In vitro activity of sulbactam-durlobactam against *Acinetobacter baumannii-calcoaceticus* complex isolates collected globally from 2016-2017

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Abstract

Acinetobacter baumannii-calcoaceticus complex (ABC) causes severe infections that are difficult to treat due to pre-existing antibiotic resistance. Sulbactam-durlobactam (formerly sulbactam-ETX2514) (SUL-DUR) is a β-lactam/β-lactamase inhibitor combination antibiotic designed to treat serious infections caused by ABC, including multidrug-resistant (MDR) strains. The *in vitro* antibacterial activity of SUL-DUR and comparator agents was determined by broth microdilution against 1722 clinical isolates of ABC collected in 2016 and 2017 from 31 countries across Asia/South Pacific, Europe, Latin America, the Middle East and North America. Over 50% of these isolates were resistant to carbapenems. Against this collection of global isolates, SUL-DUR had a MIC_{50}/MIC_{90} of 1/2 µg/ml compared to a MIC_{50}/MIC_{90} of 8/64 µg/ml for sulbactam alone. This level of activity was found to be consistent across organisms, regions, sources of infection and subsets of resistance phenotypes, including MDR and extensively drug-resistant isolates. The SUL-DUR activity was superior to that of the tested comparators, with only colistin having similar potency. Whole genome sequencing of the 39 isolates (2.3%) with a SUL-DUR MIC > 4 µg/ml revealed that these strains either encoded for the metallo-β-lactamase NDM-1, which durlobactam does not inhibit, or for single amino acid substitutions near the active site of PBP3, the primary target of sulbactam. In summary, SUL-DUR demonstrated potent antibacterial activity against recent, geographically diverse clinical isolates of ABC, including MDR isolates.

Introduction

Acinetobacter baumannii can cause severe nosocomial infections associated with high mortality rates and is increasingly being reported as extensively drug-resistant (XDR) in many parts of the world (1, 2). One of the most alarming trends is the wide-spread acquisition of class D β-lactamases that confer resistance to carbapenems in clinical isolates of *A. baumannii* (2-6). Other currently available treatment options have unacceptable toxicity profiles or may be ineffective due to their poor pharmacokinetic properties or pre-existing resistance (1).

A combination of sulbactam and durlobactam (also known as ETX2514) is currently in clinical development for the treatment of *Acinetobacter baumannii-calcoaceticus* complex (ABC) infections (6). Sulbactam is a β-lactam Ambler class A β-lactamase inhibitor (BLI) that also has intrinsic antibacterial activity against *Acinetobacter* spp., due primarily to inhibition of penicillin binding proteins (PBP1 and PBP3), which are essential components of cell wall synthesis (7). However, degradation of sulbactam by a variety of β-lactamases present in most ABC isolates limit its clinical use (6, 8, 9). Durlobactam is a non-β-lactam BLI which has a modified diazabicyclooctane (DBO) scaffold with an extended spectrum of activity compared to other DBO inhibitors. Durlobactam inhibits a broad range of class D β-lactamases with notably more potent inhibition of class A and C β-lactamases (6, 10). While durlobactam does not demonstrate antibacterial activity alone against ABC organisms, it does have intrinsic activity against certain species of *Enterobacterales*, due to inhibition of PBP2 in these organisms (6).

This study reports the *in vitro* antibacterial activity of sulbactam-durlobactam (SUL-DUR) and comparator antimicrobial agents against contemporary, clinical isolates of ABC (*A. baumannii*, *A. pittii*, *A. nosocomialis* and *A. calcoaceticus*) collected globally in 2016 and 2017. The study evaluated 1722 ABC isolates from 31 countries across Europe, North America, Latin America,

the Middle East, and Asia and the South Pacific from community- and hospital-associated infection sources. Isolates with reduced susceptibility to SUL-DUR were subjected to whole genome sequencing to identify the molecular drivers of SUL-DUR resistance.

Results

In vitro activity of SUL-DUR against global, clinical ABC isolates

A surveillance study was conducted to assess the *in vitro* activity of SUL-DUR and comparator agents against a collection of 1722 ABC isolated during 2016 (n = 843) and 2017 (n=879) from 209 medical centers in 31 countries around the world. Only one isolate per patient was collected from the following infection sources: bloodstream (13.9%); intraabdominal (3.8%); respiratory tract (61.2%); urinary tract (18.3%); skin and soft tissue (0.8%); and other or unknown sources (2.0%). Isolates were collected from Europe (41.4%), North America (USA only) (29.7%), Latin America (15.2%), Asia/South Pacific (12.8%) and the Middle East (Israel only) (0.9%). Since *A. baumannii* is the most clinically relevant and antibiotic-resistant species of the ABC complex, this collection was predominantly *A. baumannii* (82.5%). This collection contained 13.5% *A. pittii*, 3.5% *A. nosocomialis* and 0.6% *A. calcoaceticus*, reflecting their lower prevalence (11, 12).

Against all 1722 ABC isolates, addition of durlobactam to sulbactam lowered the MIC_{90} compared to sulbactam alone by 32-fold, from 64 µg/ml to 2 µg/ml (Tables 1 and 2). Over half of these isolates were non-susceptible to carbapenems, with only 47.4% and 46.0% susceptible to imipenem and meropenem, respectively. The most active compound against this set of isolates was colistin with 95.3% susceptibility (MIC_{90} of 1 µg/ml), followed by minocycline with 80.4%

susceptibility (MIC_{90} of 16 µg/ml), based on current CLSI breakpoint criteria (13). However, the colistin susceptibility results must be interpreted with caution as susceptibility breakpoint criteria are not recognized for *A. baumannii* by the FDA (14). The MIC_{90} of tigecycline was 2 µg/ml; however, the overall susceptibility to this agent cannot be defined because there are no approved breakpoints for tigecycline for the treatment of *Acinetobacter* spp.

