CLINICS

RAPID COMMUNICATION

The minimal inhibitory concentration for sulbactam was not associated with the outcome of infections caused by carbapenem-resistant *Acinetobacter* sp. treated with ampicillin/sulbactam

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OBJECTIVE: The objective of this study was to evaluate whether the outcomes of carbapenem-resistant *Acinetobacter* infections treated with ampicillin/sulbactam were associated with the in vitro susceptibility profiles.

METHODS: Twenty-two infections were treated with ampicillin/sulbactam. The median treatment duration was 14 days (range: 3-19 days), and the median daily dose was 9 g (range: 1.5-12 g). The median time between *Acinetobacter* isolation and treatment was 4 days (range: 0-11 days).

RESULTS: The sulbactam minimal inhibitory concentration (MIC) ranged from 2.0 to 32.0 mg/L, and the MIC was not associated with patient outcome, as 4 of 5 (80%) patients with a resistant infection (MIC≥16), 5 of 10 (50%) patients with intermediate isolates (MIC of 8) and only 1 of 7 (14%) patients with susceptible isolates (MIC ≤4) survived hospitalization.

CONCLUSION: These findings highlight the need to improve the correlation between in vitro susceptibility tests and clinical outcome.

KEYWORDS: Antimicrobial Susceptibility; Resistance; Treatment.

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INTRODUCTION

Infections caused by carbapenem-resistant *Acinetobacter* spp. are therapeutically challenging because treatment options are limited. The most studied of these options include polymyxins and sulbactam. Sulbactam, a synthetic beta-lactam, is mainly used as a beta-lactamase inhibitor, but it exhibits in vitro activity against *Acinetobacter* spp. (1) and has been used to treat infections caused by this organism. In vitro susceptibility testing for sulbactam and *Acinetobacter* spp. are problematic for multiple reasons. First, the minimal inhibitory concentration (MIC) breakpoint for sulbactam has not been determined. As a result, the criterion for an ampicillin/sulbactam combination is typically used instead (2). Second, unacceptably high proportions of errors associated with the disk diffusion method have been published. In one study, 196 clinical isolates of *Acinetobacter* spp. were tested using disk diffusion and broth microdilution, and unacceptably high proportions of errors occurred for ampicillin/sulbactam (A/S) (very major: 9.8%; minor: 16.1%) (3) Third, the MIC breakpoints used to interpret results are not well studied and may not predict clinical outcomes.

The objective of this study was to evaluate whether the outcomes of patients with carbapenem-resistant *Acinetobacter* infections treated with A/S were associated with the in vitro susceptibility profiles.

METHODS

This study was conducted at Hospital das Clínicas, a 1,988-bed, tertiary-care teaching hospital affiliated with the University of São Paulo. We performed a retrospective review of all patients who visited the hospital from 2000 through 2004 for carbapenem-resistant *Acinetobacter baumannii* (CRAB) bacteremia and were treated with at least 4 doses of A/S. Information was collected from the patients' medical records. Infection diagnoses were based on CDC criteria (4) and were obtained from the infection-control

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database. Patients were excluded if they had received polymyxin simultaneously.

Isolates were phenotypically identified using an automated method (Vitek; bioMerieux; Hazelwood; MO; USA) and confirmed using classical microbiological techniques. The antimicrobial activities of sulbactam were evaluated against carbapenem-resistant *Acinetobacter* sp. isolates. Carbapenem resistance was defined as resistance to imipenem by broth microdilution susceptibility testing using the Clinical and Laboratory Standards Institute (CLSI) criteria (MIC≥16 mg/L). Imipenem powder was obtained from Merck & Co., Inc. (EUA).

The sulbactam MIC was determined using the broth microdilution method according to the CLSI guidelines (5). Sulbactam powder was obtained from European Pharmacopoeia Reference Standards CRS & BRP (EDQM European for the Quality of Medicines and Healthcare; Council of Europe; Catalogue code Y0000528). The culture medium consisted of cation-adjusted Mueller-Hinton broth (BBL™ Becton Dickinson). A standardized inoculum was prepared using the direct colony suspension method. Each bacterial suspension was adjusted to the 0.5 McFarland turbidity standard (1 to 2×10^8 CFU/mL) using a photometric device (colorimeter Vitek®1, BioMérieux, Etoile, France). The adjusted inoculum suspension was diluted in broth to achieve each an approximate final concentration of 5×10^5 CFU/mL in each well. The sulbactam final concentrations were 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 mg/L. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control (QC) strains. The MIC results were evaluated by at least 3 observers.

