

Article

In Vitro Activity of Sulbactam–Durlobactam against Carbapenem-Resistant *Acinetobacter baumannii* Clinical Isolates: A Multicentre Report from Italy

Bernardetta Segatore ¹, Alessandra Piccirilli ¹, Sabrina Cherubini ¹, Luigi Principe ^{2,*} , Giovanni Alloggia ¹, Maria Lina Mezzatesta ³, Mario Salmeri ³, Stefano Di Bella ⁴ , Roberta Migliavacca ⁵, Aurora Piazza ⁵, Elisa Meroni ⁶ , Paolo Fazii ⁷, Daniela Visaggio ^{8,9}, Paolo Visca ^{8,9} , Venere Cortazzo ¹⁰, Giulia De Angelis ¹⁰, Arianna Pompilio ^{11,12}  and Mariagrazia Perilli ¹ 

- ¹ Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, 67100 L'Aquila, Italy
 - ² Clinical Pathology and Microbiology Unit, "S. Giovanni di Dio" Hospital, 88900 Crotona, Italy
 - ³ Department of Biomedical and Biotechnological Sciences, Section of Microbiology, University of Catania, 95131 Catania, Italy
 - ⁴ Clinical Department of Medical, Surgical and Health Sciences, University of Trieste, 34129 Trieste, Italy
 - ⁵ Unit of Microbiology and Clinical Microbiology, Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, University of Pavia, 27100 Pavia, Italy
 - ⁶ Clinical Microbiology and Virology Unit, "A. Manzoni" Hospital, 23900 Lecco, Italy
 - ⁷ Clinical Microbiology and Virology Unit, Spirito Santo Hospital, 65122 Pescara, Italy
 - ⁸ Department of Science, Roma Tre University, Viale G. Marconi 446, 00146 Rome, Italy
 - ⁹ Santa Lucia Foundation IRCCS, 00179 Rome, Italy
 - ¹⁰ Dipartimento di Scienze Biotechnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, 00168 Rome, Italy
 - ¹¹ Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University of Chieti-Pescara, 66100 Chieti, Italy
 - ¹² Center of Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara, 66100 Chieti, Italy
- * Correspondence: luigi.principe@gmail.com



Citation: Segatore, B.; Piccirilli, A.; Cherubini, S.; Principe, L.; Alloggia, G.; Mezzatesta, M.L.; Salmeri, M.; Di Bella, S.; Migliavacca, R.; Piazza, A.; et al. In Vitro Activity of Sulbactam–Durlobactam against Carbapenem-Resistant

Acinetobacter baumannii Clinical Isolates: A Multicentre Report from Italy. *Antibiotics* **2022**, *11*, 1136. <https://doi.org/10.3390/antibiotics11081136>

Academic Editors: Zhi Ruan, Yongcheng Wang and Hua Zhou

Received: 2 August 2022

Accepted: 19 August 2022

Published: 22 August 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: In the present study, the in vitro activity of the sulbactam–durlobactam (SUL–DUR) combination was evaluated against 141 carbapenem-resistant *A. baumannii* (CRAb) clinical strains collected from six Italian laboratories. Over half (54.6%) of these isolates were resistant to colistin. The SUL–DUR combination was active against these CRAb isolates with MIC₅₀ and MIC₉₀ values of 0.5 mg/L and 4 mg/L, respectively. Only eleven isolates were resistant to SUL–DUR with MIC values ranging from 8 to 128 mg/L. The SUL–DUR resistant *A. baumannii* exhibited several antimicrobial resistance genes (ARGs) such as *bla*_{OXA-20}, *bla*_{OXA-58}, *bla*_{OXA-66}, *bla*_{ADC-25}, *aac*(6′)-Ib3 and *aac*(6′)-Ib-cr and mutations in *gyrA* (S81L) and *parC* (V104I, D105E). However, in these isolates, mutations Q488K and Y528H were found in PBP3. Different determinants were also identified in these CRAb isolates, including *adeABC*, *adeFGH*, *adeIJK*, *abeS*, *abaQ* and *abaR*, which encode multidrug efflux pumps associated with resistance to multiple antibacterial agents. This is the first report on the antimicrobial activity of SUL–DUR against carbapenem-resistant *A. baumannii* isolates selected from multiple regions in Italy.

Keywords: durlobactam; *A. baumannii*; WGS

1. Introduction

Acinetobacter baumannii has emerged in the last two decades as one of the major causes of nosocomial infections associated with significant morbidity and mortality and it has been recognized by World Health Organization (WHO) as a “critical priority pathogen” (www.who.int, accessed on 2 August 2022) [1,2]. *A. baumannii* is ubiquitous and can be found in various environmental sources including soil, water, vegetables, meat and fish [3,4]. In hospital settings, especially in intensive care units, *A. baumannii* can cause

ventilator-associated pneumonia and bloodstream infections [5–8]. The success of this organism is attributed to its ability to survive long-term in hospital environments and its prodigious capacity to acquire new antimicrobial resistance determinants [9]. The mechanisms of resistance in *A. baumannii* include enzymatic inactivation by β -lactamases, modification of target sites (e.g., Penicillin Binding Proteins, PBPs), alterations of porin proteins that result in decreased permeability and the upregulation of the activity of multidrug efflux pumps [9]. Currently, carbapenem-resistant *A. baumannii* (CRAb) pose a global threat to human health. CRAb is emerging worldwide, and the majority of these isolates show multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) phenotypes [10–13]. Currently, few therapeutic options are available for CRAb treatment [14,15]. Generally, colistin (CST), tigecycline and aminoglycosides are used against MDR *A. baumannii*, although there are limitations due to toxicity and poor pharmacokinetic properties [16]. CST has been successfully used to treat pneumonia and, bloodstream and meningitis infections caused by CRAb [17,18]. However, colistin-resistant isolates are emerging worldwide [19]. The intravenous fosfomycin is also used in combination with colistin or tigecycline or aminoglycoside for the treatment of hospital-acquired pneumonia caused by CRAb [20]. Cefiderocol, a novel siderophore cephalosporin, has recently been approved for the treatment of MDR *A. baumannii* [21]. Durlobactam (DUR), previously called ETX2514, is a non- β -lactam diazabicyclooctane (DBO) inhibitor with activity against Ambler class A, C and D β -lactamases [22,23]. Recently, some studies have shown that sulbactam in combination with durlobactam is active against MDR *A. baumannii* [24–31]. Sulbactam (SUL) is one of the first β -lactamase inhibitors used in combination with ampicillin for the treatment of class A β -lactamase-producing pathogens. In *A. baumannii*, SUL also has antibacterial activity by targeting PBPs (i.e., PBP1a/b and PBP3), enzymes required for cell wall synthesis [32]. DUR inactivates serine- β -lactamases by forming a reversible covalent bond with the active site serine [33]. This potent activity of durlobactam allows the susceptibility of CRAb to sulbactam to be restored [22,27]. The aim of the present study was to examine the in vitro activity of sulbactam–durlobactam (SUL–DUR) against 141 CRAb clinical isolates retrospectively collected from six clinical microbiology laboratories located across the national territories representative of northern, central and southern Italy.

