In vitro activity of sulbactam-durlobactam against carbapenem-resistant *Acinetobacter baumannii* and mechanisms of resistance

Jacqueline Findlay [a,*], Laurent Poirel [a,b,c,d], Maxime Bouvier [b], Patrice Nordmann [a,b,c,d]

[a] *Department of Medical and Molecular Microbiology, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland*
[b] *National Institute for Health and Medical Research (INSERM) European Unit (IAME), University of Fribourg, Fribourg, Switzerland*
[c] *Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland*
[d] *Institute for Microbiology, University of Lausanne and University Hospital Centre, Lausanne, Switzerland*

ABSTRACT

Objectives: Multidrug-resistant *Acinetobacter baumannii* (MDR-Ab), particularly strains producing oxacillinase (OXA)-type carbapenemases, have rapidly emerged in health care settings as a frequent cause of serious infections with limited treatment options. This study evaluated the in vitro activity of sulbactam (SUL) combined with durlobactam (DUR) against a collection of carbapenemase-producing *A. baumannii*, and investigated the mechanisms of resistance.
Methods: Susceptibility testing was performed on 100 isolates by either broth microdilution or by the Epsilometer test. Isolates were screened for the insertion sequence IS*Aba1* upstream of the intrinsic chromosomal *blaADC* by polymerase chain reaction (PCR). Whole genome sequencing was performed on 25 SUL-DUR resistant isolates, and analyses were performed using the Center for Genomic Epidemiology platform. Target gene sequences were compared to *A. baumannii* American Type Culture Collection (ATCC) 17978.
Results: SUL-DUR exhibited excellent activity against *A. baumannii* isolates with susceptibility levels as follows: amikacin, 18%; colistin, 91%; cefepime, 5%; imipenem, 0%; minocycline, 46%; SUL, 3%; sulbactam-cefoperazone, 8%; SUL-DUR, 71% (based on a breakpoint at 4 mg/L). Twenty-five non-New Delhi metallo-ß–lactamase (NDM)-producing isolates had SUL-DUR MIC values >4 mg/L, amongst which 14 isolates showed substitutions in penicillin-binding protein (PBP)3, previously shown to be associated with SUL-DUR resistance. Substitutions that have not previously been described were detected in SUL-DUR targets, namely PBP1a, PBP1b, PBP2, and PBP3. By contrast, there was no evidence of the involvement of permeability or efflux.
Conclusions: SUL-DUR exhibited excellent in vitro antibacterial activity against carbapenemase-producing *A. baumannii* isolates. Amongst the 25 resistant isolates, we identified a number of mechanisms which may be contributing factors, in particular PBP substitutions and the production of specific beta-lactamases.

1. Introduction

Acinetobacter baumannii is one of the so-called 'ESKAPE' pathogens. The ESKAPE pathogens are a group of multidrug-resistant (MDR) bacteria comprised of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp. Carbapenem-resistant *A. baumannii* is labelled as "Priority 1; Critical" on the WHO's Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics [1]. Multidrug-resistant (resistance to three or more classes of antimicrobials) *A. baumannii* (MDR-Ab) have rapidly emerged in health care settings as a frequent cause of serious infections including pneumonia, bacteremia, and wound infections [2]. The incidence of infections and outbreaks involving bacterial isolates that produce oxacillinase (OXA)-type carbapenemases has increased significantly over the past two decades. As a result, most therapeutic options,

* Corresponding author. Mailing address: Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Chemin du Musée 18, CH-1700 Fribourg, Switzerland.
  E-mail address: jacqueline.findlay@unifr.ch (J. Findlay).

including last-resort drugs (e.g. carbapenems), have been rendered ineffective [2,3].