Against the 1420 *A. baumannii* isolates tested, the SUL-DUR MIC_{50}/MIC_{90} was 1/4 µg/ml (Table 1). SUL-DUR was slightly more active against the other *Acinetobacter* spp. tested, with MIC_{50}/MIC_{90} of 0.5/1 µg/ml against *A. calcoaceticus* and *A. nosocomialis* and a MIC_{50}/MIC_{90} of 0.5/2 µg/ml against *A. pittii*. Generally, the *A. baumannii* isolates were less susceptible to the comparator agents, with only 37.0% and 35.6% susceptibility to imipenem and meropenem, respectively, compared to ≥93% susceptibility to carbapenems for the other *Acinetobacter* spp. tested.

Activity of SUL-DUR was stable across all the regions examined (Tables 1 and 2). SUL-DUR had a MIC_{50}/MIC_{90} of 1/2 µg/ml against isolates from Asia and the South Pacific (n=221), the Middle East (Israel only, n=15) and North America (USA only, n=551). Against isolates from Europe (n=713) and Latin America (n=262), SUL-DUR had a MIC_{50}/MIC_{90} of 1/4 µg/ml. In comparison, susceptibility to imipenem ranged from 26.7% in the Middle East and Latin America to 65% in North America.

SUL-DUR activity was also consistent across isolates from different sources of infection (Table 1). SUL-DUR had a MIC_{50}/MIC_{90} of 1/4 µg/ml against bloodstream isolates (n=238). Against respiratory tract infection isolates (n=1056), urinary tract infection isolates (n=316), intraabdominal infection isolates (n=65) and other or unknown sources of infection isolates (n=33), the MIC_{50}/MIC_{90} for SUL-DUR was 1/2 µg/ml. For isolates from skin and soft tissue

infections (n=14), the SUL-DUR MIC_{50}/MIC_{90} was 0.5/1 µg/ml. In addition, SUL-DUR retained potency across drug-resistant ABC isolates (Table 3). The SUL-DUR MIC_{90} was 4 µg/ml for the following subsets: imipenem-non-susceptible (n=909), colistin-resistant (n=81), minocycline-non-susceptible (n=337), ciprofloxacin-non-susceptible (n=1017) and amikacin-non-susceptible isolates (n=740). SUL-DUR also maintained a MIC_{90} of 4 µg/ml against MDR (n=259) and XDR (n=32) isolates.

Characterization of SUL-DUR-resistant isolates

Of the 1722 ABC isolates tested, only 39 (2.3%) had SUL-DUR MIC values >4 µg/ml, which is the proposed breakpoint based on extensive non-clinical and clinical PK/PD analyses (15, 16). To understand the molecular drivers of SUL-DUR resistance, all 39 isolates with MIC values >4 µg/ml were subjected to whole genome sequencing. The whole genome sequencing data was analyzed for multi-locus sequence type (MLST), β-lactamase gene content and variations in the efflux systems, outer membrane porin-like proteins and PBPs. Table 4 summarizes the antibiotic susceptibility, demographic information and results from whole genome sequencing of the SUL-DUR-resistant isolates. All 39 resistant isolates were *A. baumannii*, had SUL-DUR MIC values of 8 - >64 µg/ml, and were also resistant to imipenem. Only one of these isolates was resistant to colistin. Ten of the isolates were collected in 2016 and 29 in 2017. Analysis of the genome sequences revealed three different sets of genetically identical or clonal isolates from 2017. One set of clonal isolates was comprised of three isolates collected from the same hospital in Spain. Two additional, distinct sets of clonal isolates from the same hospital in Guatemala were also identified: one set of two isolates and another set comprised of six isolates. This may reflect either the predominance of certain strains in these two countries or a clonal outbreak(s) in these two hospitals. Only one isolate from each of these groups is shown in Table 4. Based on this

analysis, there were 31 unique SUL-DUR-resistant isolates identified. The resistant isolates were collected in four different geographical regions: Asia (n=4; Vietnam and Thailand), Europe (n=10; Belgium, France, Spain, Greece, Italy and Turkey), Latin America (n=14; Mexico, Argentina, Colombia, Ecuador and Guatemala), and North America (n=3; USA).

SUL-DUR-resistant isolates either encoded for the *bla*$_{NDM-1}$ metallo-β-lactamase or for amino acid change(s) in PBP3, or both (Table 4). The 11 isolates that encoded *bla*$_{NDM-1}$ had SUL-DUR MIC values of 32- >64 mg/L. Durlobactam does not inhibit metallo-β-lactamases such as NDM-1, therefore it is not surprising that these isolates are non-susceptible to SUL-DUR (6). There were 21 isolates found to encode for amino acid changes in PBP3, which is the target of sulbactam inhibition. The most prevalent PBP3 mutant alleles were A515V (n=5) and T526S (n=10). SUL-DUR MIC values for these isolates ranged from 8 mg/L to >64 mg/L; however, for the isolates with MIC values of >64 mg/L, the *bla*$_{NDM-1}$ gene was also present. Most of the isolates with A515V or T526S PBP3 mutants had MIC values of 8-32 mg/L. Five other PBP3 mutants were also found in this surveillance study, but at much lower prevalence: T337I and G523V (n=1); K235N (n=1), F548I (n=2); V146I (n=1); and Q488K (n=1). Two incidences of a truncated PBP1A were found and some isolates encoded single amino acid changes in PBP2, MtgA or PBP6b; however, most of these variants were only found in the presence of PBP3 changes, so it remains to be determined whether these variants in other cell wall synthesis proteins affect the activity of SUL-DUR.