2. Results

2.1. Antimicrobial Susceptibility

Antimicrobial susceptibility of CRAb isolates was previously assessed by each center using commercial systems in the context of normal clinical routine. Consistently, the associated traits were as follows: imipenem and meropenem, MICs > 8 mg/L; gentamicin, MICs > 8 mg/L; ciprofloxacin, MICs > 2 mg/L and SXT, MICs > 8 mg/L (based on trimethoprim concentration). Overall, 64 CRAb isolates were XDR (MIC values for colistin \leq 2 mg/L), while 77 CRAb isolates showed a PDR phenotype (MIC values for colistin > 2 mg/L).

The in vitro activity of SUL–DUR was evaluated for 141 CRAb clinical isolates using SUL, DUR and CST as comparators. Overall, 77 out of 141 (54.6%) *A. baumannii* isolates exhibited resistance to CST with Minimal Inhibitory Concentration (MIC) values of \geq 4 mg/L. The MIC₅₀ and MIC₉₀ for CST were 4 mg/L and >4 mg/L, respectively (Table 1). As shown in Table 1, 131 out of 141 CRAb isolates exhibited MICs > 4 mg/L for SUL and the MIC₅₀ and MIC₉₀ values were 16 mg/L and 128 mg/L, respectively. DUR had MIC₅₀ and MIC₉₀ values of 64 mg/L and 128 mg/L, respectively. The SUL–DUR combination was more potent against these CRAb isolates with MIC₅₀ and MIC₉₀ values of 0.5 mg/L and 4 mg/L, respectively. Only eleven isolates exhibited MIC values > 4 mg/L, the preliminary susceptible breakpoint for SUL–DUR (Table 1) [34,35]. All of the SUL–DUR resistant CRAb isolates were from the Clinical Microbiology Laboratory of Catania University located in southern Italy (Table 2).

Table 1. In vitro activities of sulbactam–durlobactam and comparators against 141 carbapenem-resistant *Acinetobacter baumannii* collected in Italy.

Antimicrobial Agent	Number of Isolates at Each MIC (mg/L)															MIC RANGE	MIC ₅₀	MIC ₉₀
	0.06	0.125	0.25	0.5	1	2	4	>4	8	16	32	64	128	>128				
SUL	/	/	/	/	/	2	8	–	27	45	33	8	13	5	0.06–>128	16	128	
DUR	/	/	/	/	/	/	/	–	3	7	44	39	47	1	0.06–>128	64	128	
SUL–DUR	/	4	25	51	30	14	6	–	4	2	/	/	/	5	0.06/4–>128/4	0.5	4	
CST	/	7	6	12	20	19	22	55	–	–	–	–	–	–	0.06–>4	4	>4	

/ = the number of isolates equal to zero. –, no values available. In SUL–DUR combination, DUR was at fixed concentration of 4 mg/L.

Table 2. MIC distribution of sulbactam–durlobactam and comparators against 141 CRAB isolates by location of the clinical microbiology laboratory.

City (No. Isolates) Antimicrobial Agents	Number of Isolates with MIC (mg/L)															
	0.06	0.125	0.25	0.5	1	2	4	>4	8	16	32	64	128	>128		
Pavia (16)																
SUL	/	/	/	/	/	/	1	–	1	5	7	2	/	/		
DUR	/	/	/	/	/	/	/	–	/	/	/	2	13	1		
SUL–DUR	/	/	/	6	6	4	/	–	/	/	/	/	/	/		
CST	/	/	1	6	9	/	/	/	–	–	–	–	–	–		
Gemelli (26)																
SUL	/	/	/	/	/	1	2	–	4	11	7	1	/	/		
DUR	/	/	/	/	/	/	/	–	/	2	6	11	7	/		
SUL–DUR	/	/	3	16	7	/	/	–	/	/	/	/	/	/		
CST	/	/	/	/	/	/	13	13	–	–	–	–	–	–		
PE/AQ (9)																
SUL	/	/	/	/	/	/	/	–	/	1	6	1	1	/		
DUR	/	/	/	/	/	/	/	–	/	/	3	2	4	/		
SUL–DUR	/	/	1	2	2	4	/	–	/	/	/	/	/	/		
CST	/	/	/	/	/	/	/	9	–	–	–	–	–	–		
Roma Tre (20)																
SUL	/	/	/	/	/	1	3	–	3	5	7	1	/	/		
DUR	/	/	/	/	/	/	/	–	/	/	6	6	8	/		
SUL–DUR	/	/	3	7	7	1	2	–	/	/	/	/	/	/		
CST	/	/	/	/	/	7	7	6	–	–	–	–	–	–		
Catania (70)																
SUL	/	/	/	/	/	/	2	–	19	23	6	3	12	5		
DUR	/	/	/	/	/	/	/	–	3	5	29	18	15	/		
SUL–DUR	/	4	18	20	8	5	4	–	4	2	/	/	/	5		
CST	/	7	5	6	11	12	2	27	–	–	–	–	–	–		

Pavia, isolates collected from the Microbiology Laboratory of the University of Pavia. **Gemelli**, isolates collected from the teaching “Gemelli” Hospital Rome. **PE/AQ**, isolates collected from Spirito Santo Hospital Pescara and the University of L’Aquila. **Roma Tre**, isolates collected from the Clinical Microbiology Laboratory of Roma Tre University. **Catania**, isolates collected from the Clinical Microbiology Laboratory of the University of Catania. / = the number of isolates equal to zero. –, no values available. In the SUL–DUR combination, DUR was at a fixed concentration of 4 mg/L.

2.2. Whole-Genome Sequencing of SUL–DUR Resistant *A. baumannii*: Resistome and Virulome Characterization

Whole-genome sequencing of the eleven SUL–DUR resistant CRAB isolates was performed and these isolates were found to encode several antibiotic resistance genes (ARGs) (Table 3) and virulence-associated genes (VAGs) (Table 4). Among the eleven analyzed strains, all encoded for the class D β -lactamases OXA-20, OXA-58 and OXA-66, in addition to ADC-25, a chromosomally encoded class C β -lactamase. While the presence of these β -lactamases most likely confer resistance to β -lactam antibiotics such as carbapenems, durlobactam has been shown to effectively inhibit these enzymes, so are not a likely cause

of the elevated SUL–DUR MICs. Genes that confer resistance to other classes of antibiotics were also detected including aminoglycoside resistance (*aac(6′)-Ib3* and *aac(6′)-Ib-cr*) and fluoroquinolone resistance (mutations in *gyrA* (S81L) and *parC* (V104I, D105E) (Table 2). The Insertion Sequence (IS) IS26 and ISAb125 were also identified in all eleven CRAb, and the transposon Tn6018 was found in two isolates (CT24 and CT58). All of the SUL–DUR-resistant CRAb showed the same profile of virulence factors (Table 4); however, isolates CT57 and CT58 possessed, in addition, *lpsB* and *lpx* VAGs which confer resistance to CST. The eleven CRAb isolates showed the presence of AdeABC, AdeFGH, AdeIJK, *abeS*, *abaQ* and *abaR* multidrug efflux pumps. As shown in Table 4, several genes involved in the biofilm formation system were also identified. Resistance mediated by quorum sensing is represented by *abaI* and *abaR* genes. The *pbpG*, also known as PBP7, was also identified in all SUL–DUR-resistant CRAb (Table 4). The impact of these genes on SUL–DUR susceptibility is not known.

