants: ○ Introduction in *E. coli*: 8-fold higher (0.03→0.25 mg/L) MIC by SHV-2 [54]; ≥2-fold higher (≤0.125→0.25 mg/L) by SHV-2a [56]; 2- to 4-fold higher (0.063–0.125→0.25 mg/L) by SHV-1 [29]; 4- to 8-fold higher (0.063–0.125→0.5 mg/L) by SHV-4, SHV-12, and SHV-5 [29]; and ≥32-fold (≤0.125→4 mg/L) by SHV-12 in another study [56]. Up to 2-fold higher (0.063–0.125→0.125 mg/L) by SHV-3, SHV-11, SHV-26, and SHV-28 [29]. ○ Introduction in *P. aeruginosa*: 8-fold (0.5→4 mg/L) higher MIC by SHV-2a, 16-fold (0.5→8 mg/L) higher MIC by SHV-12 [29]. - Based on clinical isolates: ○ Iregui et al. [17] showed that *K. pneumoniae* and *A. baumannii* isolates expressing the SHV extended-spectrum β-lactamase (ESBL) had significantly higher MICs than isolates lacking SHV ESBL. ○ Coexpression of NDM-1 and SHV was detected in four of five cefiderocol nonsusceptible *K. pneumoniae* in one cohort [29].
AmpC variants	*Enterobacter* spp. *P. aeruginosa* * *E. coli* *	- Based on isogenic mutants: ○ 32-fold increase in cefiderocol MIC (0.06→2 mg/L) by introduction of A292_L293del AmpC in *E. coli* [43]. ○ 4-fold increase (0.5→2 mg/L) by introduction of A294_P295del AmpC in *E. coli* [43]. ○ 4-fold increase (0.25→1 mg/L) by overexpression of AmpC in *E. cloacae* [48]. ○ 2-fold increase (0.125→0.25 mg/L) by introduction of ACT-17-like (A313P) in *E. coli* [32]. - Based on clinical isolates: ○ In vivo emerging cefiderocol cross-resistance attributable to *AmpC* mutations was reported in two patients with *E. cloacae* infections being treated with ceftazidime/avibactam [43]. ○ *AmpC* E247K (emerging during treatment with ceftolozane/tazobactam) was detected in two ceftolozane/tazobactam-resistant *P. aeruginosa* isolates and was associated with 32-fold (0.25→8 mg/L) and 8-fold (0.12→1 mg/L) higher cefiderocol MIC [16]. ○ *AmpC* L147F (emerging during treatment with ceftolozane/tazobactam) in combination with mutations in *piuA* and *pirR* was detected in a ceftolozane/tazobactam-resistant *P. aeruginosa* isolates and was associated with a 4-fold (2→8 mg/L) higher cefiderocol MIC [44]. ○ ACT-17-like (A313P) was detected in an in vivo emerging cefiderocol-resistant *E. cloacae* (MIC1→4 mg/L) [21,32].

Table A1. *Cont.*

β-Lactamase	Organism(s)	Findings

OXA-427	**Enterobacterales**	• Based on isogenic mutants:
		  ○ Introduction of OXA-427 in *E. coli* resulted in only a 2-fold increase in (MIC ≤0.125→0.25 mg/L) [56].
		• Based on clinical isolates:
		  ○ Uniform cefiderocol nonsusceptibility (based on disk diffusion) was reported among n = 26 OXA-427-producing Enterobacterales from Belgium [15].

SPM-1, VIM-2, AIM-1, GIM-1 (MBLs)	*E. coli* *, *P. aeruginosa* *	• Based on isogenic mutants:
		  ○ In *E. coli*: ≥16-fold higher MIC (≤0.125→2 mg/L) by SPM-1; no change by VIM-2, AIM-1, or GIM-1 [56].
		  ○ In *P. aeruginosa*: 16-fold higher MIC (0.5→8 mg/L) by SPM-1, 2-fold higher (0.5→1 mg/L) by VIM-2, and 4-fold higher (0.5→2 mg/L) by AIM-1 and GIM-1 [56].

GES-6	*P. aeruginosa* *	• Introduction of GES-6 in *P. aeruginosa* resulted in 4-fold (0.5→2 mg/L) higher cefiderocol MICs [56].

PDC-30 (*P. aeruginosa* cephalosporinase)	***P. aeruginosa***	• Based on isogenic mutants:
		  ○ 8-fold increase (0.125→1 mg/L) by introduction of PDC-30 in *E. coli* [32]
		• Based on clinical isolates:
		  ○ PDC-30 was detected in an in vivo emerging cefiderocol-resistant *P. aeruginosa* (MIC1→4 mg/L) [32]
		  ○ PDC-30 mutation was in a *P. aeruginosa* clinical isolate after treatment with cefiderocol and was associated with an 8-fold higher cefiderocol MIC [21]

ADC variants (cephalosporinase), OXA-66, (OXA 23)	*A. baumannii*	• Based on clinical isolates:
		  ○ Acquired ADC variants and OXA-23 were detected in all six cefiderocol resistant isolates [63].
		  ○ ADC variants were detected in all 28 cefiderocol resistant isolates, OXA-23 in 15 isolates, and OXA-66 in 24 [29].
		  ○ ADC-30 homologues, OXA-23, and OXA-66 were detected in all 20 cefiderocol-resistant clinical isolates [30].
		
		Note: Based on isogenic mutants, introduction of OXA-23 in *A. baumannii* and *E. coli* was not shown to affect cefiderocol MIC [29,32,54,66]. Furthermore, cefiderocol has been shown to be stable against OXA-23 [66].

BEL *	*E. coli* *, *P. aeruginosa* *	• Based on isogenic *E. coli* mutants:
		  ○ 16-fold MIC (0.03→0.5 mg/L) by BEL-2 [54], ≥4-fold-higher (≤0.125→0.5 mg/L) by BEL-2 in another study [56], and ≥2-fold-higher (≤0.125→0.25 mg/L) by BEL-1 [56].
		• Based on isogenic *P. aeruginosa* mutants:
		  ○ 4-fold (0.5→2 mg/L) higher MIC by BEL-1 and 8-fold (0.5→4 mg/L) by BEL-2 [56].