The so-called first-generation ß-lactamase inhibitor sulbactam (SUL) has been in clinical use since its approval in the 1980s. It is usually used in combination with ampicillin or cefoperazone for the treatment of infections caused by broad-spectrum ß-lactamase-producing *Acinetobacter* spp. because of its unique antibacterial activity against this organism [4]. Hence, SUL possesses dual ß-lactam and ß-lactamase inhibitory activity, with the ability to bind to and subsequently inhibit serine ß-lactamases, but also to bind to penicillin-binding proteins (PBPs), including PBP1a, PBP1b, and PBP3 in *Acinetobacter* spp. [4]. However, the degradation of SUL by some ß-lactamases, including TEM-1 [5], and its poor inhibitory activity against some class D enzymes, has resulted in the poor activity of SUL combinations currently available (e.g. ampicillin-sulbactam) [6]. Durlobactam (DUR) is a novel non-ß-lactam diazabicyclooctane (DBO) ß-lactamase inhibitor that exhibits broad-spectrum activity against class A, C, and D ß-lactamases [7]. Compared to avibactam (AVI), another DBO inhibitor that is currently licensed for clinical use, DUR exhibits greater activity against class D ß-lactamases, including those with carbapenemase activity [7–9]. Studies have found that the mechanism of action of DUR is similar to that of AVI; the inhibitor forms a covalent bond with the active site serine, resulting in the carbamylation and inactivation of the ß-lactamase before dissociation of the intact inhibitor [7,8]. Like SUL, DUR can be considered to have dual action in *A. baumannii*, as it has been shown to exhibit binding and inhibition features with respect to PBP2 (and to a lesser extent, PBP1a), although this does not lead to sufficient antibacterial activity on its own against this organism [7].

Recently, SUL and DUR have been combined to target MDR-Ab, particularly those producing OXA-type carbapenemases, [7–9] and several recent studies have reported the excellent activity of SUL-DUR against MDR *Acinetobacter* spp. This in vitro study aimed to both evaluate the SUL-DUR combination against a predefined collection of MDR-Ab, and to explore the possible mechanisms for corresponding resistance.

2. Materials and methods

2.1. Isolates

One hundred nonduplicate clinical *A. baumannii* isolates with previously characterized resistance mechanisms were used in this study. They were selected to be representative of the MDR patterns commonly observed in *A. baumannii* among human strains of worldwide origin that contribute to infection (e.g. septicemia; pulmonary and urinary infection; and catheter infections). This collection comprised producers of OXA-23 (n = 73), OXA-72 (n = 10), OXA-40 (n = 6), OXA-58 (n = 5), OXA-24 (n = 1), and New Delhi metallo-ß–lactamase (NDM) (n = 5, NDM-1 [n = 4], and NDM-5 [n = 1]) enzymes. Within these, a subset of 42 isolates were particularly resistant because they also produced 16S rRNA methylases (which are pandrug-resistant to aminoglycosides), and 9 were colistin (COL)-resistant.

2.2. Susceptibility testing

The minimal inhibitory concentrations (MICs) for each strain and drug combination were determined following Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M100-S30, 2020) [10]. Materials included premanufactured frozen 96-well broth microdilution panels with Mueller Hinton Broth (MHBII) growth medium and antibiotics supplied by Entasis Therapeutics (Waltham, MA): amikacin (AMK), COL, cefepime (FEP), minocycline (MIN), SUL, sulbactam-cefoperazone (SUL-CEF), and SUL-DUR. SUL-

DUR was tested as a dilution of SUL in the presence of DUR at a fixed concentration of 4 mg/L. Imipenem (IMI) MICs were determined by the Epsilometer test (Biomerieux, La Balme Les Grottes, France). Breakpoints of R>4 mg/L, based upon the CLSI ampicillin-sulbactam breakpoint, were used for SUL, SUL-CEF, and SUL-DUR (for which no breakpoints have yet been established against *A. baumannii*).

2.3. Whole genome sequencing and analysis

Whole genome sequencing (WGS) was performed on all non-NDM isolates with SUL-DUR MIC values of ≥ 8 mg/L on a MiSeq instrument (Illumina, San Diego, CA) using the Nextera sample preparation method (2 × 300 bp paired-end reads and a coverage of ≥50X). Reads were assembled into contigs using the Shovill pipeline (https://github.com/tseemann/shovill) and contigs were annotated using Prokka software [11]. Sequence types (STs), the presence of resistance genes, and the confirmation of speciation were determined using MLST (Multilocus sequence typing) 2.0, ResFinder 4.1 [12], and KmerFinder 3.2 [13] software available on the Center for Genomic Epidemiology platform (https://cge.cbs.dtu.dk/services/). Mutations and substitutions in PBPs were identified by sequence extraction and comparison with the *A. baumannii* ATCC 17978 genome (GenBank Accession No. CP018664). NCBI BLAST (National Center for Biotechnology Information Basic Local Alignment Search Tool) was used to investigate the prevalence of PBP protein sequences, and variants producing <10 hits were considered 'uncommon'.

Novel *blaADC* alleles, *blaADC-259* and *blaADC-260*, were submitted to GenBank under accession numbers OK340849 and OK396701, respectively.