Table 3. Characterization of Sulbactam–Durlabactam-resistant *A. baumannii*.

Strain	Sequence Type	Ward	Sample	SUL–DUR MIC (mg/L)	SUL MIC (mg/L)	DUR MIC (mg/L)	CST MIC (mg/L)	Resistance Genes		Mobile Genetic Elements
								β-Lactamases	Other	
<i>A. baumannii</i> CT20	2	transplant	BAL	8	128	128	0.125	<i>bla</i> _{ADC-25} <i>bla</i> _{OXA-20} <i>bla</i> _{OXA-58}	<i>aac(6′)-Ib-cr</i> <i>aac(6′)-Ib3</i> <i>tetA(41)</i>	IS26, ISAb125
<i>A. baumannii</i> CT57	2	ICU	BAL	8	128	64	64	<i>bla</i> _{ADC-25} <i>bla</i> _{OXA-20} <i>bla</i> _{OXA-58}	<i>aac(6′)-Ib-cr</i> <i>aac(6′)-Ib3</i> <i>tetA(41)</i>	Tn6018, IS26, ISAb125
<i>A. baumannii</i> CT58	2	ICU	wound	8	128	32	32	<i>bla</i> _{ADC-25} <i>bla</i> _{OXA-20} <i>bla</i> _{OXA-58}	<i>aac(6′)-Ib-cr</i> <i>aac(6′)-Ib3</i> <i>tetA(41)</i>	Tn6018, IS26, ISAb125
<i>A. baumannii</i> CT68	2	ICU	blood	8	128	64	0.25	<i>bla</i> _{ADC-25} <i>bla</i> _{OXA-20} <i>bla</i> _{OXA-58}	<i>aac(6′)-Ib-cr</i> <i>aac(6′)-Ib3</i> <i>sul1</i>	IS26, ISAb125
<i>A. baumannii</i> CT24	2	ICU	blood	16	64	128	0.5	<i>bla</i> _{ADC-25} <i>bla</i> _{OXA-20} <i>bla</i> _{OXA-58} <i>bla</i> _{OXA-66}	<i>aac(6′)-Ib-cr</i> <i>aac(6′)-Ib3</i> <i>qacE</i> <i>sul1</i> <i>gyrA</i> (S81L) <i>parC</i> (V104I, D105E)	IS26, ISAb125
<i>A. baumannii</i> CT25	2	ICU	catheter	16	128	64	1	<i>bla</i> _{ADC-25} <i>bla</i> _{OXA-20} <i>bla</i> _{OXA-58} <i>bla</i> _{OXA-66}	<i>aac(6′)-Ib-cr</i> <i>aac(6′)-Ib3</i> <i>sul1</i>	IS26, ISAb125
<i>A. baumannii</i> CT26	2	surgery	bile	>128	>128	64	0.5	<i>bla</i> _{ADC-25} <i>bla</i> _{OXA-20} <i>bla</i> _{OXA-58} <i>bla</i> _{OXA-66}	<i>aac(6′)-Ib-cr</i> <i>aac(6′)-Ib3</i> <i>qacE</i> <i>sul1</i> <i>gyrA</i> (S81L) <i>parC</i> (V104I, D105E)	IS26, ISAb125
<i>A. baumannii</i> CT29	2	ICU	exudate	>128	>128	128	1	<i>bla</i> _{ADC-25} <i>bla</i> _{OXA-20} <i>bla</i> _{OXA-58} <i>bla</i> _{OXA-66}	<i>aac(6′)-Ib-cr</i> <i>aac(6′)-Ib3</i> <i>qacE</i> <i>sul1</i> <i>gyrA</i> (S81L) <i>parC</i> (V104I, D105E)	IS26, ISAb125
<i>A. baumannii</i> CT30	20	ICU	catheter	>128	>128	32	1	<i>bla</i> _{ADC-25} <i>bla</i> _{OXA-20} <i>bla</i> _{OXA-58} <i>bla</i> _{OXA-66}	<i>aac(6′)-Ib-cr</i> <i>aac(6′)-Ib3</i> <i>qacE</i> <i>sul1</i> <i>gyrA</i> (S81L) <i>parC</i> (V104I, D105E)	IS26, ISAb125

Table 3. Cont.

Strain	Sequence Type	Ward	Sample	SUL–DUR MIC (mg/L)	SUL MIC (mg/L)	DUR MIC (mg/L)	CST MIC (mg/L)	Resistance Genes		Mobile Genetic Elements
								β-Lactamases	Other	
<i>A. baumannii</i> CT31	20	ICU	pus	>128	>128	128	0.125	bla _{ADC-25} bla _{OXA-20} bla _{OXA-58} bla _{OXA-66}	aac(6′)-Ib-cr aac(6′)-Ib3 qacE sul1 gyrA (S81L) parC(V104I, D105E)	IS26, ISAba125
<i>A. baumannii</i> CT32	20	ICU	BAL	>128	>128	128	1	bla _{ADC-25} bla _{OXA-20} bla _{OXA-58} bla _{OXA-66}	aac(6′)-Ib-cr aac(6′)-Ib3 qacE sul1 gyrA (S81L) parC (V104I, D105E)	IS26, ISAba125

Table 4. Virulence factors encoded by the eleven *A. baumannii* isolates resistant to SUL–DUR.

