

Letter to the Editor

Stability of cefiderocol against clinically significant broad-spectrum oxacillinases

Sir,

Production of carbapenemases is a major concern in Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp. since these enzymes usually confer resistance to carbapenems. Three main β -lactamase classes group the carbapenemases, the most common being the KPC-type Ambler class A, class B (NDM-1, VIM-1, IMP-1 and SPM-1 metallo-enzymes) and class D (OXA-48-like, OXA-40, OXA-23) enzymes with extended geographic spread worldwide. The OXA-48-like β -lactamases are the most prevalent carbapenemases in Enterobacteriaceae in Europe. Carbapenemases are widespread in *Acinetobacter baumannii* isolates, with most multidrug-resistant strains producing OXA-40-like and OXA-23-like enzymes, with OXA-23-producers being the most frequent carbapenemase-producing *A. baumannii*.

Cefiderocol is a parenteral siderophore cephalosporin also known as S-649266 that possesses a unique mechanism for penetrating efficiently into Gram-negative pathogens. It uses a 'Trojan horse' strategy by binding free iron and then actively penetrates into the bacterial cell across the outer membrane of Gram-negative bacteria owing to the bacterial iron transport system. Once across the outer membrane, the iron dissociates and the cephalosporin binds to penicillin-binding proteins (PBPs), mainly PBP3, similar to other cephalosporins, to disrupt cell wall synthesis contributing to a potent antimicrobial activity against Gram-negative bacteria [1]. Cefiderocol exhibits potent in vitro and in vivo activity against all Gram-negative bacteria, including carbapenem-resistant Enterobacteriaceae, *Pseudomonas aeruginosa* and *A. baumannii* [2]. This study evaluated the stability of cefiderocol against the most prevalent carbapenem-hydrolysing class D β -lactamases (CHDLs) in Enterobacteriaceae and *A. baumannii*, namely OXA-48, OXA-23 and OXA-40, regardless of their bacterial hosts.

The *bla*_{OXA-48}, *bla*_{OXA-23} and *bla*_{OXA-40} genes were amplified by PCR and were cloned into the isopropyl β -D-1-thiogalactopyranoside (IPTG)-inducible vectors pET24 (Novagen, Merck, Darmstadt, Germany) and pGEX (GE Healthcare, Switzerland) giving the pET24-OXA-48, pGEX-OXA-23 and pGEX-OXA-40 recombinant plasmids, respectively, that were subsequently transformed into *Escherichia coli*. Induction of gene expression was performed by adding IPTG (1 mM) to the cultures for 5 h. Following centrifugation, the pellet of induced bacterial cells producing OXA-48 was resuspended in triethanolamine buffer pH 7.2 (20 mM) and was disrupted by sonication using a Vibra-Cell™ 75186 sonicator (Thermo Fisher Scientific). Following filtration

using a 0.22- μ m nitrocellulose filter, the crude extract was loaded onto a HiTrap™ Q HP anion exchange column (GE Healthcare) using an ÄKTAprime chromatography system (GE Healthcare). Presence of the β -lactamase in the flow through fractions was monitored using nitrocefim (200 μ M). The positive fractions were dialysed against piperazine buffer pH 9.5 (20 mM) and were loaded again onto a HiTrap™ Q HP anion exchange column and were eluted using a linear gradient of K₂SO₄ (500 mM).

Purification of OXA-23 and OXA-40 β -lactamases followed the same process except that resuspension of the pellets was done in 10 mM sodium phosphate, 140 mM NaCl and 2.7 mM KCl (pH 7.4) and elution was done using glutathione buffer (50 mM Tris-HCl, 10 mM glutathione, pH 8). All fractions containing purified β -lactamases were dialysed against sodium phosphate buffer pH 7.0 (0.1 M) in order to perform kinetic measurements.

Purified β -lactamases were used for kinetic measurements performed at 30°C in sodium phosphate buffer pH 7.0 (0.1 M). Kinetic parameters showed a significant hydrolysis rate of penicillins and carbapenems by the OXA-48, OXA-23 and OXA-40 purified enzymes, respectively, which is consistent with previous studies. Noteworthy, no enzymatic activity was detected for cefiderocol, mirroring the susceptibility test results (Table 1).

Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method in iron-depleted and calcium-adjusted Muller-Hinton broth medium (Bio-Rad). MICs showed that all CHDL-producing *E. coli* clones exhibited a high level of resistance to aminopenicillins and carboxypenicillins and intermediate resistance to imipenem (MICs of 1, 4 and 8 mg/L for OXA-48, OXA-40 and OXA-23, respectively, compared with 0.06 mg/L for the *E. coli* recipient), but remained fully susceptible to cefiderocol with no change in the MICs (respective values being all at 0.03 mg/L).

Altogether, these findings highlight the stability of cefiderocol toward the hydrolytic capacity of CHDLs. Cefiderocol is a modified cephalosporin molecule with an attached catechol moiety on the 3-position side chain that binds to ferric iron [1]. It was previously shown that CHDLs do not hydrolyse broad-spectrum cephalosporins [2]. The current data thus confirm that cefiderocol act as a potent cephalosporin, even in the presence of CHDLs.

Previous studies showed that cefiderocol is active against a wide range of carbapenemase-producers, including those belonging to classes A and B [3, 4]. Very recently, a study described the stability of cefiderocol against AmpC-producing Gram-negative isolates [5]. Here we demonstrated that the cefiderocol molecule is also stable against CHDL-producers, confirming that this molecule represents an interesting option for treating infections caused by carbapenemase-producing Gram-negative bacteria.

Table 1

Kinetic parameters of the OXA-48, OXA-40 and OXA-23 carbapenemases.

β -Lactam	OXA-48			OXA-40			OXA-23		
	K_m (μ M)	k_{cat} (s^{-1})	k_{cat}/K_m (mM^{-1}/s^{-1})	K_m (μ M)	k_{cat} (s^{-1})	k_{cat}/K_m (mM^{-1}/s^{-1})	K_m (μ M)	k_{cat} (s^{-1})	k_{cat}/K_m (mM^{-1}/s^{-1})
Benzylpenicillin	200	1750	8750	20	210	10 500	60	40	670
Ampicillin	1100	370	340	150	1.5	10	nd	<0.1	nd
Ticarcillin	90	70	780	70	4	50	70	7	100
Imipenem	15	1	70	50	3.5	70	80	0.5	6
Cefiderocol	ND	<0.1	ND	ND	<0.1	ND	ND	<0.1	ND

nd, not determined due to instability of the hydrolysis complex; ND, not determinable due to the absence of detectable hydrolytic activity.

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Competing interests

None declared.

Ethical approval

Not required.

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