2.4. Screening of ISAba1

Isolates were screened by PCR for the presence of the insertion sequence *ISAba1* upstream of *blaADC* as previously described [14].

3. Results and discussion

3.1. Susceptibility testing

Susceptibility testing (Table 1) showed the susceptibility levels to the tested antibiotics as follows: AMK, 18%; COL, 91%; FEP, 5%; IMI, 0%; MIN, 46%; SUL, 3%; SUL-CEF, 8%; and SUL-DUR, 71%. SUL-DUR exhibited greater levels of susceptibility, with the exception of COL, to all tested antibiotics, most notably when compared with imipenem. It is important to note that, largely due to colistin-heteroresistance, COL treatment of MDR-Ab infections has been associated with the emergence of resistance during therapy and unfavourable outcomes [15]. Low levels of susceptibility to the aminoglycoside AMK could be attributed to the production of the 16S rRNA aminoglycoside resistance methylase (ArmA) for 42 isolates in this study. Overall, a comparison of SUL, SUL-CEF, and SUL-DUR showed a significant ß-lactamase antibacterial effect of DUR against most of the strains, including those producing class D carbapenemases (OXA-23, OXA-24, OXA-40, OXA-58, and OXA-72), and one out of five NDM-producing isolates. This result is consistent with the known inability of DUR, as with all clinically available inhibitors, to inhibit the activity of class B metallo-ß–lactamases [7]. Twenty-five isolates that did not produce NDM-type enzymes had SUL-DUR MIC values above the preliminary breakpoint of 4 mg/L [16,17]. Nine strains were resistant to COL, and in four out of these nine strains, SUL-DUR may offer a possibility of treatment (MIC <8 mg/L). Approximately 46% of the isolates were susceptible to MIN, which resembles previously reported results for tetracycline derivatives versus *Acinetobacter* spp. [18]. The

Table 1
Susceptibility testing results of 100 clinical *Acinetobacter baumannii*

Antibiotic/ Carbapenemase	Range Tested	Breakpoints, ≤S/>R (mg/L)	Number of isolates with MIC (mg/L)											%S
			≤0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	
Amikacin – all	0.06-64	≤8/>32					1	5	7	5	2	3	77	18
OXA-23 (n=73)							1	5	6	2	1	2	56	19.2
OXA-72 (n=10)									1	1		1	7	20
OXA-40 (n=6)													6	0
OXA-58 (n=5)										1	1		3	20
NDM (n=5)										1			4	20
OXA-24 (n=1)													1	0
Colistin – all	0.06-64	≤2/>2			2	34	43	12		1	4	2	2	91
OXA-23 (n=73)					1	26	28	11			4	2	1	90.4
OXA-72 (n=10)						3	6			1				90
OXA-40 (n=6)						2	4							100
OXA-58 (n=5)						3	2							100
NDM (n=5)					1		3	1						100
OXA-24 (n=1)													1	0
Cefepime – all	0.06-64	≤8/>16								5	10	13	72	5
OXA-23 (n=73)										3	4	7	59	4.1
OXA-72 (n=10)										1	1	5	3	10
OXA-40 (n=6)											4	1	1	0
OXA-58 (n=5)										1	1		3	20
NDM (n=5)													5	0
OXA-24 (n=1)													1	0
Imipenem – all	0.02-32	≤2/>4							1			99		0
OXA-23 (n=73)									1			72		0
OXA-72 (n=10)												10		0
OXA-40 (n=6)												6		0
OXA-58 (n=5)												5		0
NDM (n=5)												5		0
OXA-24 (n=1)												1		0
Minocycline – all	0.06-64	≤4/>8			2	3	8	10	6	17	14	33	7	46
OXA-23 (n=73)							2	8	3	14	10	29	7	37
OXA-72 (n=10)					1	1	2	1	1	3	1			60
OXA-40 (n=6)							1	1	2	1	1			67
OXA-58 (n=5)					1	2	2							100
NDM (n=5)					1		3				1			80
OXA-24 (n=1)											1			0
Sulbactam – all	0.06-64	≤4/>4							3	14	34	34	15	3
OXA-23 (n=73)										8	26	29	10	0
OXA-72 (n=10)									1	1	3	1		16.7
OXA-40 (n=6)									1	2	2			20
OXA-58 (n=5)									1	3	2	3	1	10
NDM (n=5)												1	4	0
OXA-24 (n=1)											1			0
Sulbactam/Cefperazone – all	0.06-64	≤4/>4						1	7	16	24	42	10	8
OXA-23 (n=73)									2	10	21	36	4	2.7
OXA-72 (n=10)										4	2	3	1	40
OXA-40 (n=6)							1			2	1	2		16.7
OXA-58 (n=5)										1	4			20
NDM (n=5)												1	4	0
OXA-24 (n=1)													1	0
Sulbactam/Durlobactam - all	0.06-64	≤4/>4		1	1	6	18	20	25	14	7	3	5	71
OXA-23 (n=73)						6	12	16	20	10	6	1	2	74
OXA-72 (n=10)							2		2	4	1			50
OXA-40 (n=6)							1	3	2					100
OXA-58 (n=5)						1	3	1						100
NDM (n=5)									1			1	3	20
OXA-24 (n=1)													1	0