SUL–DUR-Resistant <i>A. baumannii</i> (Strains No.: CT20, CT24, CT25, CT26, CT29, CT30, CT31, CT32, CT57, CT58, CT68)	
Virulence-Associated Genes	Virulence Factors
<i>adeA, adeC, adeF, adeG, adeH, adeI, adeK, adeL, adeN, adeJ, adeR, abeS, abaQ, abaF, plc, plcD, lpsB</i> (only in CT57 and CT58)	RND efflux pump <i>AdeABC, AdeFGH</i> and <i>AdeIJK</i>
<i>lpxA, lpxB, lpxC, lpxD, lpxL, lpxM</i> (only in CT57 and CT58)	SMR family of transporter efflux pumps
<i>barA, barB</i>	MFS transporters
<i>basA, basB, basC, basD, basF, basG, basH, basI, basJ, bauA, bauB, bauC, bauD, bauE, bauF, entE, hemO</i>	Phospholipase
<i>bap, pgaA, pgaB, pgaC, pgaD, csuA, csuB, csuC, csuD, csuE, bfnR, bfnS, abaI, abaR, pbpG</i> (or PBP7) and PBP3 ^{Q488K} and PBP3 ^{Y528H}	Lipopolysaccharide synthesis (mutations are involved in CST resistance)
<i>katA</i>	Biosynthesis of lipid A (mutations are involved in CST resistance)
	Iron uptake: acinetobactin and heme utilization
	Biofilm formation system and cell–cell adhesion
	Quorum sensing
	Penicillin-binding protein
	A secondary catalase/peroxidase

RND, resistant-nodulation division. SMR, small multidrug resistance. MFS, major facilitator superfamily.

2.3. Molecular Analysis of PBP-3 Gene

The amplification of the PBP-3 gene of the eleven SUL–DUR-resistant *A. baumannii* isolates gave an amplicon of about 1800 bp which was entirely sequenced. In all *A. baumannii* analyzed, the PBP3 showed the following amino acid substitutions: Q488K and Y528H.

3. Discussion

The production of carbapenem-hydrolysing β-lactamases is one of the most common mechanisms responsible for carbapenem resistance in *A. baumannii* [10]. Several carbapenemases have been identified in *A. baumannii*, in particular, serine β-lactamases, belonging to class D and metallo-β-lactamases (class B enzymes). Nevertheless, metallo-β-lactamases are very rare in this microorganism [10]. However, in addition to β-lactamases, other mechanisms of resistance to carbapenems, including overexpression of efflux pumps, the reduction or inactivation of the expression of porins and the modification in the expression or synthesis of new PBPs, are found in *A. baumannii* isolates [10]. In the 141 *A. baumannii* analyzed in the present study, class C and D β-lactamases such as OXA-23, OXA-58, OXA-66 (OXA-51-like enzyme), OXA-82 (OXA-51-like enzyme) and ADC-25 were previously identified [36–47]. The bla_{OXA}-types are, usually, flanked by one or two copies of

the insertion sequences (i.e., *ISAbal*, *ISAbal25*) which are located in opposite orientations. These *ISAbal* insertion sequences enhance the expression of *bla*_{OXA} genes and they give genetic plasticity to *A. baumannii* species [48]. Herein, we have demonstrated potent in vitro activity of the SUL–DUR combination against 92% of the CRAb clinical isolates collected from a range of geographical regions within Italy. Of note, SUL–DUR demonstrated antimicrobial activity against both OXA-23- and OXA-58-producing isolates. Moreover, all but two of the colistin-resistant isolates (more than 50% of total isolates) were susceptible to the SUL–DUR combination. Eleven isolates (all from the Microbiology Clinical Laboratory of Catania University) were resistant to SUL–DUR, with most of them belonging to ST2 ($n = 8$) and showing a MIC range of 8–>128 mg/L. Two of these isolates were also resistant to CST. The whole genome analysis of the eleven SUL–DUR-resistant CRAb showed the presence of several ARGs, including *bla*_{OXA-20} (*bla*_{OXA-58} like gene), *bla*_{OXA-58}, *bla*_{ADC-25}, *aac(6′)Ib-cr*, *aac(6′)-Ib3*, and *tetA(41)*, a tetracycline efflux pump protein closely related to *Tet(39)* often found in *Acinetobacter* spp. [49]. Multiple VAGs were also found in these isolates. The multidrug efflux systems (RND, SMR, MFS families) are associated with multiple resistance mechanisms which are capable of extruding a broad range of structurally unrelated compounds [50–52]. The contribution of these ARGs and VAGs to the SUL–DUR-resistant phenotype seen in these isolates is not known. However, in the eleven SUL–DUR-resistant *A. baumannii* we found Q488K and Y528H mutations in PBP3. To date, reports of SUL–DUR resistance have been rare and resistance is usually attributed to the presence of metallo- β -lactamases, which DUR does not inhibit, or to mutations near the active site of PBP3, the target of sulbactam [24–26]. Few therapeutic choices are available to treat CRAb isolates [14,16,53]. CRAb pneumonia is a major clinical issue with unmet therapeutic needs; in fact, both colistin and tigecycline did not reach a satisfactory epithelial lining fluid concentration and cefiderocol showed disappointing clinical outcomes [54]. DUR displayed an acceptable ratio of epithelial lining fluid to plasma concentrations of 0.37 while SUL reached a 0.5 ratio [34,55]. Another important clinical issue to consider is that the SUL–DUR combination is expected to have a lower degree of nephrotoxicity compared to CST (ATTACK Trial. Available online: <https://investors.entasistx.com/news-releases/news-release-details/entasis-therapeutics-announces-positive-topline-results>, accessed on 2 August 2022), and in general be more safe. Taking into account the significant MIC reduction reached upon adding DUR to SUL and the promising clinical data from the phase 3 clinical trial comparing the safety and efficacy of SUL–DUR to CST for the treatment of infections caused by CRAb (SUL–DUR mortality 19%, CST mortality 32%, 95% CI: –30.0, 3.5), if approved, SUL–DUR may be an important option for CRAb treatment regimens. Further studies are needed to elucidate the molecular mechanisms responsible for resistance to SUL–DUR and to explore its therapeutic potential. It will also be necessary to combine in vitro findings with additional pharmacokinetic and pharmacodynamic data in order to provide more meaningful predictions of the in vivo efficacy of SUL–DUR combination in clinical practice.

4. Materials and Methods

4.1. Antibiotics and Inhibitors

SUL and DUR (ETX2514) were kindly provided by Dr. Alita A. Miller, Entasis Therapeutics, Waltham, MA, USA.

4.2. Bacterial Strains Selection

A total of 141 non duplicate CRAb strains, which were previously characterized for their mechanisms of resistance, were included in this study [36–47]. In particular, we retrospectively selected 64 XDR and 77 PDR previously characterized CRAb clinical isolates collected in six centers from Italy. Isolates were selected on the basis of this extensive resistance although resistance determinants were different (mostly including OXA-type enzymes, as reported in related publications). Most of the isolates (121 out of 141) were collected from five clinical microbiology laboratories distributed throughout northern,