CTX-M-27 *	*E. coli* *	Introduction of CTX-M-27 in *E. coli* was associated with a 4- to 8-fold higher cefiderocol MIC (0.063–0.125→0.5) [29].

In **bold** are mechanisms of resistance of which the role has been confirmed in isogenic mutant experiments (group 5 studies, see "Eligibility criteria" in Methods) and that have been detected in cefiderocol-resistant clinical isolates (group 1–3 studies, see "Eligibility criteria" in Methods). * Based only on in vitro isogenic mutant experiment (group 5 studies, see "Eligibility criteria" in Methods).

Table A2. Resistance mediated by mutations affecting siderophore receptors.

Target Gene	Organism(s)	Findings
piuA, *piuD*, *pirA*	*P. aeruginosa*, *A. baumannii*	• Based on isogenic *P. aeruginosa* mutants: ○ Deficiency of PiuA resulted in 16-fold higher cefiderocol MIC (0.125→2 mg/L) [47]. In the same strain, deficiency of *pirA* (either alone or in addition to *piuA*) did not affect cefiderocol MIC [47]. ○ Deletion of *piuA* resulted in a 16-fold higher MIC (0.5→8 mg/L) and a 32-fold higher MIC (0.5→16 mg/L) when combined with deletion of *pirA* [50]. Deletion of *pirA* alone in the same strain had no effect on cefiderocol MIC [50]. ○ Deletion of *piuD* resulted in a 32-fold higher cefiderocol MIC (0.06→2 mg/L) and 64-fold a higher MIC (0.06→4 mg/L) when combined with deletion of *pirA* [50]. Deletion of *pirA* alone in the same strain resulted in a 2-fold higher MIC (0.06→0.125 mg/L) [50]. • Based on clinical isolates: ○ *AmpC* L147F (emerging during treatment with ceftolozane/tazobactam) in combination with mutations in *piuA* and *pirR* was detected in a ceftolozane/tazobactam-resistant *P. aeruginosa* isolate and was associated with a 4-fold (2→8 mg/L) higher cefiderocol MIC [44]. ○ In a collection of six cefiderocol-resistant *A. baumannii* clinical isolates, expression of both PirA and PiuA was absent in three. In one, only expression of PiuA was absent (in combination with measurable but reduced PirA expression), and in the remaining three, mutations in both *pirA* and *piuA* were detected [52]. ○ In another collection of 12 cefiderocol-resistant *A. baumannii* clinical isolates, deficiency of PiuA was detected in 12 [30].
fecI **	*P. aeruginosa* **	• In an <u>in vitro derived cefiderocol-resistant strain</u> with a 4-fold higher MIC (0.5→2 mg/dL), mutations in *fecI* were identified. *fecI* regulates the synthesis of the iron transporter FecA, contributing to transport of iron citrate. *fecA* expression was 9-fold higher in the *fecI* mutant [46].
cirA, *fiu* *	*E. coli*, *K. pneumoniae*, *E. cloacae* **	• Based on isogenic mutants: ○ *E. coli*: Deletion of *fiu* alone resulted in a 2-fold higher cefiderocol MIC (0.063→0.125 mg/L) and a 16-fold higher MIC when combined with deletion of *cirA* (0.063→1 mg/L) [47]. Deletion of *cirA* alone did not affect the MIC [47]. ○ *K. pneumoniae*: Loss of CirA resulted in 4-fold higher cefiderocol MIC (0.5→2 mg/L) [57]. • Based on in vitro derived (after serial passaging in the presence of cefiderocol) <u>cefiderocol-resistant mutants</u>: ○ In all five resistant *E. clocae* mutants (MIC 4→>128 mg/L), various mutations affecting the *cirA* gene were detected and were not associated with fitness cost [40]. ○ A mutated *cirA* gene was detected in a *K. pneumoniae* mutant (MIC 2→>128 mg/L) [57]. • Based on clinical isolates: ○ Comparing a cefiderocol-resistant *K. pneumoniae* isolate (emerging in vivo after cefiderocol treatment) with its parental strain, various mutations affecting *cirA* were detected. *cirA* was the only gene mutating between the two strains [24]. ○ *CirA* mutations were also detected in six cefiderocol-resistant *E. coli* clinical isolates (in combination with NDM and PMB-3 mutations) [59]. ○ CirA deficiency was detected in a cefiderocol-resistant *K. pneumoniae* (in combination with NDM-5) [64].
fhuA, *fepA*, *fecA* **, *fbpA* **, *efeO* **, *exbD* **	*K. pneumoniae*	• Based on in vitro derived cefiderocol-resistant mutants: ○ Mutations were detected in *efeo* (iron uptake system component), *fecA* (tonB-dependent receptor), and *fbpA* (ferric iron ABC transporter) [28]. ○ Mutation of *exbD* (an accessory protein related to iron transport) was associated with a >8-fold increase in cefiderocol MIC (4→>32 mg/L) [31]. • Based on clinical isolates: ○ Mutations/deletions in *fhuA* (ferrichrome iron receptor) or *fepA* (ferric enterobactin receptor) were detected by whole genome sequencing in two clinical isolates with cefiderocol MIC 2 mg/L [61].

Table A2. *Cont.*

Target Gene	Organism(s)	Findings
tonB **, *exbD* **, *smlat1148* **, *cirA* **	*S. maltophilia* **	• Based on in vitro derived cefiderocol-resistant mutants: ○ Among 31 mutants, 25 had mutations in *tonB*, and 3 had mutations in exbD. These mutations were associated with fitness cost and were reversible [58]. ○ *tonB*, *cirA*, and *smlat1148* mutations were detected separately in three mutants [60]

In bold are mechanisms of resistance of which the role has been confirmed in isogenic mutant experiments (group 5 studies, see "Eligibility criteria" in Methods) and that have been detected in cefiderocol-resistant clinical isolates (group 1–3 studies, see "Eligibility criteria" in Methods). * Based only on in vitro isogenic mutant experiments (group 5 studies, see "Eligibility criteria" in Methods). ** Based only on in vitro derived mutants (group 4 studies, see "Eligibility criteria" in Methods).

Table A3. Resistance mediated by mutations affecting function/expression of porins and efflux pumps.