NOTE: Broken vertical lines indicate intermediate breakpoints and the continuous vertical lines indicate resistant breakpoints.
NDM, New Delhi metallo-ß–lactamase; OXA, oxacillinase.

MIC values of FEP were very high (95% ≥16 mg/L), indicating that this antibiotic cannot be considered as a possible alternative for treating infections caused by MDR-Ab isolates. As expected, the MIC values of SUL-CEF were also much higher than those of SUL-DUR. Overall these results are similar to those found in previous studies assessing the in vitro activity of SUL-DUR [19–21]

3.2. Analysis and characteristics of SUL-DUR-resistant isolates

Twenty-five isolates with SUL-DUR MIC values of ≥ 8 mg/L that did not harbour any *blaNDM* genes were subject to WGS in order to define the molecular mechanism of resistance. The characteristics of these strains are shown in Table 2.

3.2.1. Beta-lactamases

The acquired OXA-type carbapenemase encoding genes that were identified among all SUL-DUR resistant isolates were as follows: *blaOXA-23* (n = 19), *blaOXA-72* (n = 5), and *blaOXA-24* (n = 1). Amongst the intrinsic *blaOXA-51*-like genes, most isolates had *blaOXA-66* (n = 18), followed by *blaOXA-68* (n = 2) and *blaOXA-90* (n = 2), and single isolates each had either *blaOXA-64, blaOXA-69*, or *blaOXA-94*. Three isolates harboured extended

Table 2

Characteristics of the 25 SUL-DUR isolates that were subject to whole genome sequencing (WGS)