central and southern Italy. Specifically, 16 *A. baumannii* were collected from the Microbiology Laboratory of the University of Pavia (Northern Italy) during 2018, 26 isolates were collected from the teaching “Gemelli” Hospital Rome (central Italy) between 2020 and 2022, 8 isolates were collected from Spirito Santo Hospital Pescara (Central Italy) in 2020–2021, 1 isolate was from the University of L’Aquila (Central Italy) and 70 isolates were collected from the Clinical Microbiology Laboratory of the University of Catania (Southern Italy) between 2008 and 2018. In addition, 20 *A. baumannii* were kindly given by Professor Visca, Clinical Microbiology Laboratory of Roma Tre University (Central Italy). These strains were collected in 2004–2014 from different countries during the project “Carbapenem-resistant *Acinetobacter baumannii*: whole-genomic and phenomic investigation of the traits that favored the predominance and shift to OXA-23-producing IC2 isolates”; funded by ESCMID in 2017. The antimicrobial susceptibility of CRAB isolates was previously assessed by participating centers using commercial systems in the context of normal clinical routine. Tested antibiotics were imipenem, meropenem, gentamicin, ciprofloxacin and colistin. All strains were collected from different wards including intensive care units, infectious diseases units, neurosurgery, pneumology, thoracic surgery and internal medicine. All *A. baumannii* were isolated from clinical sources, including sputum, blood, urine, wounds, peritoneal fluid, liquor and stool. The *A. baumannii* isolates belonged to the following sequence types: ST2 ($n = 121$), ST1 ($n = 6$), ST4 ($n = 1$), ST20 ($n = 5$), ST78 ($n = 2$), ST81 ($n = 1$), ST95 ($n = 1$), ST109 ($n = 1$), ST196 ($n = 1$) and ST197 ($n = 1$). In these *A. baumannii* strains, the carbapenem resistance was mainly mediated by the presence of OXA-23 (80 isolates), OXA-58 (48 isolates), OXA-66 (4 isolates) and OXA-82 (4 isolates). The simultaneous presence of OXA-23 and OXA-58 was found in 11 isolates and, in addition, ADC-25, a chromosomal AmpC enzyme, was also identified [36–47].

MDR isolates were defined as those with acquired non-susceptibility to at least one agent in three or more antimicrobial categories. XDR isolates were defined as those with acquired non-susceptibility to all antibiotics, except for one or two. PDR isolates were defined as those with acquired non-susceptibility to all antibiotics.

4.3. Bacterial Strains Identification

A. baumannii isolates were collected by standard methods, followed by isolation in pure culture on MacConkey agar plates, identified by the Vitek 2 system (bioMérieux, Marcy l’Etoile, France) and stored in Brain Heart Infusion broth with 15% glycerol and frozen at $-80\text{ }^{\circ}\text{C}$.

4.4. MIC Determination

The MIC experiments were performed by conventional broth microdilution procedures in Mueller Hinton broth supplemented with calcium and magnesium to physiological concentrations (CAMHB), using an inoculum of 5×10^5 CFU/mL according to the Clinical and Laboratory Standards Institute (CLSI) [56]. One hundred and forty-one non-duplicate *A. baumannii* isolates were tested against DUR alone as well as SUL alone, plus SUL–DUR and CST. For SUL, a susceptibility breakpoint of 4 mg/L was used, based on the CLSI ampicillin–sulbactam susceptible breakpoint of 8/4 mg/L for *Acinetobacter spp* [56]. SUL–DUR MICs were performed as 2-fold dilutions of SUL with DUR at a fixed concentration of 4 mg/L [56]. MICs were interpreted using CLSI breakpoints where available. Concurrent quality control (QC) procedures were performed by testing *Escherichia coli* ATCC 25922, examined for each MIC run. Following 18 to 20 h of aerobic incubation at $37\text{ }^{\circ}\text{C}$, the microplates were examined for growth. The determination of all MICs was performed in three separate sets of experiments.

4.5. Whole-Genome Sequencing

Total nucleic acid was extracted using MagMax Microbiome Ultra Nucleic Acid Isolation kit (Applied Biosystems and ThermoFisher Scientific, Monza, Italy). Genomic libraries were prepared using Swift 2S Turbo DNA Library kit (Swift Biosciences, Ann Arbor, MI,

USA) as previously reported [57,58]. WGS was performed on an Illumina MiSeq using v3 reagent kits generating 2×300 bp paired-end reads (Illumina, San Diego, CA, USA). DRAGEN FastQC + MultiQC v3.9.5 (<https://basespace.illumina.com/apps/10562553/DRAGEN-FastQC-MultiQC>, accessed on 24 May 2022) were used for quality control and sequences filtering. Paired-end reads were assembled with Velvet de novo Assembly v1.0.0 (<https://basespace.illumina.com/apps/8556549/Velvet-de-novo-Assembly>, accessed on 5 June 2022). Multi-Locus Sequence Typing (MLST) on assembled *A. baumannii* genomes was performed according to the Pasteur scheme. ResFinder4.1 and MobileElementFinder 1.0.3 were used to detect acquired antimicrobial resistance genes and mobile genetic elements, respectively. ResFinder and MobileElementFinder 1.0.3 databases were synchronized with databases from Center for Genomic Epidemiology (<http://www.genomicepidemiology.org/>, accessed on 10 June 2022). Virulence Factor Database (VFDB) was used for the detection of virulence genes (<http://www.mgc.ac.cn/VFs/>, accessed on 2 August 2022).

4.6. PBP-3 Amplification and Sequencing

The amplification of the PBP-3 gene was performed in PCR using the total genome of the SUL–DUR-resistant *A. baumannii* (CT20, CT24, CT25, CT26, CT29, CT30, CT31, CT32, CT57, CT58, CT68) and the following external primers: PBP-3_F 5'TTACCTGCGAATAGGATTTTCTG and PBP-3_R 5' ATGTGGCGGTTTTATCTGCT. The amplicons obtained were purified and directly sequenced on both strands by using a BigDye Sequencing Reaction Kit and an ABI PRISM 3500 capillary automated sequencer (Applied Biosystem, Monza, Italy).

5. Conclusions

In the present study, SUL–DUR demonstrated good in vitro antimicrobial activity against XDR and PDR *A. baumannii* clinical isolates, collected from different regions across Italy. These data confirmed the results from recent studies showing good activity of the SUL–DUR combination against MDR, XDR and PDR *A. baumannii* [59]. To the best of our knowledge, this study also represents the first report on SUL–DUR activity against a large number of carbapenem-resistant, and largely colistin-resistant, *A. baumannii* isolates from Italy.