The SUL-DUR-resistant isolates were also analyzed for mutations in efflux systems and outer membrane porins. Nine out of the 31 unique SUL-DUR-resistant isolates lacked *adeC*, the outer membrane component of one of the RND efflux systems in *Acinetobacter*, which is found in about 20% of clinical isolates (17, 18). Several different single amino acid changes in

components of the AdeABC, AdeFGH, and AdeIJK RND efflux systems were also observed. One isolate was found to encode for a frameshift mutation in the CarO porin, which has been associated with carbapenem resistance (2). Additionally, an isolate with a transposon insertion in *adeS*, part of the AdeRS two-component regulatory system for the regulation of the AdeABC efflux system, was found. Mutations in AdeRS can lead to overexpression of AdeABC and an increase in antibiotic efflux (19). For each of these mutations in efflux systems or porins, the isolate also encoded for variations in the target of sulbactam (PBP3) and/or for $bla_{\text{NDM-1}}$, making it difficult to define the extent to which these changes affect SUL-DUR activity.

Discussion

SUL-DUR demonstrated potent *in vitro* activity against clinical ABC isolates collected in 2016-2017 from around the globe. Of the isolates tested, 97.7% had a sulbactam-durlobactam MIC ≤ 4 µg/ml, the proposed SUL-DUR breakpoint (15, 16). In contrast, only 45.1% of isolates had MIC values ≤ 4 µg/ml for sulbactam alone, indicating that durlobactam effectively restores *in vitro* antibacterial activity to sulbactam. Susceptibility to SUL-DUR was higher than all comparator agents tested against the ABC in this study. The activity of SUL-DUR was consistent across geographical regions. This contrasts with activity observed for some of the comparator agents such as imipenem, where susceptibility varied by region from 65% susceptible in North America to <35% susceptible in Latin America, Asia and the Middle East. This variability in carbapenem resistance is consistent with other surveillance studies, which report higher rates of carbapenem resistance in Europe, Latin America and some countries in Asia than in North America (2).

The activity of SUL-DUR was also stable across isolates from a variety of infection sources, including bloodstream and respiratory tract infections. Notably, SUL-DUR maintained potency

against a variety of antibiotic-resistant subsets including imipenem-non-susceptible, colistin-resistant, minocycline-non-susceptible, ciprofloxacin-non-susceptible, and amikacin-non-susceptible as well as MDR and XDR isolates.

A small percentage (2.3%) of isolates had SUL-DUR MIC values above the proposed breakpoint of 4 µg/ml. Of the SUL-DUR-non-susceptible isolates, two previously identified mechanisms of resistance were identified (6, 7, 20), which constitute the majority of resistant isolates found in this study. Most of the isolates encoded for either the NDM-1 metallo β-lactamase, which is not inhibited by durlobactam, or for amino acid change(s) in PBP3, the target of sulbactam (6, 7). Of note is that in this study, the prevalence of NDM-1 was less than 1% of all isolates, similar to what has been found in other ABC surveillance studies (6, 21). The most common PBP3 mutant alleles were the A515V and T526S variants. We have previously shown that *in vitro* frequency of spontaneous resistance to sulbactam alone and SUL-DUR maps to PBP3 at low frequencies resulting in mutants with reduced affinity for sulbactam (7, 20). None of these PBP3 mutants isolated during spontaneous mutant selection *in vitro* were identified in this surveillance study; however, all of the amino acid substitutions in PBP3 identified in the current study are also located near the active site serine (S336) (22), suggesting they may have reduced affinity for sulbactam.

Currently there are limited treatment options for infections caused by *A. baumannii* due to antibiotic resistance, resulting in >50% mortality (1, 2). A phase 3 study to evaluate the efficacy and safety of intravenous SUL-DUR in the treatment of patients with infections caused by ABC complex is on-going (23). The potent activity of SUL-DUR against recent, global clinical isolates of ABC organisms shown in this study suggests that SUL-DUR may be useful for the treatment of infections caused by *A. baumannii* for which there is a great unmet medical need.

Materials and Methods

Bacterial isolates

A total of 1722 ABC isolates were collected from 2016 to 2017 from 209 medical centers in 31 countries around the world. Each site was requested to collect clinical isolates of ABC that were limited to one isolate per patient per year from hospitalized patients and to submit them to International Health Management Associates, Inc. (IHMA) in Schaumburg, IL, USA for confirmatory identification and antimicrobial susceptibility testing. Due to allocation of each of the ABC species from different geographical regions, this study did not evaluate the prevalence of each of these species in the regions examined. The identity of each isolate was confirmed using matrix-assisted laser desorption ionization-time of flight spectrometry (MALDI-TOF) (Bruker Daltonics, Billerica, MA, USA). The composition of ABC by species was as follows: *A. baumannii*, n= 1420 (82.4%); *A. calcoaceticus*, n=10 (0.6%); *A. nosocomialis*, n=60 (3.5%); and *A. pittii*, n=232 (13.5%).

The ABC isolates were collected from five geographical regions: Asia/South Pacific, n= 221 (12.8%) (Australia, Japan, Philippines, South Korea, Taiwan, Thailand, Vietnam); Europe, n=713 (41.4%) (Belgium, Czech Republic, France, Germany, Greece, Hungary, Italy, Portugal, Russia, Spain, Turkey, United Kingdom); Latin America, n=262 (15.2%) (Argentina, Brazil, Chile, Colombia, Ecuador, Guatemala, Mexico, Panama, Puerto Rico, Venezuela); Middle East, n=15 (0.9%) (Israel) and North America, n=511 (29.7%) (USA). Isolates were taken from the following infection sources: bloodstream, n=238 (13.9%); intraabdominal, n=65 (3.8%); respiratory tract, n=1056 (61.2%); urinary tract, n=316 (18.3%); skin and soft tissue, n=14 (0.8%); and other or unknown sources, n=33 (2.0%).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed at IHMA using broth microdilution panels prepared in house following standardized CLSI methods (24). Quality control testing was performed each day of testing as specified by CLSI using *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *A. baumannii* NCTC 13304 (13). The SUL-DUR broth MIC quality control range is 0.5/4 – 2/4 µg/ml for *A. baumannii* NCTC 13304 (13). MIC values were interpreted using CLSI breakpoints for all antimicrobial agents except those for which CLSI breakpoints are not available (13). SUL-DUR was tested as 2-fold dilutions of sulbactam in combination with a fixed concentration of 4 µg/ml durlobactam. Isolates were categorized as MDR or XDR based on the criteria for *Acinetobacter* spp. outlined by Magiorakos *et al*. (25), which defines MDR as non-susceptible to ≥1 agent in ≥3 classes and XDR as non-susceptible to ≥1 agent in all but ≤2 classes of antimicrobials. The antimicrobial agents used for the MDR analysis were: imipenem, minocycline and amikacin. The antimicrobial agents used for the XDR analysis were: imipenem, minocycline, amikacin, ciprofloxacin, colistin, cefepime and sulbactam. For sulbactam a susceptibility breakpoint of 4 µg/ml was used, which is based on the ampicillin-sulbactam (2:1) breakpoint of 8/4 µg/ml where sulbactam comprises the active component of the combination for *Acinetobacter* spp. (13).