Isolate	Country/Year of Isolation	Site of Isolation	ST (Ox/Pa)	SUL-DUR MIC	OXA Carbapenemase	ADC Variant	ISAba1 upstream of blaADC	Other beta-lactamases	PBPs	Efflux Genes
N1026	Switzerland/2019	Faeces	ST436/ST2	64	OXA-23	ADC-188	Y	OXA-66*, TEM-1	PBP1b [P112S]; PBP3 [A515V][b]	
R3397	France/2017	Unknown	ST360/ST2	64	OXA-24	ADC-260	Y	OXA-66*	PBP1b [P112S]; PBP3 [I517N][b]	
R3401	France/2017	Unknown	ST391/ST157	64	OXA-23	ADC-91	Y	OXA-68*	PBP2 [P665A, I108V]; PBP3 [T526S] [b]	No adeRS; adeA truncated
N233	Switzerland/2018	Urine	ST436/ST2	32	OXA-23	ADC-188	Y	OXA-66*, TEM-1	PBP1b [P112S]; PBP3 [A515V][b]	
N224	Switzerland/2018	Skin	ST1816/ST2	16	OXA-23	ADC-73	Y	OXA-66*	PBP1b [P112S]; PBP3 [A515V] [b]	
N715	Switzerland/2019	Skin	ST1806/ST2	16	OXA-23	ADC-73	Y	OXA-66*, TEM-1	PBP1b [P112S]; PBP3 [A515V] [b]	adeS truncated; adeA truncated
N758	Switzerland/2019	Skin	ST1816/ST2	16	OXA-23	ADC-73	Y	OXA-66*	PBP1b [P112S, P545L] [b]; PBP3 [A515V] [b]	
N800	Switzerland/2019	Respiratory	ST1816/ST2	16	OXA-23	ADC-73	Y	OXA-66*	PBP1b [P112S]; PBP3 [A515V] [b]	
N1188	Switzerland/2020	Respiratory	ST1816/ST2	16	OXA-23	ADC-25	Y	OXA-66*	PBP3 [A515V] [b]	
R3393	France/2017	Unknown	ST1808/ST2	16	OXA-72	ADC-30	Y	OXA-66*	PBP1a [G181S] [b]; PBP1b [P112S]	
R3396	France/2017	Unknown	ST1803/ST25	16	OXA-23	ADC-259	Y	OXA-64*		No adeC
N14	Switzerland/2017	Respiratory	ST944/ST78	8	OXA-72	ADC-152	Y	OXA-90*, CTX-M-15, TEM-1	PBP1b [M726V]; PBP2 [Q106L] [b]	No adeC
N457	Switzerland/2018	Faeces	ST1809/ST2	8	OXA-23	ADC-73	Y	OXA-66*, TEM-1	PBP1b [P112S]; PBP3 [A515V] [b]	
N612	Switzerland/2019	Faeces	ST1962/ST2	8	OXA-23	ADC-30	Y	OXA-66*	PBP1b [P112S]	
N688	Switzerland/2019	Faeces	ST1809/ST2	8	OXA-23	ADC-73	Y	OXA-66*, TEM-1	PBP1b [P112S]; PBP3 [A515V] [b]	
N854	Switzerland/2019	Faeces	ST2054/ST636	8	OXA-72	ADC-74	Y	OXA-66*	PBP1a [T38A, A244T, Q644K,[b] T776A] ; PBP1b [P112S]	
N883	Switzerland/2019	Blood	ST1837/ST2	8	OXA-23	ADC-25	Y	OXA-66*	PBP3 [N392T] [b]	
N933	Switzerland/2019	Skin	ST2322/ST636	8	OXA-72	ADC-74	Y	OXA-66*	PBP1a [T38A, A244T, Q644K,[b] T776A] ; PBP1b [P112S] PBP6b [Tn ins][b]	
N957	Switzerland/2019	Faeces	ST1104/ST78	8	OXA-72	ADC-152	Y	OXA-90*, CARB-16, CTX-M-115	PBP1b [M726V]; PBP2 [Q106L] [b]	No adeRS; no adeC
N1172	Switzerland/2020	Skin	ST2461/ST2	8	OXA-23	ADC-73	Y	OXA-66*	PBP1b [P112S]; PBP3 [A515V] [b]	
N1183	Switzerland/2020	Respiratory	ST1816/ST2	8	OXA-23	ADC-73	Y	OXA-66*	PBP1b [P112S]; PBP3 [A515V][b]	adeA truncated
N1230	Switzerland/2020	Faeces	ST2325/ST85	8	OXA-23	ADC-80	N	OXA-94*, GES-11	PBP2 [P662T][b]	No adeC; no adeH
N1357	Switzerland/2020	Faeces	ST436/ST2	8	OXA-23	ADC-188	Y	OXA-66*, TEM-1	PBP1b [P112S]; PBP3 [A515V][b]	
R627	Bahrain/2008	Blood	ST449/ST20	8	OXA-23	ADC-74	Y	OXA-69*	PBP1b [N513H]; PBP2 [P665A]; PBP3 [V565L] [b]	
R3400	France/2017	Unknown	ST391/ST157	8	OXA-23	ADC-91	Y	OXA-68*	PBP2 [P665A, I108V]; PBP3 [T526S] [b]	No adeRS; adeA truncated

OXA, oxacillinase; PBP, penicillin-binding protein; ST, sequence type.

[a]Naturally occurring intrinsic *blaOXA-51*-like gene.

[b] Mutations that were found to be relatively uncommon (<10 BLAST hits).

spectrum ß-lactamase (ESBLs) genes, namely *blaGES-11* (n =1) or *blaCTX-M-115* (n = 2); one isolate also harboured *blaCARB-16*. Seven isolates harboured *blaTEM-1*, which is known to confer resistance to SUL [5].

3.2.2. Sequence types

Seven and 17 different STs were identified according to the Pasteur (Pa) [22] and Oxford (Ox) [23] MLST schemes, respectively. The most represented STs were ST2Pa (n = 19) and ST1816Ox (n = 5). ST2Pa belongs to global clone 2 (GC2) and is the most dominant ST worldwide [3]. ST2Pa is commonly associated with *blaOXA* carbapenemase gene carriage and has been frequently reported as the cause of most nosocomial outbreaks [3].