Author Contributions: Conceptualization, B.S., A.P. (Alessandra Piccirilli), M.P. and L.P.; methodology, B.S., A.P. (Arianna Pompilio), G.A., M.S., R.M., A.P. (Aurora Piazza), E.M., D.V., V.C. and G.D.A.; investigation, B.S., A.P. (Alessandra Piccirilli), M.P., S.C. and A.P. (Arianna Pompilio); data curation, B.S., S.D.B., L.P., M.L.M. and M.P.; writing—original draft preparation, L.P., M.L.M., A.P. (Alessandra Piccirilli) and M.P.; writing—review and editing, L.P., M.L.M., A.P. (Alessandra Piccirilli), P.F., P.V. and M.P.; visualization, B.S., A.P. (Arianna Pompilio), G.A., M.S., R.M., A.P. (Aurora Piazza), E.M., D.V., V.C., L.P., M.L.M., A.P. (Alessandra Piccirilli) and G.D.A.; supervision, M.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors wish to thank Anna Toso (Toronto Catholic District School Board, Toronto, Canada) for the language revision of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Health Organization. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. Available online: http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1 (accessed on 1 August 2022).
2. Mancuso, G.; Midiri, A.; Gerace, E.; Biondo, C. Bacterial antibiotic resistance: The most critical pathogens. *Pathogens* **2021**, *10*, 1310. [CrossRef] [PubMed]

3. Carvalheira, A.; Silva, J.; Teixeira, P. *Acinetobacter* spp. in food and drinking water—A review. *Food Microbiol.* **2021**, *95*, 103675. [[CrossRef](#)] [[PubMed](#)]
4. Kittinger, C.; Kirschner, A.; Lipp, M.; Baumert, R.; Mascher, F.; Farnleitner, A.H.; Zarfel, G.E. Antibiotic resistance of *Acinetobacter* spp. Isolates from the River Danube: Susceptibility stays high. *Int. J. Environ. Res. Public Health* **2017**, *15*, 52. [[CrossRef](#)] [[PubMed](#)]
5. Salehi, M.; Jafari, S.; Ghafouri, L.; Ardakani, H.M.; Abdollahi, A.; Beigmohammadi, M.T.; Manshadi, S.A.D.; Feizabadi, M.M.; Ramezani, M.; Abtahi, H.; et al. Ventilator-associated pneumonia: Multidrug resistant *Acinetobacter* vs. extended Spectrum β -Lactamase-producing *Klebsiella*. *J. Infect. Dev. Ctries.* **2020**, *14*, 660–663. [[CrossRef](#)] [[PubMed](#)]
6. Russo, A.; Gavaruzzi, F.; Ceccarelli, G.; Borrazzo, C.; Oliva, A.; Alessandri, F.; Magnanini, E.; Pugliese, F.; Venditti, M. Multidrug-resistant *Acinetobacter baumannii* infections in COVID-19 patients hospitalized in intensive care unit. *Infection* **2022**, *50*, 83–92. [[CrossRef](#)]
7. Andersen, C.T.; Langendorf, C.; Garba, S.; Sayinzonga-Makombe, N.; Mambula, C.; Mouniaman, I.; Hanson, K.E.; Grais, R.F.; Isanaka, S. Risk of community- and hospital-acquired bacteremia and profile of antibiotic resistance in children hospitalized with severe acute malnutrition in Niger. *Int. J. Infect. Dis.* **2022**, *119*, 163–171. [[CrossRef](#)]
8. Manyahi, J.; Kibwana, U.; Mgimba, E.; Majigo, M. Multi-drug resistant bacteria predict mortality in bloodstream infection in a tertiary setting in Tanzania. *PLoS ONE* **2020**, *15*, e0220424. [[CrossRef](#)]
9. Kyriakidis, I.; Vasileiou, E.; Pana, Z.D.; Tragiannidis, A. *Acinetobacter baumannii* antibiotic resistance mechanisms. *Pathogens* **2021**, *10*, 373. [[CrossRef](#)]
10. Ramirez, M.S.; Bonomo, R.A.; Tolmasky, M.E. Carbapenemases: Transforming *Acinetobacter baumannii* into a yet more dangerous menace. *Biomolecules* **2020**, *10*, 720. [[CrossRef](#)]
11. Nodari, C.S.; Cayô, R.; Streling, A.P.; Lei, F.; Wille, J.; Almeida, M.S.; de Paula, A.I.; Pignatari, A.C.C.; Seifert, H.; Higgins, P.G.; et al. Genomic analysis of carbapenem-resistant *Acinetobacter baumannii* isolates belonging to major endemic clones in South America. *Front. Microbiol.* **2020**, *11*, 584603. [[CrossRef](#)]
12. Hammer, P.; Nguyen, N.H.; Nguyen, T.N.M.; Splettstoesser, W.D.; Makarewicz, O.; Neubauer, H.; Sprague, L.D.; Pletz, M.W. Phenotypic and WGS-derived antimicrobial resistance profiles of clinical and non-clinical *Acinetobacter baumannii* isolates from Germany and Vietnam. *Int. J. Antimicrob. Agents* **2020**, *56*, 106127.
13. Zarrilli, R.; Bagattini, M.; Migliaccio, A.; Esposito, E.P.; Triassi, M. Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* in Italy. *Ann. Ig.* **2021**, *33*, 401–409. [[PubMed](#)]
14. Vrancianu, C.O.; Gheorghe, I.; Czobor, I.B.; Chifiriuc, M.C. Antibiotic resistance profiles, molecular mechanisms and innovative treatment strategies of *Acinetobacter baumannii*. *Microorganisms* **2020**, *8*, 935. [[CrossRef](#)] [[PubMed](#)]
15. Tacconelli, E.; Carrara, E.; Savoldi, A.; Harbarth, S.; Mendelson, M.; Monnet, D.L.; Pulcini, C.; Kahlmeter, G.; Kluytmans, J.; Carmeli, Y.; et al. WHO Pathogens Priority List Working Group. Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect. Dis.* **2018**, *18*, 318–327. [[CrossRef](#)]
16. Asif, M.; Alvi, I.A.; Rehman, S.U. Insight into *Acinetobacter baumannii*: Pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. *Infect. Drug Resist.* **2018**, *11*, 1249–1260. [[CrossRef](#)]
17. Bartal, C.; Rolston, K.V.I.; Neshler, L. Carbapenem-resistant *Acinetobacter baumannii*: Colonization, Infection and Current Treatment Options. *Infect. Dis. Ther.* **2022**, *11*, 683–694. [[CrossRef](#)]
18. Garnacho-Montero, J.; Ortiz-Leyba, C.; Jiménez-Jiménez, F.J.; Barrero-Almodóvar, A.E.; García-Garmendia, J.L.; Bernabeu-Wittell, M.; Gallego-Lara, S.L.; Madrazo-Osuna, J. Treatment of multidrug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia (VAP) with intravenous colistin: A comparison with imipenem-susceptible VAP. *Clin. Infect. Dis.* **2003**, *36*, 1111–1118. [[CrossRef](#)]
19. Pormohammad, A.; Mehdinejadani, K.; Gholizadeh, P.; Nasiri, M.J.; Mohtavinejad, N.; Dadashi, M.; Karimaei, S.; Safari, H.; Azimi, T. Global prevalence of colistin resistance in clinical isolates of *Acinetobacter baumannii*: A systematic review and meta-analysis. *Microb. Pathog.* **2020**, *139*, 103887. [[CrossRef](#)]
20. Russo, A.; Bassetti, M.; Bellelli, V.; Bianchi, L.; Marincola Cattaneo, F.; Mazzocchetti, S.; Paciacconi, E.; Cottini, F.; Schiattarella, A.; Tufaro, G.; et al. Efficacy of a fosfomicin-containing regimen for treatment of severe pneumonia caused by multidrug-resistant *Acinetobacter baumannii*: A prospective, observational study. *Infect. Dis. Ther.* **2021**, *10*, 187–200. [[CrossRef](#)]
21. 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). *Int. J. Antimicrob. Agents* **2019**, *53*, 177–184. [[CrossRef](#)]
22. Barnes, M.D.; Kumar, V.; Bethel, C.R.; Moussa, S.H.; O'Donnell, J.; Rutter, J.D.; Good, C.E.; Hujer, K.M.; Hujer, A.M.; Marshall, S.H.; 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. [[CrossRef](#)] [[PubMed](#)]
23. Shapiro, A.B.; Gao, N. Interactions of the diazabicyclooctane serine β -lactamase inhibitor ETX1317 with target enzymes. *ACS Infect. Dis.* **2021**, *7*, 114–122. [[CrossRef](#)] [[PubMed](#)]
24. Granata, G.; Taglietti, F.; Schiavone, F.; Petrosillo, N. Durlobactam in the treatment of multidrug-resistant *Acinetobacter baumannii* infections: A systematic review. *J. Clin. Med.* **2022**, *11*, 3258. [[CrossRef](#)] [[PubMed](#)]