Whole genome sequencing and analysis

Extraction of chromosomal DNA, whole genome sequencing and subsequent analysis of genomic content of each isolate with a SUL-DUR MIC > 4 µg/ml was performed at Entasis Therapeutics. Chromosomal DNA was extracted from each isolate using the Promega Maxwell 16 instrument and Maxwell 16 Cell DNA Purification kit following the manufacturer's protocol (Promega, Madison, WI). DNA was quantified with a Qubit 2.0 fluorometer using the dsDNA

Broad Range Assay kit (Life Technologies, Grand Island, NY). DNA was diluted to 0.2 ng/µl, and 5 µl was used for library generation using the Nextera XT DNA sample preparation kit and Nextera XT index primers (Illumina, San Diego, CA). The recommended procedure was followed except the library normalization step was omitted in favor of qPCR library quantification. qPCR was performed on a BioRad CFX96 cycler using the Kappa Biosystems Library quantification kit (KK4824) (Woburn, MA). Libraries were diluted to a standard concentration of 4 nM of DNA, and 2.5 µl of each sample (8-12 samples, targeted 25-50-fold coverage) were combined and denatured with 0.1N NaOH (final) for 5 minutes. The sample was diluted to 600 µl to provide a 15-20 pM multiplex library. Samples were sequenced on an Illumina MiSeq instrument using the V2 chemistry in a 2 x 150 paired-end read format.

Assembly and analysis of whole genome sequencing was performed using CLC Genomics Workbench v9.5 (CLCBio, Cambridge, MA). Fastq files were processed and analyzed as follows: duplicate sequence reads were removed, and remaining reads were trimmed for quality and minimum length (50 bp). Reads were *de novo* assembled at high stringency (fraction length = 0.9, similarity fraction = 0.99) using default mismatch/insertion/deletion costs. Detection of SNPs and indels was accomplished through mapping to a parent reference assembly using the same parameters. Quality-based SNPs were detected at a minimum frequency of 80% using default criteria.

Selected strains with reduced susceptibility to sulbactam-durlobactam were selected for sequence analysis of cell wall synthesis, efflux, and porin genes. The corresponding amino acid sequences were compared with the reference sequence of *A. baumannii* strain ATCC 17978 (Genbank accession number CP000521.1). The resultant variations in the amino acids of the proteins are listed in Table 4. The β-lactamase content of each strain was determined by BLAST within the

CLC Genomics Workbench against an assembled database of genes curated at Entasis Therapeutics, with sequences originating from the NCBI Bacterial Antimicrobial Resistance Reference Gene Database (accession number PRJNA313047). For MLST determination, assembled contigs were exported from CLC Genomics Workbench and uploaded into the PubMLST database (https://pubmlst.org/databases/). For *Acinetobacter*, PubMLST hosts two different MLST schemes, Oxford and Institute Pasteur. The Oxford scheme (ST_{ox}) assigns sequence types using the following genes: *gltA, gyrB, gdhB, recA, cpn60, gpi,* and *rpoD*. Alternatively, the Institute Pasteur scheme (ST_{IP}) assigns sequence types using alleles of *cpn60, fusA, gltA, pyrG, recA, rplB,* and *rpoB*. Sequence types from both schemes are reported when available.

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364 **Table 1** *In vitro* activities of sulbactam-durlobactam and comparator antimicrobial agents
365 tested against 1722 clinical isolates of ABC collected globally from 2016-2017

Region, category (no. of isolates)	Antimicrobial agent	MIC (µg/ml)			Percent susceptible[a]
		MIC_{50}	MIC_{90}	Range	
Global, all (1722)	Sulbactam-durlobactam	1	2	≤0.03 - >64	NA
	Sulbactam	8	64	0.25 - >64	NA
	Cefepime	16	>16	≤0.12 - >16	41.3
	Imipenem	16	64	0.06 - >64	47.4
	Meropenem	16	>64	0.06 - >64	46.0
	Amikacin	4	>64	≤0.5 - >64	57.0
	Ciprofloxacin	>4	>4	≤0.12 - >4	40.9
	Colistin	0.5	1	≤0.25 - >8	95.3
	Minocycline	0.5	16	≤0.12 - >16	80.4
	Tigecycline	0.5	2	≤0.015 - 32	NA
Global, *A. baumannii* (1420)	Sulbactam-durlobactam	1	4	≤0.03 - >64	NA
	Sulbactam	16	64	0.25 - >64	NA
	Cefepime	>16	>16	≤0.12 - >16	31.2
	Imipenem	32	64	0.06 - >64	37.0
	Meropenem	32	>64	0.06 - >64	35.6
	Amikacin	32	>64	≤0.5 - >64	48.7
	Ciprofloxacin	>4	>4	≤0.12 - >4	30.1
	Colistin	0.5	1	≤0.25 - >8	94.4
	Minocycline	1	16	≤0.12 - >16	76.3
	Tigecycline	0.5	2	≤0.015 - 32	NA
Global, *A. calcoaceticus* (10)	Sulbactam-durlobactam	0.5	1	0.12 - 1	NA
	Sulbactam	2	4	1 - 4	NA
	Cefepime	4	8	4 - 8	100
	Imipenem	0.12	0.25	0.12 - 0.25	100
	Meropenem	0.25	0.5	0.12 - 0.5	100
	Amikacin	≤0.5	1	≤0.5 - 2	100
	Ciprofloxacin	≤0.12	0.25	≤0.12 - 0.25	100
	Colistin	0.5	1	≤0.25 - 1	100
	Minocycline	≤0.12	≤0.12	≤0.12 - ≤0.12	100
	Tigecycline	0.06	0.12	0.06 - 0.12	NA
Global, *A. nosocomialis* (60)	Sulbactam-durlobactam	0.5	1	0.12 - 4	NA
	Sulbactam	2	8	0.5 - 64	NA
	Cefepime	2	>16	1 - >16	85.0
	Imipenem	0.25	0.25	0.06 - >64	93.3
	Meropenem	0.25	1	0.12 - 64	93.3
	Amikacin	2	16	≤0.5 - >64	91.7
	Ciprofloxacin	0.25	2	≤0.12 - >4	85.0