3.2.3. ADC variants and ISAba1

The presence or absence of insertion sequence IS*Aba1* upstream of the intrinsic cephalosporinase, *blaADC*, was investigated to identify any correlation with SUL or SUL-DUR resistance. Indeed, it was previously shown that the presence of this insertion sequence upstream of *blaADC* results in overexpression of this Ambler class C ß-lactamase gene, and therefore leads to increased MICs of penicillins and cephalosporins [14]. Polymerase chain reaction screening identified the presence of IS*Aba1* upstream of *blaADC* in 78 of 100 isolates, therefore indicating that the *blaADC* gene was likely to be overexpressed in those strains, but with no obvious correlation with MICs of SUL. Most (24 of 25) of the SUL-DUR resistant isolates harboured the IS*Aba1* element upstream of the *blaADC* gene. Ten different *blaADC* variants were identified, two of which correspond to novel alleles. In a previous study [24], carriage of an overexpressed *blaADC-30* or *blaADC-73* gene was suggested to contribute to SUL-resistance; this was observed in 2 and 8 isolates in this study, respectively.

3.2.4. Penicillin binding proteins

Within the 25 SUL-DUR-resistant isolates, 17 (68%) were found to encode PBP3 and exhibit a series of substitutions comparable to those of the wildtype sequences; PBP3 was the primary target of SUL in *A. baumannii* [25]. Five different PBP3 substitutions, relative to *A. baumannii* ATCC 17978, were identified, two of which were identified in 2 (T526S) and 12 (A515V) isolates, respectively. The latter substitutions have been previously shown to be associated with resistance to SUL-DUR [19–21, 26]. The remaining three isolates harbored N392T, I517N, and V565L substitutions in their PBP3 sequences which, to our knowledge, have not been previously described as a cause or contributing factor to antibiotic resistance.

Because no substitutions could be identified within the PBP3 sequence compared with the reference sequence in eight isolates, the sequences of other PBPs were analyzed accordingly, and substitutions were investigated both by comparison with the reference genome ATCC 17978 and by BLAST analyses. Six strains had substitutions in PBP2, a target for DUR [7], including Q106L (n = 2), P665A (n = 3), and P662T (n = 1). However, BLAST analyses revealed that only P662T could be considered an 'uncommon' substitution if we consider <10 BLAST hits to be 'uncommon'. Within PBP1a, another SUL target, one isolate harbored a G181S substitution and two isolates had T38A, A244T, Q644K, and T776A changes, but only the G181S and Q644K were found to be 'uncommon' when compared with sequences in GenBank. Nineteen isolates carried substitutions within PBP1b: 15 with P112S; 2 with M726V; 1 with N513H; and one isolate showing both P112S and P545L substitutions. However, only the P545L substitution could be considered potentially significant. Apart from the known PBP targets (PBP1a, 1b, 2, and 3) for SUL and DUR, one isolate (N933) harbored a transposon insertion after T302 in PBP6b.

The role of these substitutions, excepting the previously reported PBP3 mutations (T526S and A515V), have yet to be con-

firmed biochemically with respect to resistance to SUL-DUR. However, if we consider only 'uncommon' or known substitutions within the known SUL (PBPs 1a, 1b and 3) or DUR (PBP2) targets, then only two isolates, N612 and R3396, did not show significant PBP substitutions relative to the reference genome *A. baumannii* ATCC 17978.

3.2.5. Outer membrane proteins

No significant mutations or disruptions in major porin encoding genes (*ompA, carO, ompW,* and *oprD*) were detected by analysis of sequences obtained by WGS (data not shown). Of particular note was the high level of sequence conservation between SUL-DUR-resistant and SUL-DUR-susceptible strains for *ompA*, despite this porin being reported to be involved in DUR permeation into *A. baumannii* cells [27]. This suggests that SUL-DUR resistance in the isolates from this study is not related to *ompA*-mediated uptake.

3.2.6. Efflux

The sequences of efflux genes, belonging to the Resistance-Nondulation–Division (RND) family of efflux pumps, were investigated for any common genotype amongst the SUL-DUR resistant strains. Notably, four and one isolates were missing the *adeC* and *adeH* genes, respectively, which both encode the outer membrane components of the AdeABC and AdeFGH efflux pumps. However, the absence of the *adeC* gene has been reported to be relatively common amongst *A. baumannii* isolates [28]. In three isolates, the two-component system *adeRS*, which regulates the expression of *adeAB*, was absent and the *adeS* gene was truncated in another isolate. The *adeA* gene was also truncated in four isolates. The mutations or sequence variations observed in the efflux components in these isolates are unlikely to play a role in SUL-DUR resistance because such mutations or variants are usually associated with increased susceptibility to antimicrobials [29]. While this work was in progress, a recent study reported that efflux systems likely play no role in SUL-DUR resistance [30].