We described the characteristics of the patients, infections, treatments, mortality outcomes during treatment, in-hospital mortality and clinical failure (defined as death or persistent signs and symptoms of infection, persistent isolation of *Acinetobacter* or a change in the antibiotic between day 3 and 7 of A/S treatment).

Bivariate analysis was performed for 2 outcomes (mortality during treatment and in-hospital mortality). Multivariate analysis using logistic regression was performed for in-hospital mortality. Data were analyzed using EpiInfo 3.5.2 (CDC, Atlanta, GA).

RESULTS

Sixty-three CRAB infections occurred in 58 patients; of these, 20 received no treatment, 22 received A/S, 10 received colistin, 4 received colistin + A/S, and records were not available for 2 patients. The mean age of the studied patients was 48 years (SD: 23.3). Of the total patients, 64% were male, 21 (96%) used central venous catheters, 16 (73%) used urinary catheters, and 12 (55%) required mechanical ventilation. The median treatment duration was 14 days (range: 3-19 days), and the median daily dose was 9 g (range: 1.5-12 g). The median time between *Acinetobacter* isolation and treatment was 4 days (range: 0-11 days). Eight patients (36%) received simultaneous carbapenems, and 13 (59%) received vancomycin. A description of the studied cases is shown in Table 1.

The sulbactam MICs ranged from 2.0 to 32.0 mg/L. Five (23%) patients were classified as resistant, 7 (32%) were susceptible, and 10 (45%) were intermediate. Clinical failure occurred in 7 (33%) patients. Seven (33%) patients died during treatment, and 12 patients (55%) died during hospitalization. The outcomes stratified by the sulbactam MICs are depicted in Figure 1. Bivariate analyses showed that male sex and ICU admission were risk factors for in-hospital mortality (Table 2).

Multivariate analysis revealed that male sex (OR 15.16; 95% CI: 1.15-200.41) and admission to the ICU (OR: 15.20; 95% CI: 1.15-200.40) were associated with in-hospital mortality. The MICs for sulbactam and simultaneous treatment with carbapenems were not associated with patient outcome.

DISCUSSION

Sulbactam, a beta-lactamase inhibitor, also exhibits intrinsic activity against *Acinetobacter* spp., including carbapenem-resistant strains, and therefore represents an alternative to treatment with polymyxins (6). However, the optimal treatment for multidrug-resistant *Acinetobacter* infections has not been established (7).

Our case series involved 22 patients with mainly catheter-associated bloodstream infections. Surprisingly, patients infected with *Acinetobacter* who demonstrated higher MICs were more severely ill but had lower in-hospital mortality rates. Correlating in vitro antimicrobial susceptibility profiles with in vivo clinical outcomes can be difficult. First, appropriate breakpoints should be set. Breakpoints may originally be defined as the concentrations that distinguish subpopulations based on the MIC distribution, although these determinations are also used to guide therapy. Therefore, the use of breakpoints originally created to distinguish microbiological subpopulations to predict clinical success may be problematic. The EUCAST defined the latter breakpoint as a ''clinical breakpoint'' and the former as a ''microbiological or epidemiological breakpoint''. However, this terminology is not universal, and several current guidelines do not make these distinctions (8). To our knowledge, these differences have not been evaluated for A/S, and in our case-series, it appeared that the breakpoints did not adequately predict patient outcomes.

It is always difficult to define the exact cause of death in patients with multi-resistant infections because they often exhibit several underlying diseases, receive invasive procedures and have been hospitalized for long periods (9). In our study, only admission to the ICU and sex were independently associated with in-hospital mortality. Admission to the ICU reflects the severity of the patient's condition, but we cannot explain the influence of gender on mortality.