Region, category (no. of isolates)	Antimicrobial agent	MIC (µg/ml)			Percent susceptible[a]
		MIC_{50}	MIC_{90}	Range	
	Colistin	0.5	2	≤0.25 - >8	98.3
	Minocycline	≤0.12	0.5	≤0.12 - 8	98.3
	Tigecycline	0.12	1	0.03 - 2	NA
Global, *A. pittii* (232)	Sulbactam-durlobactam	0.5	2	0.12 - 4	NA
	Sulbactam	2	4	0.5 - 64	NA
	Cefepime	4	8	0.25 - >16	90.1
	Imipenem	0.25	0.25	0.12 - 64	96.6
	Meropenem	0.5	1	0.06 - 64	95.7
	Amikacin	1	2	≤0.5 - >64	97.4
	Ciprofloxacin	≤0.12	0.5	≤0.12 - >4	93.5
	Colistin	0.5	1	≤0.25 - 2	100
	Minocycline	≤0.12	0.25	≤0.12 - 4	100
	Tigecycline	0.12	0.5	0.03 - 2	NA
Asia/South Pacific, All (221)	Sulbactam-durlobactam	1	2	0.06 - 64	NA
	Sulbactam	32	64	0.5 - >64	NA
	Cefepime	>16	>16	0.5 - >16	30.3
	Imipenem	32	64	0.06 - >64	31.2
	Meropenem	64	>64	0.13 - >64	31.7
	Amikacin	>64	>64	≤0.5 - >64	42.1
	Ciprofloxacin	>4	>4	≤0.12 - >4	30.3
	Colistin	0.5	1	≤0.25 - >8	97.3
	Minocycline	2	8	≤0.12 - >16	77.8
	Tigecycline	1	2	0.03 - 8	NA
Europe, All (713)	Sulbactam-durlobactam	1	4	≤0.03 - 64	NA
	Sulbactam	8	64	0.25 - >64	NA
	Cefepime	16	>16	≤0.12 - >16	43.8
	Imipenem	16	64	0.06 - >64	47.8
	Meropenem	8	>64	0.06 - >64	47.5
	Amikacin	4	>64	≤0.5 - >64	57.5
	Ciprofloxacin	>4	>4	≤0.12 - >4	41.4
	Colistin	0.5	1	≤0.25 - >8	93.4
	Minocycline	0.5	16	≤0.12 - >16	74.1
	Tigecycline	0.5	2	0.03 - 8	NA
Latin America, All (262)	Sulbactam-durlobactam	1	4	0.12 - >64	NA
	Sulbactam	16	64	0.5 - >64	NA
	Cefepime	>16	>16	0.5 - >16	23.3
	Imipenem	32	>64	0.06 - >64	26.7
	Meropenem	64	>64	0.12 - >64	25.6
	Amikacin	32	>64	≤0.5 - >64	37.4

Region, category (no. of isolates)	Antimicrobial agent	MIC (µg/ml)			Percent susceptible[a]
		MIC_{50}	MIC_{90}	Range	
	Ciprofloxacin	>4	>4	≤0.12 - >4	22.5
	Colistin	0.5	1	≤0.25 - >8	98.9
	Minocycline	0.5	8	≤0.12 - >16	87.4
	Tigecycline	0.5	2	≤0.015 - 4	NA
Middle East (Israel), All (15)	Sulbactam-durlobactam	1	2	0.25 - 2	NA
	Sulbactam	8	16	2 - 32	NA
	Cefepime	>16	>16	2 - >16	6.7
	Imipenem	16	32	0.25 - 64	26.7
	Meropenem	32	64	0.25 - 64	26.7
	Amikacin	32	64	1 - >64	20.0
	Ciprofloxacin	>4	>4	0.25 - >4	6.7
	Colistin	0.5	1	0.5 - 1	100
	Minocycline	0.5	16	≤0.12 - 16	86.7
	Tigecycline	1	1	0.12 - 2	NA
North America (USA), All (511)	Sulbactam-durlobactam	1	2	≤0.03 - 8	NA
	Sulbactam	4	32	0.25 ->64	NA
	Cefepime	8	>16	0.5 - >16	53.0
	Imipenem	0.25	64	0.06 - >64	65.0
	Meropenem	1	>64	0.12 - >64	61.2
	Amikacin	2	>64	≤0.5 - >64	74.0
	Ciprofloxacin	0.5	>4	≤0.12 - >4	55.4
	Colistin	0.5	1	≤0.25 - >8	95.1
	Minocycline	0.25	8	≤0.12 - >16	86.7
	Tigecycline	0.5	2	0.03 - 32	NA
Global, Bloodstream infections (238)	Sulbactam-durlobactam	1	4	0.06 - 8	NA
	Sulbactam	4	32	0.5 - >64	NA
	Cefepime	8	>16	0.5 - >16	53.8
	Imipenem	0.25	64	0.06 - >64	59.7
	Meropenem	1	>64	0.12 - >64	58.8
	Amikacin	2	>64	≤0.5 - >64	67.6
	Ciprofloxacin	0.5	>4	≤0.12 - >4	55.0
	Colistin	0.5	1	≤0.25 - >8	95.0
	Minocycline	0.25	16	≤0.12 - >16	84.0
	Tigecycline	0.25	2	0.03 - 4	NA
Global, Respiratory tract infections (1056)	Sulbactam-durlobactam	1	2	≤0.03 - >64	NA
	Sulbactam	8	64	0.25 - >64	NA
	Cefepime	>16	>16	≤0.12 - >16	36.0
	Imipenem	32	64	0.06 - >64	41.2
	Meropenem	32	>64	0.06 - >64	39.5