4. Conclusions

SUL-DUR was shown in this study to have notable in vitro antibacterial activity against a representative set of MDR-Ab isolates. It was found to be superior to all comparator agents tested with the exception of colistin. Twenty-five isolates were resistant to SUL-DUR in this study, mechanisms of which remain to be fully elucidated. However, we did identify mechanisms which may be contributing factors, such as PBP substitutions and the production of specific ß-lactamases, namely *blaTEM-1* and *blaADC* variants. These results suggest that SUL-DUR could be an effective treatment option for infections caused by MDR-Ab, although the mechanisms of SUL-DUR resistance that were observed in 25 isolates remain to be fully elucidated. Therefore, further study of resistance mechanisms to SUL-DUR is imperative.

Competing interests

None declared

Ethical approval

Not required

Acknowledgments

References

[1] World Health Organization Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. World Health Organisation; 2017. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed [accessed 20.04.20].

[2] Ramirez MS, Bonomo RA, Tolmasky ME. Carbapenemases: transforming *Acinetobacter baumannii* into a yet more dangerous menace. Biomolecules 2020;10:720. doi:10.3390/biom10050720.

[3] Hamidian M, Nigro SJ. Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii*. Microb Genom 2019;5:e000306. doi:10.1099/mgen.0.000306.

[4] Akova M. Sulbactam-containing beta-lactamase inhibitor combinations. Clin Microbiol Infect 2008;14:185–8. doi:10.1111/j.1469-0691.2007.01847.x.

[5] Krizova L, Poirel L, Nordmann P, Nemec A. TEM-1 ß-lactamase as a source of resistance to sulbactam in clinical strains of *Acinetobacter baumannii*. J Antimicrob Chemother 2013;68:2786–91. doi:10.1093/jac/dkt275.

[6] Shapiro AB. Kinetics of sulbactam hydrolysis by β-lactamases, and kinetics of β-lactamase inhibition by sulbactam. Antimicrob Agents Chemother 2017;61:e01612–17. doi:10.1128/AAC.01612-17.

[7] Durand-Réville T, Guler S, Comita-Prevoir J, Chen B, Bifulco N, Huynh H, et al. ETX2514 is a broad-spectrum β-lactamase inhibitor for the treatment of drug-resistant Gram-negative bacteria including *Acinetobacter baumannii*. Nat Microbiol 2017;2:17104. doi:10.1038/nmicrobiol.2017.104.

[8] Shapiro AB, Gao N, Jahić H, Carter NM, Chen A, Miller AA. Reversibility of covalent, broad-spectrum serine β-lactamase inhibition by the diazabicyclooctenone ETX2514. ACS Infect Dis 2017;3:833–44. doi:10.1021/acsinfecdis.7b00113.

[9] Barnes MD, Kumar V, Bethel CR, Moussa SH, O'Donnell J, Rutter JD, et al. Targeting multidrug-resistant *Acinetobacter* spp.: sulbactam and the diazabicyclooctenone β-lactamase inhibitor ETX2514 as a novel therapeutic agent. mBio 2019;10:e00159–19. doi:10.1128/mBio.00159-19.

[10] Clinical and Laboratory Standards Institute. Document M100-S30. In: Performance standards for antimicrobial susceptibility testing: twenty-seventh informational supplement, Wayne, Pennsylvania; 2020.

[11] Seeman T. Prokka: rapid prokaryotic genome annotation. Bioinf 2014;30:2068–9. doi:10.1093/bioinformatics/btu153.

[12] Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67:2640–4. doi:10.1093/jac/dks261.

[13] Larsen MV, Cosentino S, Lukjancenko O, Saputra D, Rasmussen S, Hasman H, et al. Benchmarking of methods for genomic taxonomy. J Clin Microbiol 2014;52:1529–39. doi:10.1128/JCM.02981-13.

[14] Rodríguez-Martínez J-M, Nordmann P, Ronco E, Poirel L. Extended-spectrum cephalosporinase in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2010;54:3484–8. doi:10.1128/AAC.00050-10.

[15] Cai Y, Chai D, Wang R, Liang B, Bai N. Colistin resistance of *Acinetobacter baumannii*: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother 2012;67:1607–15. doi:10.1093/jac/dks084.

[16] Rodvold KA, Gotfried MH, Isaacs RD, O'Donnell JP, Stone E. Plasma and intrapulmonary concentrations of ETX2514 and sulbactam following intravenous administration of ETX2514SUL to healthy adult subjects. Antimicrob Agents Chemother 2018;62:e01018–89. doi:10.1128/AAC.01089-18.