25. 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.* **2022**, *11*, 814530. [[CrossRef](#)]
26. Findlay, J.; Poirel, L.; Bouvier, M.; Nordmann, P. In-Vitro activity of sulbactam-durlobactam against carbapenem-resistant *Acinetobacter baumannii* and mechanisms of resistance. *J. Glob. Antimicrob. Resist.* **2022**, *9*, 32. [[CrossRef](#)]
27. Durand-Réville, T.F.; Guler, S.; Comita-Prevoir, J.; Chen, B.; Bifulco, N.; Huynh, H.; Lahiri, S.; Shapiro, A.B.; McLeod, S.M.; Carter, N.M.; 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. [[CrossRef](#)]
28. McLeod, S.M.; Moussa, S.H.; Hackel, M.A.; Miller, A.A. In Vitro activity of sulbactam-durlobactam against *Acinetobacter baumannii-calcoaceticus* complex isolates collected globally in 2016 and 2017. *Antimicrob. Agents Chemother.* **2020**, *64*, e02534-19. [[CrossRef](#)]
29. Yang, Q.; Xu, Y.; Jia, P.; Zhu, Y.; Zhang, J.; Zhang, G.; Deng, J.; Hackel, M.; Bradford, P.A.; Reinhart, H.J. In Vitro activity of sulbactam/durlobactam against clinical isolates of *Acinetobacter baumannii* collected in China. *J. Antimicrob. Chemother.* **2020**, *75*, 1833–1839. [[CrossRef](#)]
30. Nodari, C.S.; Santos, F.F.; Kurihara, M.N.L.; Valiatti, T.B.; Cayô, R.; Gales, A.C. In Vitro activity of sulbactam/durlobactam against extensively drug-resistant *Acinetobacter baumannii* isolates belonging to South American major clones. *J. Glob. Antimicrob. Resist.* **2021**, *25*, 363–366. [[CrossRef](#)]
31. Seifert, H.; Müller, C.; Stefanik, D.; Higgins, P.G.; Miller, A.; Kresken, M. In Vitro activity of sulbactam/durlobactam against global isolates of carbapenem-resistant *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* **2020**, *75*, 2616–2621. [[CrossRef](#)]
32. Penwell, W.F.; Shapiro, A.B.; Giacobbe, R.A.; Gu, R.F.; Gao, N.; Thresher, J.; McLaughlin, R.E.; Huband, M.D.; DeJonge, B.L.; Ehmann, D.E.; et al. Molecular mechanisms of sulbactam antibacterial activity and resistance determinants in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **2015**, *59*, 1680–1689. [[CrossRef](#)] [[PubMed](#)]
33. Shapiro, A.B.; Gao, N.; Jahic, H.; Carter, N.M.; Chen, A.; Miller, A.A. Reversibility of covalent, broad-spectrum serine β -lactamase inhibition by the diazabicyclooctenone ETX2514. *ACS Infect. Dis.* **2017**, *3*, 833–844. [[CrossRef](#)] [[PubMed](#)]
34. Rodvold, K.A.; Gotfried, M.H.; Isaacs, R.D.; O'Donnell, J.P.; Stone, E. Plasma and intrapulmonary concentrations of ETX2514 and sulbactam following intravenous administration of ETX2514SUL to healthy adult subjects. *Antimicrob. Agents Chemother.* **2018**, *62*, e01089-18. [[CrossRef](#)]
35. O'Donnell, J.; Rubino, C.; Onufrak, N.J.; Bhaunani, S.M.; Ambrose, P.G.; Tommasi, R.; Stone, E.; Rodvold, K.; Mueller, J.; Isaacs, R.; et al. *Pharmacokinetics/Pharmacodynamics and Phase 3 Dose Projection for the Novel β -Lactamase Inhibitor ETX2514 in Combination with Sulbactam against *Acinetobacter baumannii-calcoaceticus* Complex (ABC), Poster AAR-LB-14*; Microbe: San Francisco, CA, USA, 2019.
36. Mezzatesta, M.L.; D'Andrea, M.M.; Migliavacca, R.; Giani, T.; Gona, F.; Nucleo, E.; Fugazza, G.; Pagani, L.; Rossolini, G.M.; Stefani, S. Epidemiological characterization and distribution of carbapenem-resistant *Acinetobacter baumannii* clinical isolates in Italy. *Clin. Microbiol. Infect.* **2012**, *18*, 160–166. [[CrossRef](#)]
37. Caio, C.; Maugeri, G.; Zingali, T.; Gona, F.; Stefani, S.; Mezzatesta, M.L. Extensively drug-resistant *ArmA*-producing *Acinetobacter baumannii* in an Italian intensive care unit. *New Microbiol.* **2018**, *41*, 159–161. [[PubMed](#)]
38. Mezzatesta, M.L.; Caio, C.; Gona, F.; Cormaci, R.; Salerno, I.; Zingali, T.; Denaro, C.; Gennaro, M.; Quattrone, C.; Stefani, S. Carbapenem and multidrug resistance in Gram-negative bacteria in a single centre in Italy: Considerations on In Vitro assay of active drugs. *Int. J. Antimicrob. Agents* **2014**, *44*, 112–116. [[CrossRef](#)]
39. Carcione, D.; Siracusa, C.; Sulejmani, A.; Migliavacca, R.; Mercato, A.; Piazza, A.; Principe, L.; Clementi, N.; Mancini, N.; Leoni, V.; et al. In Vitro antimicrobial activity of the siderophore cephalosporin cefiderocol against *Acinetobacter baumannii* strains recovered from clinical samples. *Antibiotics* **2021**, *10*, 1309. [[CrossRef](#)]
40. Migliavacca, R.; Espinal, P.; Principe, L.; Drago, M.; Fugazza, G.; Roca, I.; Nucleo, E.; Bracco, S.; Vila, J.; Pagani, L.; et al. Characterization of resistance mechanisms and genetic relatedness of carbapenem-resistant *Acinetobacter baumannii* isolated from blood, Italy. *Diagn. Microbiol. Infect. Dis.* **2013**, *75*, 180–186. [[CrossRef](#)]
41. Principe, L.; Piazza, A.; Giani, T.; Bracco, S.; Caltagirone, M.S.; Arena, F.; Nucleo, E.; Tammara, F.; Rossolini, G.M.; Pagani, L.; et al. AMCLI-CRAB Survey Participants. Epidemic diffusion of OXA-23-producing *Acinetobacter baumannii* isolates in Italy: Results of the first cross-sectional countrywide survey. *J. Clin. Microbiol.* **2014**, *52*, 3004–3010. [[CrossRef](#)]
42. Nucleo, E.; Steffanoni, L.; Fugazza, G.; Migliavacca, R.; Giacobbone, E.; Navarra, A.; Pagani, L.; Landini, P. Growth in glucose-based medium and exposure to subinhibitory concentrations of imipenem induce biofilm formation in a multidrug-resistant clinical isolate of *Acinetobacter baumannii*. *BMC Microbiol.* **2009**, *9*, 270. [[CrossRef](#)]
43. Cherubini, S.; Perilli, M.; Segatore, B.; Fazii, P.; Parruti, G.; Frattari, A.; Amicosante, G.; Piccirilli, A. Whole-genome sequencing of ST2 *A. baumannii* causing bloodstream infections in COVID-19 patients. *Antibiotics* **2022**, *11*, 955. [[CrossRef](#)] [[PubMed](#)]
44. D'Arezzo, S.; Principe, L.; Capone, A.; Petrosillo, N.; Petrucca, A.; Visca, P. Changing carbapenemase gene pattern in an epidemic multidrug-resistant *Acinetobacter baumannii* lineage causing multiple outbreaks in central Italy. *J. Antimicrob. Chemother.* **2011**, *66*, 54–61. [[CrossRef](#)] [[PubMed](#)]
45. Higgins, P.G.; Prior, K.; Harmsen, D.; Seifert, H. Development and evaluation of a core genome multilocus typing scheme for whole-genome sequence-based typing of *Acinetobacter baumannii*. *PLoS ONE* **2017**, *12*, e0179228. [[CrossRef](#)] [[PubMed](#)]