Region, category (no. of isolates)	Antimicrobial agent	MIC (µg/ml)			Percent susceptible[a]
		MIC_{50}	MIC_{90}	Range	
	Amikacin	16	>64	≤0.5 - >64	50.8
	Ciprofloxacin	>4	>4	≤0.12 - >4	36.0
	Colistin	0.5	1	≤0.25 - >8	94.2
	Minocycline	1	16	≤0.12 - >16	78.3
	Tigecycline	0.5	2	0.03 - 32	NA
Global, Urinary tract infections (316)	Sulbactam-durlobactam	1	2	0.06 - >64	NA
	Sulbactam	4	32	0.25 - >64	NA
	Cefepime	16	>16	0.25 - >16	49.7
	Imipenem	0.5	64	0.06 - >64	58.5
	Meropenem	1	>64	0.06 - >64	57.6
	Amikacin	2	>64	≤0.5 - >64	69.3
	Ciprofloxacin	>4	>4	≤0.12 - >4	47.5
	Colistin	0.5	1	≤0.25 - >8	97.8
	Minocycline	0.25	16	≤0.12 - >16	83.2
	Tigecycline	0.5	2	0.03 - 8	NA
Global, Intraabdominal infections (65)	Sulbactam-durlobactam	1	2	0.12 - 8	NA
	Sulbactam	16	32	1 - >64	NA
	Cefepime	>16	>16	1 - >16	32.3
	Imipenem	32	64	0.12 - >64	35.4
	Meropenem	32	>64	0.12 - >64	35.4
	Amikacin	32	>64	≤0.5 - >64	47.7
	Ciprofloxacin	>4	>4	≤0.12 - >4	29.2
	Colistin	0.5	1	≤0.25 - >8	98.5
	Minocycline	0.5	8	≤0.12 - 16	83.1
	Tigecycline	0.5	2	0.03 - 4	NA
Global, Skin and soft tissue infections (14)	Sulbactam-durlobactam	0.5	1	0.5 - 1	NA
	Sulbactam	2	32	0.5 - 64	NA
	Cefepime	4	>16	1 - >16	71.4
	Imipenem	0.25	1	0.12 - 64	85.7
	Meropenem	0.25	32	0.12 - >64	85.7
	Amikacin	2	>64	≤0.5 - >64	85.7
	Ciprofloxacin	≤0.12	>4	≤0.12 - >4	71.4
	Colistin	0.5	1	≤0.25 - 1	100
	Minocycline	0.25	0.5	≤0.12 - 4	100
	Tigecycline	0.25	0.5	≤0.015 - 1	NA
Global, Other and unknown sources of infections (33)	Sulbactam-durlobactam	1	2	0.12 - 32	NA
	Sulbactam	4	32	0.5 - 64	NA
	Cefepime	16	>16	0.5 - >16	48.5
	Imipenem	1	64	0.12 - >64	57.6

Region, category (no. of isolates)	Antimicrobial agent	MIC (µg/ml)			Percent susceptible[a]
		MIC_{50}	MIC_{90}	Range	
	Meropenem	1	>64	0.12 - >64	57.6
	Amikacin	4	>64	1 - >64	67.7
	Ciprofloxacin	>4	>4	≤0.12 - >4	45.5
	Colistin	0.5	1	≤0.25 - 1	100
	Minocycline	0.25	8	≤0.12 - 16	81.8
	Tigecycline	0.25	1	0.06 - 2	NA

366 [a]Percent susceptible according to CLSI M100S 2019; NA, no available breakpoints.
367

368

Table 2 MIC cumulative frequency distribution for sulbactam-durlobactam (SUL-DUR) and sulbactam (SUL) against clinical isolates of ABC collected globally from 2016-2017

Category (n) and drug[a]	% Frequency distribution by MIC (µg/ml)[b]												
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
All ABC (1722)													
SUL-DUR	0.2	0.3	2.8	13.0	42.0	71.8	**90.9**	97.7	98.6	98.8	99.4	99.9	100
SUL	0	0	0	0.1	1.3	13.5	35.8	45.1	54.9	74.2	89.2	**97.1**	100
Asia/SP (221)													
SUL-DUR	0	0.5	2.3	9.0	44.3	78.7	**94.6**	98.2	98.2	98.6	99.1	100	
SUL	0	0	0	0	1.8	10.0	26.2	31.2	36.2	49.8	80.5	**97.3**	100
Europe (713)													
SUL-DUR	0.1	0.3	2.4	13.0	40.1	67.6	87.8	**98.3**	99.2	99.2	99.4	100	
SUL	0	0	0	0.1	1.3	13.3	37.0	44.0	52.0	71.0	87.5	**97.2**	100
Lat. Amer. (262)													
SUL-DUR	0	0	1.5	9.5	32.4	62.6	86.6	**92.4**	94.7	95.4	98.5	99.2	100
SUL	0	0	0	0	0.4	6.9	19.8	26.0	36.3	72.9	85.5	**92.0**	100
Middle East (15)													
SUL-DUR	0	0	0	6.7	26.7	73.3	**100**						
SUL	0	0	0	0	0	0	6.7	20.0	66.7	**93.3**	100		
N. Amer. (511)													
SUL-DUR	0.4	0.6	4.5	16.4	49.1	79.3	**95.7**	99.4	100				
SUL	0	0	0	0.2	1.6	19.0	47.4	63.2	76.1	89.4	**97.1**	99.4	100

[a]SUL-DUR, sulbactam-durlobactam; SUL, sulbactam; Asia/SP, Asia and South Pacific; Lat. Amer., Latin America; N. Amer., North America; n, number of isolates tested. [b]MIC_{90} values are in bold for each MIC distribution.