[17] O'Donnell J, Preston RA, Mamikonyan G, Stone E, Isaacs R. Pharmacokinetics, safety, and tolerability of intravenous durlobactam and sulbactam in subjects with renal impairment and healthy matched control subjects. Antimicrob Agents Chemother 2019;63:e00719–94. doi:10.1128/AAC.00794-19.

[18] Diekema DJ, Hsueh P-R, Mendes RE, Pfaller MA, Rolston KV, Sader HS, et al. The microbiology of bloodstream infection: 20-year trends from the SENTRY antimicrobial surveillance program. Antimicrob Agents Chemother 2019;63:e00319–55. doi:10.1128/AAC.00355-19.

[19] McLeod SM, Moussa SH, Hackel MA, Miller AA. In vitro activity of sulbactam-durlobactam against *Acinetobacter baumannii*-calcoaceticus complex isolates collected globally in 2016 and 2017. Antimicrob. Agents Chemother 2020;64:e02519–34. doi:10.1128/AAC.02534-19.

[20] Seifert H, Mueller C, Stefanik D, Higgins PG, Miller A, Kresken M. In vitro activity of sulbactam/durlobactam against global isolates of carbapenem-resistant *Acinetobacter baumannii*. J Antimicrob Chemother 2020;75:2616–21. doi:10.1093/jac/dkaa208.

[21] Petropoulou D, Siopi M, Vourli S, Pournaras S. Activity of sulbactam-durlobactam and comparators against a national collection of carbapenem-resistant *Acinetobacter baumannii* isolates from Greece. Front Cell Infect Microbiol 2021;11:814530. doi:10.3389/fcimb.2021.814530.

[22] Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One 2010;5:e10034. doi:10.1371/journal.pone.0010034.

[23] Bartual SG, Seifert H, Hippler C, Dominguez Luzon MA, Wisplinghoff H, Rodriguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 2005;43:4382–90. doi:10.1128/JCM.43.9.4382-4390.2005.

[24] Yang Y, Fu Y, Lan P, Xu Q, Jiang Y, Chen Y, et al. Molecular epidemiology and mechanism of sulbactam resistance in *Acinetobacter baumannii* isolates with diverse genetic backgrounds in China. Antimicrob Agents Chemother 2018;62:e01917–47. doi:10.1128/AAC.01947-17.

[25] Penwell WF, Shapiro AB, Giacobbe RA, Gu R-F, Gao N, Thresher J, et al. Molecular mechanisms of sulbactam antibacterial activity and resistance determinants in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2015;59:1680–9. doi:10.1128/AAC.04808-14.

[26] Naha A, Vijayakumar S, Lal B, Shankar BA, Chandran S, Ramaiah S, et al. Genome sequencing and molecular characterisation of XDR *Acinetobacter baumannii* reveal complexities in resistance: novel combination of sulbactam-durlobactam holds promise for therapeutic intervention. J Cell Biochem 2021;122:1946–57. doi:10.1002/jcb.30156.

[27] Iyer R, Moussa SH, Durand-Reville TF, Tommasi R, Miller AA. *Acinetobacter baumannii* OmpA is a selective antibiotic permeant porin. ACS Infect Dis 2018;4:373–81. doi:10.1021/acsinfecdis.7b00168.

[28] Nemec A, Maixnerová M, van der Reijden TJK, van den Broek PJ, Dijkshoorn L. Relationship between the AdeABC efflux system gene content, netilmicin susceptibility and multidrug resistance in a genotypically diverse collection of *Acinetobacter baumannii* strains. J Antimicrob Chemother 2007;60:483–9. doi:10.1093/jac/dkm231.

[29] Richmond GE, Evans LP, Anderson MJ, Wand ME, Bonney LC, Ivens A, et al. The *Acinetobacter baumannii* two-component system AdeRS regulates genes required for multidrug efflux, biofilm formation, and virulence in a strain-specific manner. mBio 2016;7:e00416–30. doi:10.1128/mBio.00430-16.

[30] Carter NM, Moussa SH, McLeod SM, Miller AA, Ramkumar I. Outer membrane permeability and efflux do not limit antibacterial activity of sulbactam-durlobactam in *Acinetobacter baumannii*, https://www.entasistx.com/application/files/5916/2488/9830/WMF21-0322_SUL-DUR_Efflux_Poster.pdf; 2021 [accessed 20.04.20].