Target Genes/Involved Porins/Efflux Pumps	Organism(s)	Findings
ompK35, ompK36, ompK37 (porins)	**K. pneumoniae**	• Based on isogenic mutants: ○ 4-fold higher MIC (0.031→0.125 mg/L) by deletion of *ompK35* and 2-fold higher MIC (0.031→0.063 mg/L) by deletion of *ompK36* (with or without concurrent deletion of *ompK35*) [47]. • Based on clinical isolates: ○ *ompK37* mutations were identified in all n = 7 cefiderocol-resistant *K. pneumoniae* isolates (in combination with *ompK36* mutations in 6) [37] ○ Truncation of *ompK35* was detected in two cefiderocol-resistant clinical isolates in combinations with KPC variants known to contribute to cefiderocol resistance [55]. ○ In two cefiderocol-resistant *K. pneumoniae* isolates, disruption of OmpK35, OmpK36, and OmpK37 was detected in combination with various β-lactamases [53].
ompC, ompF (porins)	*Enterobacter* spp.	• Based on clinical isolates: ○ Alterations in *ompC* and *ompF* (in combination with AmpC and ESBLs) were detected in four cefiderocol-resistant *Enterobacter* spp. isolates [53]. ○ *ompC* deletions/truncation were found in three of six cefiderocol-resistant *Enterobacter* spp. in one study [37].
oprD (porin)	**P. aeruginosa**	• Based on isogenic mutants: ○ Mutation in porin *oprD* was associated with a 2-fold higher MIC (0.125→0.25 mg/L) [47]. • Based on clinical isolates: ○ Opr-D truncation was detected in two in vivo emerging (after cefiderocol treatment) cefiderocol-resistant clinical *P. aeruginosa* isolates (MIC 0.25→4 mg/L in both) [21].
ChrA (heavy metal iron transporter), *SugE* (efflux pump)	*K. pneumoniae*	n = 7 cefiderocol-resistant (MIC > 2 mg/L) and CR *K. pneumoniae* clinical isolates were compared by WGS with n = 8 cefiderocol-susceptible CR *K. pneumoniae* isolates. ChrA expression was detected in five of seven cefiderocol-resistant isolates but only one of eight cefiderocol-susceptible isolates [37]. SugE expression was detected in two of seven cefiderocol-resistant isolates but none of the cefiderocol-susceptible isolates [37].
mexR or *nalD* (repressors of MexAB–OprM efflux pump) *	*P. aeruginosa* *	Based on isogenic mutants: mutations in mexR or nalD leading to overexpression of the MexAB–OprM efflux pump were associated with 2-fold higher cefiderocol MIC (0.125→0.25 mg/L) in *P. aeruginosa*, while mutations in *mexB* or *oprM* (resulting in loss of function of the MexAB–OprM efflux pump) resulted in a 2-fold lower cefiderocol MIC [47].
smeT **	*S. maltophilia* **	SmeT promoter mutation (resulting in overexpression of the efflux pump smeDEF) was detected in an in vitro derived cefiderocol-resistant mutant [60].
AxyABM (efflux pump) *	*A. xylosoxidans* *	Overexpression of AxyABM was associated with a 3-fold higher cefiderocol MIC when comparing two isogenic *A. xylosoxidans* isolates (0.032→0.094 mg/L). Disruption of the efflux pump was associated with 2- to 23.5-fold lower cefiderocol MICs [51].

In bold are mechanisms of resistance of which the role has been confirmed in isogenic mutant experiments (group 5 studies, see "Eligibility criteria" in Methods) and that have been detected in cefiderocol-resistant clinical isolates (group 1–3 studies, see "Eligibility criteria" in Methods). * Based only on in vitro isogenic mutant experiments (group 5 studies, see "Eligibility criteria" in Methods). ** Based only on in vitro derived mutants (group 4 studies, see "Eligibility criteria" in Methods).

Table A4. Other mutations associated with cefiderocol resistance.

Target Gene	Organism(s)	Findings

PBP-3 (=target of cefiderocol)	***E. coli*** , ***A. baumannii***	• Based on isogenic *E. coli* mutants:
		  ○ Introduction of either YRIN or YRIK insertion in PBP-3 resulted in 2-fold higher cefiderocol MIC (0.063→0.125 mg/L) [31].
		• Based on clinical isolates:
		  ○ In a collection of nine *E. coli* isolates (all from a single hospital in Turkey) with specific PBP-3 mutations (YRIN insertion after position P333 and I532L substitution), a raised cefiderocol MIC was observed (MIC 5 mg/L in two isolates, 2 mg/L in four isolates, and 4 mg/L in two isolates) [31].
		  ○ PBP3 mutations (including YRIN insertion at position P33) were also detected in six other cefiderocol-resistant *E. coli* (in combination with NDM and *cirA* mutations) [59].
		  ○ A mutation (predicted to have a moderate likelihood of affecting functionality) in PBP-3 was detected in one of six cefiderocol-resistant *A. baumannii* clinical isolates in one study [52].
		  ○ Mutations in PBP-3 were found in four cefiderocol-resistant *A. baumannii* isolates (in combination with various β-lactamases) [30].
		  ○ Mutation in PBP-3 (H370Y) was found in a cefiderocol-resistant *A. baumannii* emerging in vivo after cefiderocol treatment [21,32].