374

375 **Table 3** MIC cumulative frequency distribution for sulbactam-durlobactam (SUL-DUR)
376 and sulbactam (SUL) against drug-resistant clinical isolates of ABC

Category (n) and drug[a]	% Frequency distribution by MIC (μg/ml)[b]												
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
All ABC (1722)													
SUL-DUR	0.2	0.3	2.8	13.0	42.0	71.8	**90.9**	97.7	98.6	98.8	99.4	99.9	100
SUL	0	0	0	0.1	1.3	13.5	35.8	45.1	54.9	74.2	89.2	**97.1**	100
IPM-NS (909)													
SUL-DUR	0	0	0.4	5.1	22.1	56.5	84.2	**95.7**	97.4	97.7	98.9	99.8	100
SUL	0	0	0	0	0	0.1	1.0	5.2	19.3	53.2	80.1	**94.6**	100
CST-R (81)													
SUL-DUR	0	0	0	4.9	16.0	46.9	80.2	**98.8**	100				
SUL	0	0	0	0	0	2.5	6.2	12.3	33.3	61.7	82.7	**97.5**	100
MIN-NS (337)													
SUL-DUR	0	0	0	1.5	8.0	32.3	73.0	**95.8**	97.9	98.2	98.8	100	
SUL	0	0	0	0	0	0	0.6	3.6	12.8	39.2	76.9	**94.1**	100
CIP-NS (1017)													
SUL-DUR	0	0	1.0	6.1	23.5	57.8	85.9	**96.8**	98.1	98.4	99.0	99.8	100
SUL	0	0	0	0	0.1	1.5	4.9	11.6	26.0	57.7	82.8	**95.8**	100
AMK-NS (740)													
SUL-DUR	0	0	0.5	4.3	20.8	53.5	83.5	**96.5**	98.0	98.2	99.1	99.7	100
SUL	0	0	0	0	0	0.1	0.8	5.4	18.1	48.9	78.6	**94.7**	100
MDR (259)													
SUL-DUR	0	0	0	1.2	6.2	30.1	69.5	**95.0**	97.3	97.7	98.5	100	
SUL	0	0	0	0	0	0	0	1.2	6.2	30.1	72.2	**93.1**	100
XDR (32)													
SUL-DUR	0	0	0	0	0	25.0	65.6	**100**					
SUL	0	0	0	0	0	0	0	0	3.1	25.0	65.6	**93.8**	100

377 [a]n, number of isolates tested SUL-DUR, sulbactam-durlobactam; SUL, sulbactam; IPM-NS,
378 imipenem-non-susceptible; CST-R, colistin-resistant; MIN-NS, minocycline-non-susceptible;
379 CIP-NS, ciprofloxacin-non-susceptible; AMK-NS, amikacin-non-susceptible; MDR, multidrug-
380 resistant (IPM-NS, MIN-NS, AMK-NS); XDR, extensively drug-resistant (IPM-NS, MIN-NS,
381 AMK-NS, CIP-NS, CST-R, SUL-NS, FEP-NS). [b]MIC_{90} values are in bold for each MIC
382 distribution.

383 **Table 4** Antibiotic susceptibility and demographic information for *A. baumannii* isolates with reduced susceptibility to
384 sulbactam-durlobactam

Isolate ID	Year	Country	MIC (µg/ml)[a]						MLST[b] (Oxford/ Institute Pasteur)	Efflux/ porin variants[c]	*bla* variants	PBP variants
			SUL-DUR	IPM	AMK	CIP	CST	MIN				
1308777	2016	Vietnam	16	64	>64	>4	0.5	16	ST_{OX}1806, 208/ ST_{IP}2	ND	ADC-82; OXA-23; OXA-66	PBP3 [T526S]
1465642	2016	Belgium	64	64	4	>4	≤0.25	≤0.12	ST_{OX}1089/ ST_{IP}85	ND	OXA-94; NDM-1; subclass B3 MBL	ND
1435703	2016	France	32	>64	2	>4	≤0.25	0.5	ST_{OX}164/--	ND	CARB-2; OXA-58; OXA-91; NDM-1	ND
1407634	2016	Spain	8	>64	4	>4	≤0.25	1	ST_{OX}106/ ST_{IP}3	AdeI [E30K]	ADC-7-like; OXA-24; OXA-71	PBP3 [T337I, G523V]
1407635	2016	Spain	8	64	>64	>4	0.5	16	ST_{OX}218/ ST_{IP}2	ND	ADC-30; OXA-23; OXA-66	ND
1420327	2016	Mexico	32	16	>64	>4	≤0.25	8	ST_{OX}1806, 208/ ST_{IP}2	ND	ADC-30; TEM-1; OXA-83	PBP3 [A515V]
1386786	2016	Argentina	32	>64	>64	>4	0.5	1	ST_{OX}229/ ST_{IP}25	ND	ADC-26; OXA-64; PER-7; NDM-1	ND
1457337	2016	Argentina	16	32	16	>4	0.5	4	ST_{OX}1806, 208/ ST_{IP}2	ND	ADC-25; OXA-23; OXA-66	PBP3 [T526S]
1484127	2016	USA	8	64	>64	>4	0.5	8	ST_{OX}1806, 208/ ST_{IP}2	ND	ADC-30; OXA-23; OXA-66	PBP3 [T526S]
1483449	2016	USA	8	64	>64	>4	0.5	16	ST_{OX}1806, 208/ ST_{IP}2	ND	ADC-73; OXA-23; OXA-66	PBP3 [A515V]
1683261	2017	Thailand	64	>64	2	>4	≤0.25	≤0.12	ST_{OX}355/--	No *adeC*	ADC-169; CARB-2; OXA-402; NDM-1	ND
1683267	2017	Thailand	64	>64	>64	>4	1	8	ST_{OX}1838, 349/ $ST2_{IP}$	ND	ADC-73; TEM-1; OXA-23; OXA-66; NDM-1	ND