46. Iacono, M.; Villa, L.; Fortini, D.; Bordoni, R.; Imperi, F.; Bonnal, R.J.; Sicheritz-Ponten, T.; Bellis, G.D.; Visca, P.; Cassone, A.; et al. Whole-genome pyrosequencing of an epidemic multidrug-resistant *Acinetobacter baumannii* strain belonging to the European clone II group. *Antimicrob. Agents Chemother.* **2008**, *52*, 2616–2625. [[CrossRef](#)]
47. Vranić-Ladavac, M.; Bedenić, B.; Minandri, F.; Ištok, M.; Bošnjak, Z.; Frančula-Zaninović, S.; Ladavac, R.; Visca, P. Carbapenem resistance and acquired class D β -lactamases in *Acinetobacter baumannii* from Croatia 2009–2010. *Eur. J. Clin. Microbiol. Infect. Dis.* **2014**, *33*, 471–478. [[CrossRef](#)] [[PubMed](#)]
48. Corvec, S.; Poirel, L.; Naas, T.; Drugeon, H.; Nordmann, P. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene blaOXA-23 in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **2007**, *51*, 1530–1533. [[CrossRef](#)] [[PubMed](#)]
49. Agersø, Y.; Petersen, A. The tetracycline resistance determinant Tet 39 and the sulphonamide resistance gene *sulII* are common among resistant *Acinetobacter* spp. isolated from integrated fish farms in Thailand. *J. Antimicrob. Chemother.* **2007**, *59*, 23–27. [[CrossRef](#)]
50. Piddock, L.J. Multidrug-resistance efflux pumps-not just for resistance. *Nat. Rev. Microbiol.* **2006**, *4*, 629–636. [[CrossRef](#)]
51. Pérez-Varela, M.; Corral, J.; Aranda, J.; Barbé, J. Functional Characterization of AbaQ, a novel efflux pump mediating quinolone resistance in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **2018**, *62*, e00906-18. [[CrossRef](#)]
52. Srinivasan, V.B.; Rajamohan, G.; Gebreyes, W.A. Role of AbeS, a novel efflux pump of the SMR family of transporters, in resistance to antimicrobial agents in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **2009**, *53*, 5312–5316. [[CrossRef](#)]
53. Piperaki, E.T.; Tzouveleakis, L.S.; Miriagou, V.; Daikos, G.L. Carbapenem-resistant *Acinetobacter baumannii*: In pursuit of an effective treatment. *Clin. Microbiol. Infect.* **2019**, *25*, 951–957. [[CrossRef](#)]
54. 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](#)]
55. Drwiega, E.N.; Rodvold, K.A. Penetration of antibacterial agents into pulmonary epithelial lining fluid: An update. *Clin. Pharmacokinet.* **2022**, *61*, 17–46. [[CrossRef](#)] [[PubMed](#)]
56. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 11th ed.; Approved Standard M07-A11; CLSI: Wayne, PA, USA, 2018.
57. Piccirilli, A.; Cherubini, S.; Azzini, A.M.; Tacconelli, E.; Lo Cascio, G.; Maccacaro, L.; Bazaj, A.; Naso, L.; Amicosante, G.; Ltf-Veneto Working Group; et al. Whole-genome sequencing (WGS) of carbapenem-resistant *K. pneumoniae* isolated in Long-Term Care Facilities in the Northern Italian Region. *Microorganisms* **2021**, *9*, 1985. [[CrossRef](#)] [[PubMed](#)]
58. Cherubini, S.; Perilli, M.; Azzini, A.M.; Tacconelli, E.; Maccacaro, L.; Bazaj, A.; Naso, L.; Amicosante, G.; LTCF-Veneto Working Group; Lo Cascio, G.; et al. Resistome and virulome of multi-drug resistant *E. coli* ST131 isolated from residents of Long-Term Care Facilities in the Northern Italian Region. *Diagnostics* **2022**, *12*, 213. [[CrossRef](#)] [[PubMed](#)]
59. Shapiro, A.B.; Moussa, S.H.; McLeod, S.M.; Durand-Réville, T.; Miller, A.A. Durlabactam, a new diazabicyclooctane β -lactamase inhibitor for the treatment of *Acinetobacter* infections in combination with sulbactam. *Front. Microbiol.* **2021**, *12*, 709974. [[CrossRef](#)]