baeS (a sensor of a two-component regulation system)	*K. pneumoniae*	• In three <u>in vitro derived mutants</u>, mutations of *baeS* were associated with 4- to 32-fold increases in cefiderocol MIC (0.063→2 mg/L, 4→16 mg/L, and 4→32 mg/L) [31]. • *baeS* mutations were identified in all seven cefiderocol-resistant (MIC > 2 mg/L) <u>clinical isolates</u> in a single-centre study [37].
envZ (a sensor of a two-component regulation system) **	*K. pneumoniae* **, *E. coli*	• In two <u>in vitro derived mutants</u>, mutations of *envZ* (a sensor of a two-component regulation system) were associated with 4-fold increases in cefiderocol MIC (4→16 mg/L) [31]. • In one case, in vivo emerging resistance was described in an *E. coli* <u>clinical isolate</u> associated with increased copy numbers of NDM-5 in combination with *envZ* mutations (but the additional role of *envZ* appeared to be minor).
yicM (putative membrane transport protein)	*K. pneumoniae*	Mutations in yicM were detected in two of six cefiderocol-resistant *K. pneumoniae* clinical isolates [37].
tolQ (membrane transporter), smf-1 (affects fimbriae and surface adhesion) **	*S. maltophilia* **	*tolQ* and *smf-1* mutations were each found in two separate <u>in vitro derived mutants</u> [60].
PmrB, mcr-10		A higher prevalence of colistin resistance (29% vs. 0%) was reported in cefiderocol-resistant than in susceptible *K. pneumoniae* clinical isolates [37]. Furthermore, *PmrB* mutations (known to be involved in cefiderocol resistance [69]) were identified in four of seven (57%) cefiderocol-resistant *K. pneumoniae* isolates, while the *mcr-10* gene was identified in half (three of six) of cefiderocol-resistant *E. cloacae* isolates [37]. A reduction in the net negative charge (associated with cefiderocol resistance) could also affect cefiderocol, but future studies are necessary to confirm this hypothetical mechanism [37].

In bold are mechanisms of resistance of which the role has been confirmed in isogenic mutant experiments (group 5 studies, see "Eligibility criteria" in Methods) and that have been detected in cefiderocol-resistant clinical isolates (group 1–3 studies, see "Eligibility criteria" in Methods). ** Based only on in vitro derived mutants (group 4 studies, see "Eligibility criteria" in Methods).

References

1. Karakonstantis, S.; I Kritsotakis, E.; Gikas, A. Pandrug-resistant Gram-negative bacteria: A systematic review of current epidemiology, prognosis and treatment options. *J. Antimicrob. Chemother.* **2020**, *75*, 271–282. [CrossRef]
2. Karakonstantis, S.; Gikas, A.; Astrinaki, E.; Kritsotakis, E.I. Excess mortality due to pandrug-resistant Acinetobacter baumannii infections in hospitalized patients. *J. Hosp. Infect.* **2020**, *106*, 447–453. [CrossRef]
3. Karakonstantis, S.; I Kritsotakis, E.; Gikas, A. Treatment options for K. pneumoniae, P. aeruginosa and A. baumannii co-resistant to carbapenems, aminoglycosides, polymyxins and tigecycline: An approach based on the mechanisms of resistance to carbapenems. *Infection* **2020**, *48*, 835–851. [CrossRef]

4. Karakonstantis, S.; Ioannou, P.; Samonis, G.; Kofteridis, D.P. Systematic Review of Antimicrobial Combination Options for Pandrug-Resistant *Acinetobacter baumannii*. *Antibiotics* **2021**, *10*, 1344. [CrossRef]

5. Karakonstantis, S.; Ioannou, P.; Kofteridis, D.D. In search for a synergistic combination against pandrug-resistant A. baumannii; methodological considerations. *Infection* **2022**, 1–13. [CrossRef]

6. Sato, T.; Yamawaki, K. Cefiderocol: Discovery, Chemistry, and In Vivo Profiles of a Novel Siderophore Cephalosporin. *Clin. Infect. Dis.* **2019**, *69*, S538–S543. [CrossRef]

7. Aoki, T.; Yoshizawa, H.; Yamawaki, K.; Yokoo, K.; Sato, J.; Hisakawa, S.; Hasegawa, Y.; Kusano, H.; Sano, M.; Sugimoto, H.; et al. Cefiderocol (S-649266), A new siderophore cephalosporin exhibiting potent activities against Pseudomonas aeruginosa and other gram-negative pathogens including multi-drug resistant bacteria: Structure activity relationship. *Eur. J. Med. Chem.* **2018**, *155*, 847–868. [CrossRef]

8. Karlowsky, J.A.; Hackel, M.A.; Takemura, M.; Yamano, Y.; Echols, R.; Sahm, D.F. In Vitro Susceptibility of Gram-Negative Pathogens to Cefiderocol in Five Consecutive Annual Multinational SIDERO-WT Surveillance Studies, 2014 to 2019. *Antimicrob. Agents Chemother.* **2022**, *66*, e0199021. [CrossRef]

9. Candel, F.J.; Henriksen, A.S.; Longshaw, C.; Yamano, Y.; Oliver, A. In vitro activity of the novel siderophore cephalosporin, cefiderocol, in Gram-negative pathogens in Europe by site of infection. *Clin. Microbiol. Infect.* **2022**, *28*, 447.e1–447.e6. [CrossRef]

10. Hackel, M.A.; Tsuji, M.; Yamano, Y.; Echols, R.; Karlowsky, J.A.; Sahm, D.F. In Vitro Activity of the Siderophore Cephalosporin, Cefiderocol, against a Recent Collection of Clinically Relevant Gram-Negative Bacilli from North America and Europe, Including Carbapenem-Nonsusceptible Isolates (SIDERO-WT-2014 Study). *Antimicrob. Agents Chemother.* **2017**, *61*, e00093-17. [CrossRef]

11. Karlowsky, J.A.; Hackel, M.A.; Tsuji, M.; Yamano, Y.; Echols, R.; Sahm, D.F. 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* **2018**, *53*, 456–466. [CrossRef]

12. Kazmierczak, K.M.; Tsuji, M.; Wise, M.G.; Hackel, M.; Yamano, Y.; Echols, R.; Sahm, D.F. In vitro activity of cefiderocol, a siderophore cephalosporin, against a recent collection of clinically relevant carbapenem-non-susceptible Gram-negative bacilli, including serine carbapenemase- and metallo-β-lactamase-producing isolates (SIDERO-WT-2014 Study). *Int. J. Antimicrob. Agents* **2018**, *53*, 177–184. [CrossRef]

13. Golden, A.R.; Adam, H.J.; Baxter, M.; Walkty, A.; Lagacé-Wiens, P.; Karlowsky, J.A.; Zhanel, G.G. In Vitro Activity of Cefiderocol, a Novel Siderophore Cephalosporin, against Gram-Negative Bacilli Isolated from Patients in Canadian Intensive Care Units. *Diagn. Microbiol. Infect. Dis.* **2020**, *97*, 115012. [CrossRef]