Isolate ID	Year	Country	MIC (µg/ml)[a]						MLST[b] (Oxford/ Institute Pasteur)	Efflux/ porin variants[c]	*bla* variants	PBP variants
			SUL-DUR	IPM	AMK	CIP	CST	MIN				
1662094	2017	Thailand	32	>64	32	>4	≤0.25	1	ST_{OX}355/ ST_{IP}16	No *adeC*	ADC-169; OXA-58; OXA-402; VEB-1; NDM-1	ND
1589408	2017	Belgium	64	64	4	>4	≤0.25	≤0.12	ST_{OX}1089/ ST_{IP}85	No *adeC*	ADC-80 [V119E]; ADC-176; OXA-94; NDM-1	ND
1620872	2017	Greece	8	64	>64	>4	1	8	ST_{OX}1816, 195/ ST_{IP}2	*adeA::Tn*	ADC-73; OXA-23; OXA-66	PBP1A [W343*] ; PBP3 [A515V]
1629634	2017	Italy	8	64	>64	>4	0.5	16	ST_{OX}1806, 208/ ST_{IP}2	ND	ADC-73; TEM-1; OXA-23; OXA-66	PBP3 [A515V]
1562926[d]	2017	Spain	64	64	64	>4	0.5	>16	ST_{OX}1489/ ST_{IP}25	AdeJ [A290S]; No *adeC*; *adeS::Tn*	ADC-5 [G239S, N341T]; OXA-23; OXA-64	PBP3 [T526S]; PBP6b [T188S]
1557168	2017	Spain	8	64	2	>4	≤0.25	16	ST_{OX}218/ ST_{IP}2	ND	OXA-23; OXA-66	PBP1A [W465*] ; PBP3 [K235N]
1557526	2017	Turkey	8	64	>64	>4	>8	2	ST_{OX}448/ ST_{IP}2	AdeA [Q202L]	ADC-30; TEM-1; OXA-23; OXA-66	PBP3 [F548I]
1625487	2017	Argentina	8	64	64	>4	1	2	ST_{OX}1806, 208/ ST_{IP}2	ND	ADC-25; OXA-23; OXA-66	PBP3 [T526S]
1647876	2017	Argentina	8	64	4	>4	≤0.25	4	ST_{OX}1806, 208/ ST_{IP}2	ND	ADC-25; OXA-23; OXA-66	PBP3 [T526S]
1647764	2017	Argentina	8	64	64	>4	0.5	2	ST_{OX}1806, 208/ ST_{IP}2	AdeH [Q79R]	ADC-25; OXA-23; OXA-66	PBP3 [T526S]
1660516	2017	Colombia	>64	>64	>64	>4	≤0.25	4	--/ST_{IP}2	ND	ADC-73; OXA-23; OXA-66; NDM-1	PBP3 [A515V]

Isolate ID	Year	Country	MIC (µg/ml)[a]						MLST[b] (Oxford/ Institute Pasteur)	Efflux/ porin variants[c]	*bla* variants	PBP variants
			SUL-DUR	IPM	AMK	CIP	CST	MIN				
1660477	2017	Colombia	8	>64	>64	>4	0.5	1	ST_{OX}124/ ST_{IP}79	AdeC [Q93*]	ADC-5; TEM-1: OXA-23; OXA-65	PBP1A [T117S]
1692917	2017	Ecuador	32	64	4	>4	≤0.25	0.25	--/ ST_{IP}126	No *adeC*	ADC-50; OXA-58; OXA-64; NDM-1	ND
1699512[e]	2017	Guatemala	64	>64	>64	>4	0.5	4	--/ ST_{IP}108	No *adeC*	ADC-152 [S341T]; TEM-1; OXA-132; NDM-1	PBP3 [V146I]
1699517[f]	2017	Guatemala	32	>64	2	0.25	0.5	≤0.12	ST_{OX}1736/ ST_{IP}734	AdeT [Q63P]; CarO [W179fs]	ADC-99-like; OXA-24; OXA-69	PBP2 [A133T]; PBP3 [N377Y, T526S]; PBP6b [A183V]
1699549	2017	Guatemala	16	64	>64	>4	0.5	2	--/ ST_{IP}108	No *adeC*	ADC-152 [S341T]; OXA-24; OXA-132	PBP3 [Q488K]
1699548	2017	Guatemala	8	>64	>64	>4	0.5	1	--/ ST_{IP}1	AdeC [P29L]	ADC-53 [A236V]; TEM-1; CTX-M-15; OXA-24; OXA-69	PBP3 [T526S]
1699513	2017	Guatemala	>64	>64	64	>4	0.5	0.25	ST_{OX}514/ ST_{IP}103	No *adeC*	ADC-97-like; TEM-1; OXA-70; NDM-1	MtgA [F12I]; PBP3 [N377Y, T526S]
1558650	2017	USA	8	32	>64	>4	≤0.25	8	ST_{OX}1701/ ST_{IP}2	ND	ADC-25; OXA-23; OXA-66	PBP3 [F548I]

[a]SUL-DUR, sulbactam-durlobactam; IPM, imipenem; AMK, amikacin; CIP, ciprofloxacin; CST, colistin; MIN, minocycline. [b]MLST, multi-locus sequence type determined by whole genome sequencing. ST_{OX}/ST_{IP}, sequence type Oxford/ Pasteur schemes. [c]ND, none detected. [d]Two additional clonal isolates identified. [e]One additional clonal isolate identified. [f]Five additional clonal isolates identified.

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