14. Bianco, G.; Boattini, M.; Comini, S.; Iannaccone, M.; Bondi, A.; Cavallo, R.; Costa, C. In vitro activity of cefiderocol against ceftazidime-avibactam susceptible and resistant KPC-producing Enterobacterales: Cross-resistance and synergistic effects. *Eur. J. Clin. Microbiol.* **2021**, *41*, 63–70. [CrossRef]

15. Jacob, A.-S.; Chong, G.-L.; Lagrou, K.; Depypere, M.; Desmet, S. No in vitro activity of cefiderocol against OXA-427-producing Enterobacterales. *J. Antimicrob. Chemother.* **2021**, *76*, 3317–3318. [CrossRef] [PubMed]

16. Simner, P.J.; Beisken, S.; Bergman, Y.; E Posch, A.; E Cosgrove, S.; Tamma, P.D. Cefiderocol Activity Against Clinical *Pseudomonas aeruginosa* Isolates Exhibiting Ceftolozane-Tazobactam Resistance. *Open Forum Infect. Dis.* **2021**, *8*, ofab311. [CrossRef]

17. Iregui, A.; Khan, Z.; Landman, D.; Quale, J. Activity of Cefiderocol Against *Enterobacterales*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* Endemic to Medical Centers in New York City. *Microb. Drug Resist.* **2020**, *26*, 722–726. [CrossRef]

18. Morris, C.P.; Bergman, Y.; Tekle, T.; Fissel, J.A.; Tamma, P.D.; Simner, P.J. 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. [CrossRef]

19. Mushtaq, S.; Sadouki, Z.; Vickers, A.; Livermore, D.M.; Woodford, N. In Vitro Activity of Cefiderocol, a Siderophore Cephalosporin, against Multidrug-Resistant Gram-Negative Bacteria. *Antimicrob. Agents Chemother.* **2020**, *64*, 12. [CrossRef]

20. E Choby, J.; Ozturk, T.; Satola, S.W.; Jacob, J.T.; Weiss, D.S. Widespread cefiderocol heteroresistance in carbapenem-resistant Gram-negative pathogens. *Lancet Infect. Dis.* **2021**, *21*, 597–598. [CrossRef]

21. Takemura, M.; Yamano, Y.; Matsunaga, Y.; Ariyasu, M.; Echols, R.; Nagata, T.D. 1266. Characterization of Shifts in Minimum Inhibitory Concentrations During Treatment with Cefiderocol or Comparators in the Phase 3 CREDIBLE-CR and APEKS-NP Studies. *Open Forum Infect. Dis.* **2020**, *7*, S649–S650. [CrossRef]

22. Wunderink, R.G.; Matsunaga, Y.; Ariyasu, M.; Clevenbergh, P.; Echols, R.; Kaye, K.S.; Kollef, M.; Menon, A.; Pogue, J.M.; Shorr, A.F.; et al. Cefiderocol versus high-dose, extended-infusion meropenem for the treatment of Gram-negative nosocomial pneumonia (APEKS-NP): A randomised, double-blind, phase 3, non-inferiority trial. *Lancet Infect. Dis.* **2020**, *21*, 213–225. [CrossRef]

23. Bassetti, M.; Echols, R.; Matsunaga, Y.; Ariyasu, M.; Doi, Y.; Ferrer, R.; Lodise, T.P.; Naas, T.; Niki, Y.; Paterson, D.L.; et al. Efficacy and safety of cefiderocol or best available therapy for the treatment of serious infections caused by carbapenem-resistant Gram-negative bacteria (CREDIBLE-CR): A randomised, open-label, multicentre, pathogen-focused, descriptive, phase 3 trial. *Lancet Infect. Dis.* **2021**, *21*, 226–240. [CrossRef]

24. Klein, S.; Boutin, S.; Kocer, K.; O Fiedler, M.; Störzinger, D.; A Weigand, M.; Tan, B.; Richter, D.; Rupp, C.; Mieth, M.; et al. Rapid Development of Cefiderocol Resistance in Carbapenem-resistant *Enterobacter cloacae* During Therapy Is Associated With Heterogeneous Mutations in the Catecholate Siderophore Receptor *cirA*. *Clin. Infect. Dis.* **2021**, *74*, 905–908. [CrossRef]

25. Simner, P.J.; Mostafa, H.H.; Bergman, Y.; Ante, M.; Tekle, T.; Adebayo, A.; Beisken, S.; Dzintars, K.; Tamma, P.D. Progressive Development of Cefiderocol Resistance in *Escherichia coli* During Therapy is Associated With an Increase in blaNDM-5 Copy Number and Gene Expression. *Clin. Infect. Dis.* **2021**, ciab888. [CrossRef]

26. Meschiari, M.; Volpi, S.; Faltoni, M.; Dolci, G.; Orlando, G.; Franceschini, E.; Menozzi, M.; Sarti, M.; Del Fabro, G.; Fumarola, B.; et al. Real-life experience with compassionate use of cefiderocol for difficult-to-treat resistant *Pseudomonas aeruginosa* (DTR-P) infections. *JAC-Antimicrob. Resist.* **2021**, *3*, dlab188. [CrossRef]

27. Ballesté-Delpierre, C.; Ramírez, Á.; Muñoz, L.; Longshaw, C.; Roca, I.; Vila, J. Assessment of In Vitro Cefiderocol Susceptibility and Comparators against an Epidemiologically Diverse Collection of *Acinetobacter baumannii* Clinical Isolates. *Antibiotics* **2022**, *11*, 187. [CrossRef]

28. Bao, J.; Xie, L.; Ma, Y.; An, R.; Gu, B.; Wang, C. Proteomic and Transcriptomic Analyses Indicate Reduced Biofilm-Forming Abilities in Cefiderocol-Resistant Klebsiella pneumoniae. *Front. Microbiol.* **2022**, *12*, 778190. [CrossRef]

29. Kohira, N.; Hackel, M.A.; Ishioka, Y.; Kuroiwa, M.; Sahm, D.F.; Sato, T.; Maki, H.; Yamano, Y. Reduced susceptibility mechanism to cefiderocol, a siderophore cephalosporin, among clinical isolates from a global surveillance programme (SIDERO-WT-2014). *J. Glob. Antimicrob. Resist.* **2020**, *22*, 738–741. [CrossRef]

30. Yamano, Y.; Ishibashi, N.; Kuroiwa, M.; Takemura, M.; Sheng, W.-H.; Hsueh, P.-R. Characterisation of cefiderocol-non-susceptible Acinetobacter baumannii isolates from Taiwan. *J. Glob. Antimicrob. Resist.* **2021**, *28*, 120–124. [CrossRef]

31. Sato, T.; Ito, A.; Ishioka, Y.; Matsumoto, S.; Rokushima, M.; Kazmierczak, K.M.; Hackel, M.; Sahm, D.F.; Yamano, Y. *Escherichia coli* strains possessing a four amino acid YRIN insertion in PBP3 identified as part of the SIDERO-WT-2014 surveillance study. *JAC-Antimicrob. Resist.* **2020**, *2*, dlaa081. [CrossRef] [PubMed]

32. Nordmann, P.; Shields, R.K.; Doi, Y.; Takemura, M.; Echols, R.; Matsunaga, Y.; Yamano, Y. Mechanisms of Reduced Susceptibility to Cefiderocol Among Isolates from the CREDIBLE-CR and APEKS-NP Clinical Trials. *Microb. Drug Resist.* **2022**, *28*, 398–407. [CrossRef] [PubMed]

33. E Choby, J.; Ozturk, T.; Satola, S.W.; Jacob, J.T.; Weiss, D.S. Does cefiderocol heteroresistance explain the discrepancy between the APEKS-NP and CREDIBLE-CR clinical trial results? *Lancet Microbe* **2021**, *2*, 648–649. [CrossRef]

34. Falcone, M.; Tiseo, G.; Leonildi, A.; Della Sala, L.; Vecchione, A.; Barnini, S.; Farcomeni, A.; Menichetti, F. Cefiderocol-Compared to Colistin-Based Regimens for the Treatment of Severe Infections Caused by Carbapenem-Resistant Acinetobacter baumannii. *Antimicrob. Agents Chemother.* **2022**, *66*, 0214221. [CrossRef]

35. Falcone, M.; Tiseo, G.; Nicastro, M.; Leonildi, A.; Vecchione, A.; Casella, C.; Forfori, F.; Malacarne, P.; Guarracino, F.; Barnini, S.; et al. Cefiderocol as Rescue Therapy for Acinetobacter baumannii and Other Carbapenem-resistant Gram-negative Infections in Intensive Care Unit Patients. *Clin. Infect. Dis.* **2021**, *72*, 2021–2024. [CrossRef]

36. Gatti, M.; Bartoletti, M.; Cojutti, P.G.; Gaibani, P.; Conti, M.; Giannella, M.; Viale, P.; Pea, F. A descriptive case series of pharmacokinetic/pharmacodynamic target attainment and microbiological outcome in critically ill patients with documented severe extensively drug-resistant Acinetobacter baumannii bloodstream infection and/or ventilator-associated pneumonia treated with cefiderocol. *J. Glob. Antimicrob. Resist.* **2021**, *27*, 294–298. [CrossRef]

37. Simner, P.J.; Beisken, S.; Bergman, Y.; Ante, M.; Posch, A.E.; Tamma, P.D. Defining Baseline Mechanisms of Cefiderocol Resistance in the Enterobacterales. *Microb. Drug Resist.* **2022**, *28*, 161–170. [CrossRef]

38. Perez, C.G.; Maillart, E.; Deyi, V.M.; Huang, T.-D.; Kamgang, P.; Dernier, Y.; Clevenbergh, P. Compassionate use of cefiderocol in a pancreatic abscess and emergence of resistance. *Infect. Dis. Now* **2020**, *51*, 399–401. [CrossRef]

39. Kufel, W.D.; Steele, J.M.; Riddell, S.W.; Jones, Z.; Shakeraneh, P.; Endy, T.P. Cefiderocol for treatment of an empyema due to extensively drug-resistant Pseudomonas aeruginosa: Clinical observations and susceptibility testing considerations. *IDCases* **2020**, *21*, e00863. [CrossRef]

40. 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. [CrossRef]

41. Hobson, C.A.; Cointe, A.; Jacquier, H.; Choudhury, A.; Magnan, M.; Courroux, C.; Tenaillon, O.; Bonacorsi, S.; Birgy, A. Cross-resistance to cefiderocol and ceftazidime–avibactam in KPC β-lactamase mutants and the inoculum effect. *Clin. Microbiol. Infect.* **2021**, *27*, 1172.e7–1172.e10. [CrossRef] [PubMed]

42. Tiseo, G.; Falcone, M.; Leonildi, A.; Giordano, C.; Barnini, S.; Arcari, G.; Carattoli, A.; Menichetti, F. Meropenem-Vaborbactam as Salvage Therapy for Ceftazidime-Avibactam-, Cefiderocol-Resistant ST-512 *Klebsiella pneumoniae*–Producing KPC-31, a D179Y Variant of KPC-3. *Open Forum Infect. Dis.* **2021**, *8*, ofab141. [CrossRef] [PubMed]

43. Shields, R.K.; Iovleva, A.; Kline, E.G.; Kawai, A.; McElheny, C.L.; Doi, Y. Clinical Evolution of AmpC-Mediated Ceftazidime-Avibactam and Cefiderocol Resistance in *Enterobacter cloacae* Complex Following Exposure to Cefepime. *Clin. Infect. Dis.* **2020**, *71*, 2713–2716. [CrossRef] [PubMed]

44. Streling, A.P.; Al Obaidi, M.M.; Lainhart, W.D.; Zangeneh, T.; Khan, A.; Dinh, A.Q.; Hanson, B.; A Arias, C.; Miller, W.R. Evolution of Cefiderocol Non-Susceptibility in *Pseudomonas aeruginosa* in a Patient Without Previous Exposure to the Antibiotic. *Clin. Infect. Dis.* **2021**, *73*, 4472–4474. [CrossRef] [PubMed]

45. Gupta, A.; Landman, D.; Quale, J. Relationship of TonB-dependent receptors with susceptibility to cefiderocol in clinical isolates of *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* **2022**, *77*, 1282–1285. [CrossRef] [PubMed]

46. Ito, A.; Nishikawa, T.; Ishii, R.; Kuroiwa, M.; Ishioka, Y.; Kurihara, N.; Sakikawa, I.; Ota, T.; Rokushima, M.; Tsuji, M.; et al. 696. Mechanism of Cefiderocol high MIC mutants obtained in non-clinical FoR studies. *Open Forum Infect. Dis.* **2018**, *5*, S251. [CrossRef]

47. Ito, A.; Sato, T.; Ota, M.; Takemura, M.; Nishikawa, T.; Toba, S.; Kohira, N.; Miyagawa, S.; Ishibashi, N.; Matsumoto, S.; et al. In Vitro Antibacterial Properties of Cefiderocol, a Novel Siderophore Cephalosporin, against Gram-Negative Bacteria. *Antimicrob. Agents Chemother.* **2018**, *62*, e01454-17. [CrossRef]

48. Ito, A.; Nishikawa, T.; Ota, M.; Ito-Horiyama, T.; Ishibashi, N.; Sato, T.; Tsuji, M.; Yamano, Y. Stability and low induction propensity of cefiderocol against chromosomal AmpC β-lactamases of Pseudomonas aeruginosa and Enterobacter cloacae. *J. Antimicrob. Chemother.* **2018**, *73*, 3049–3052. [CrossRef]

49. Kawai, A.; McElheny, C.L.; Iovleva, A.; Kline, E.G.; Sluis-Cremer, N.; Shields, R.K.; Doi, Y. Structural Basis of Reduced Susceptibility to Ceftazidime-Avibactam and Cefiderocol in *Enterobacter cloacae* Due to AmpC R2 Loop Deletion. *Antimicrob. Agents Chemother.* **2020**, *64*, 7. [CrossRef]

50. Luscher, A.; Moynié, L.; Auguste, P.S.; Bumann, D.; Mazza, L.; Pletzer, D.; Naismith, J.H.; Köhler, T. TonB-Dependent Receptor Repertoire of Pseudomonas aeruginosa for Uptake of Siderophore-Drug Conjugates. *Antimicrob. Agents Chemother.* **2018**, *62*, e00097-18. [CrossRef]

51. Magallon, A.; Amoureux, L.; Garrigos, T.; Sonois, M.; Varin, V.; Neuwirth, C.; Bador, J. Role of AxyABM overexpression in acquired resistance in Achromobacter xylosoxidans. *J. Antimicrob. Chemother.* **2022**, *77*, 926–929. [CrossRef] [PubMed]

52. Malik, S.; Kaminski, M.; Landman, D.; Quale, J. Cefiderocol Resistance in Acinetobacter baumannii: Roles of β-Lactamases, Siderophore Receptors, and Penicillin Binding Protein 3. *Antimicrob. Agents Chemother.* **2020**, *64*, e01221-20. [CrossRef] [PubMed]

53. Rolston, K.V.I.; Gerges, B.; Shelburne, S.; Aitken, S.L.; Raad, I.; Prince, R.A. Activity of Cefiderocol and Comparators against Isolates from Cancer Patients. *Antimicrob. Agents Chemother.* **2020**, *64*, e01955-19. [CrossRef] [PubMed]

54. Poirel, L.; Sadek, M.; Nordmann, P. Contribution of PER-Type and NDM-Type β-Lactamases to Cefiderocol Resistance in Acinetobacter baumannii. *Antimicrob. Agents Chemother.* **2021**, *65*, e0087721. [CrossRef] [PubMed]

55. Poirel, L.; Sadek, M.; Kusaksizoglu, A.; Nordmann, P. Co-resistance to ceftazidime-avibactam and cefiderocol in clinical isolates producing KPC variants. *Eur. J. Clin. Microbiol.* **2022**, *41*, 677–680. [CrossRef]

56. Poirel, L.; de la Rosa, J.-M.O.; Sadek, M.; Nordmann, P. Impact of Acquired Broad-Spectrum β-Lactamases on Susceptibility to Cefiderocol and Newly Developed β-Lactam/β-Lactamase Inhibitor Combinations in Escherichia coli and Pseudomonas aeruginosa. *Antimicrob. Agents Chemother.* **2022**, *66*, e0003922. [CrossRef]

57. McElheny, C.L.; Fowler, E.L.; Iovleva, A.; Shields, R.K.; Doi, Y. In Vitro Evolution of Cefiderocol Resistance in an NDM-Producing Klebsiella pneumoniae Due to Functional Loss of CirA. *Microbiol. Spectr.* **2021**, *9*, e0177921. [CrossRef]

58. Gill, C.M.; Abdelraouf, K.; Oota, M.; Nakamura, R.; Kuroiwa, M.; Gahara, Y.; Takemura, M.; Yamano, Y.; Nicolau, D.P. Discrepancy in sustained efficacy and resistance emergence under human-simulated exposure of cefiderocol against *Stenotrophomonas maltophilia* between in vitro chemostat and in vivo murine infection models. *J. Antimicrob. Chemother.* **2021**, *76*, 2615–2621. [CrossRef]

59. Price, T.K.; Davar, K.; Contreras, D.; Ward, K.W.; Garner, O.B.; Simner, P.J.; Yang, S.; Chandrasekaran, S. Case Report and Genomic Analysis of Cefiderocol-Resistant Escherichia coli Clinical Isolates. *Am. J. Clin. Pathol.* **2021**, *157*, 257–265. [CrossRef]

60. Werth, B.J.; Ashford, N.K.; Penewit, K.; Waalkes, A.; Holmes, E.A.; Bryan, A.; Salipante, S.J. Evolution of cefiderocol resistance in *Stenotrophomonas maltophilia* using in vitro serial passage techniques. *JAC-Antimicrob. Resist.* **2022**, *4*, dlac011. [CrossRef]

61. Zhang, Q.; Neidig, N.; Chu, T.-Y.; Divoky, C.; Carpenter, J.; Lee-Hsiao, C.; Threatt, H.; Sultana, R.; Bush, K. In vitro antibacterial activity of cefiderocol against recent multidrug-resistant carbapenem-nonsusceptible Enterobacterales isolates. *Diagn. Microbiol. Infect. Dis.* **2022**, *103*, 115651. [CrossRef] [PubMed]

62. Longshaw, C.; Manissero, D.; Tsuji, M.; Echols, R.; Yamano, Y. In vitro activity of the siderophore cephalosporin, cefiderocol, against molecularly characterized, carbapenem-non-susceptible Gram-negative bacteria from Europe. *JAC-Antimicrob. Resist.* **2020**, *2*, dlaa060. [CrossRef] [PubMed]

63. Abdul-Mutakabbir, J.C.; Nguyen, L.; Maassen, P.T.; Stamper, K.C.; Kebriaei, R.; Kaye, K.S.; Castanheira, M.; Rybak, M.J. In Vitro Antibacterial Activity of Cefiderocol against Multidrug-Resistant Acinetobacter baumannii. *Antimicrob. Agents Chemother.* **2021**, *65*, e0264620. [CrossRef]

64. Lan, P.; Lu, Y.; Chen, Z.; Wu, X.; Hua, X.; Jiang, Y.; Zhou, J.; Yu, Y. Emergence of High-Level Cefiderocol Resistance in Carbapenem-Resistant Klebsiella pneumoniae from Bloodstream Infections in Patients with Hematologic Malignancies in China. *Microbiol. Spectr.* **2022**, *10*, e0008422. [CrossRef] [PubMed]

65. Bogaerts, P.; Naas, T.; Saegeman, V.; Bonnin, R.A.; Schuermans, A.; Evrard, S.; Bouchahrouf, W.; Jove, T.; Tande, D.; de Bolle, X.; et al. OXA-427, a new plasmid-borne carbapenem-hydrolysing class D β-lactamase in Enterobacteriaceae. *J. Antimicrob. Chemother.* **2017**, *72*, 2469–2477. [CrossRef]

66. Poirel, L.; Kieffer, N.; Nordmann, P. Stability of cefiderocol against clinically significant broad-spectrum oxacillinases. *Int. J. Antimicrob. Agents* **2018**, *52*, 866–867. [CrossRef] [PubMed]

67. Stainton, S.M.; Monogue, M.L.; Tsuji, M.; Yamano, Y.; Echols, R.; Nicolau, D.P. Efficacy of Humanized Cefiderocol Exposures over 72 Hours against a Diverse Group of Gram-Negative Isolates in the Neutropenic Murine Thigh Infection Model. *Antimicrob. Agents Chemother.* **2019**, *63*, e0104018. [CrossRef]

68. Karakonstantis, S.; Saridakis, I. Colistin heteroresistance in *Acinetobacter* spp.: Systematic review and meta-analysis of the prevalence and discussion of the mechanisms and potential therapeutic implications. *Int. J. Antimicrob. Agents* **2020**, *56*, 106065. [CrossRef]

69. Karakonstantis, S. A systematic review of implications, mechanisms, and stability of in vivo emergent resistance to colistin and tigecycline in *Acinetobacter baumannii*. *J. Chemother.* **2020**, *33*, 1–11. [CrossRef]

70. Andersson, D.I.; Nicoloff, H.; Hjort, K. Mechanisms and clinical relevance of bacterial heteroresistance. *Nat. Rev. Genet.* **2019**, *17*, 479–496. [CrossRef]

71. Bassetti, M.; Echols, R.; Koren, A.; Karas, A.; Longshaw, C.; Yamano, Y.; Nagata, T.D. Placing in-vitro heteroresistance in the context of clinical results. *Lancet Infect. Dis.* **2021**, *21*, 908–909. [CrossRef]

72. El-Halfawy, O.M.; Valvano, M.A. Antimicrobial Heteroresistance: An Emerging Field in Need of Clarity. *Clin. Microbiol. Rev.* **2015**, *28*, 191–207. [CrossRef] [PubMed]

73. Brukner, I.; Oughton, M. A Fundamental Change in Antibiotic Susceptibility Testing Would Better Prevent Therapeutic Failure: From Individual to Population-Based Analysis. *Front. Microbiol.* **2020**, *11*, 1820. [CrossRef] [PubMed]

74. Pereira, C.; Larsson, J.; Hjort, K.; Elf, J.; Andersson, D.I. The highly dynamic nature of bacterial heteroresistance impairs its clinical detection. *Commun. Biol.* **2021**, *4*, 521. [CrossRef]

75. Bassetti, M.; Ariyasu, M.; Binkowitz, B.; Nagata, T.D.; Echols, R.M.; Matsunaga, Y.; Toyoizumi, K.; Doi, Y. Designing A Pathogen-Focused Study To Address The High Unmet Medical Need Represented By Carbapenem-Resistant Gram-Negative Pathogens-The International, Multicenter, Randomized, Open-Label, Phase 3 CREDIBLE-CR Study. *Infect. Drug Resist.* **2019**, *12*, 3607–3623. [CrossRef] [PubMed]

76. Le, C.; Pimentel, C.; Pasteran, F.; Tuttobene, M.R.; Subils, T.; Escalante, J.; Nishimura, B.; Arriaga, S.; Carranza, A.; Mezcord, V.; et al. Human Serum Proteins and Susceptibility of *Acinetobacter baumannii* to Cefiderocol: Role of Iron Transport. *Biomedicines* **2022**, *10*, 600. [CrossRef]

77. Ouzzani, M.; Hammady, H.; Fedorowicz, Z.; Elmagarmid, A. Rayyan—A web and mobile app for systematic reviews. *Syst. Rev.* **2016**, *5*, 210. [CrossRef]

78. Mc Gann, P.; Geringer, M.R.; Hall, L.R.; Lebreton, F.; Markelz, E.; Kwak, Y.I.; Johnson, S.; Ong, A.C.; Powell, A.; Tekle, T.; et al. Pan-drug resistant Providencia rettgeri contributing to a fatal case of COVID-19. *J. Med. Microbiol.* **2021**, *70*, 001406. [CrossRef]

79. Oinuma, K.I.; Suzuki, M.; Sakiyama, A.; Tsubouchi, T.; Saeki, K.; Sato, K.; Niki, M.; Yamada, K.; Shibayama, K.; Kakeya, H.; et al. Genomic characterization of triple-carbapenemase-producing Acinetobacter baumannii. *JAC-Antimicrob. Resist.* **2021**, *3*, dlab191. [CrossRef]