Clinical efficacy is also influenced by the pharmacokinetics/pharmacodynamics of antimicrobials, which may be altered in critically ill patients. A recent study in an ex vivo human model showed that doses of 0.5 and 1 g of sulbactam infused over 30 minutes resulted in bactericidal serum levels at 2 hours after treatment. However, net regrowth and a trend to regrowth occurred, which suggests that a desirable length of time above a MIC>50% would not be achieved with the common dosage regime (1 g every 6 hours) (10).

In addition, in vitro susceptibility tests for sulbactam against *Acinetobacter* spp. may not be reliable. In one study, 196 clinical isolates of *Acinetobacter* spp. were tested by disk diffusion and broth microdilution, and unacceptably high proportions of errors occurred for A/S (very major: 9.8%; minor: 16.1%) (3). Another study evaluated the activity of sulbactam-containing combinations by broth microdilution against 469 *Acinetobacter* isolates and concluded that testing

Table 1 - Summary of clinical characteristics and outcome of 22 patients with carbapenem-resistant *Acinetobacter* spp. infections treated with ampicillin-sulbactam. Hospital das Clínicas, University of São Paulo, Brazil.

Gender	Age (y)	Apache II score (points)	Underlying diseases	Mechanical ventilation	Urinary catheter	Central venous catheter	*Acinetobacter* infection	Daily ampicillin-sulbactam dose (g)	Highest creatinine level during treatment (mg/dl)	Treatment duration (days)	Days to initiate treatment	Clinical outcome	In- hospital death	Sulbactam MIC (mg/L)
M	55	19	Renal transplant	No	No	Yes	BSI	6	2.5	14	9	Success	No	2
M	59	9	Cancer of the larynx	Yes	Yes	Yes	BSI	3	5.3	15	4	Success	Yes	2
M	84	23	Stroke	Yes	Yes	Yes	Pneumonia	NA	1.6	15	7	Success	Yes	2
M	46	13	Burn	Yes	Yes	Yes	Pneumonia	12	0.6	14	5	Success	Yes	4
F	16	14	Acute abdomen	Yes	Yes	Yes	BSI	9	1.1	16	4	Success	No	4
F	74	15	Endometrial cancer	Yes	Yes	Yes	BSI	9	2.7	14	3	Success	Yes	4
M	63	13	Lymphoma	No	Yes	Yes	BSI	6	2,2	3	3	Failure	Yes	4
M	58	22	Cirrhosis and dialytic renal failure	Yes	Yes	Yes	BSI	6	3.6	10	4	Failure	Yes	8
M	23	18	Trauma	Yes	Yes	Yes	BSI	12	0.7	11	6	Success	No	8
M	70	19	Chronic obstructive pulmonary disease	Yes	Yes	Yes	BSI	3	3.8	14	2	Success	Yes	8
M	7	19	Burn	Yes	Yes	Yes	BSI	4	0.7	9	3	Failure	Yes	8
M	24	11	Leukemia	No	No	No	BSI	12	3.3	8	3	Failure	Yes	8
F	54	7	Breast cancer	No	No	Yes	BSI	12	NA	13	36	Success	No	8
M	56	NA	Acute abdomen	No	No	Yes	BSI	6	4.3	11	4	Failure	Yes	8
F	36	11	Hemangioblastoma	Yes	Yes	Yes	Pneumonia	12	0.6	15	5	Sucess	No	8
M	60	10	Stomach cancer	No	Yes	Yes	BSI	12	NA	3	4	Success	No	8
F	7	9	Heart failure	Yes	Yes	Yes	BSI	1.5	0.9 0.8	3	3	Failure	Yes	8
M	81	15	Heart failure	No	No	Yes	BSI	12	2.5	19	0	Success	No	16
F	23	18	Systemic erythematous lupus	Yes	Yes	Yes	BSI	3	4.4	16	11	Success	No	16
F	33	13	Kidney and pancreas transplant	No	Yes	Yes	Surgical site	3	2.5	14	5	Success	No	16
M	67	22	Multiple myeloma	No	No	Yes	BSI	12	NA	10	1	Failure	Yes	16
F	61	17	Leukemia	No	Yes	Yes	BSI	9	1.6	15	1	Success	No	16

M: male, F: female, BSI: blood stream infection, NA: not available; MIC: minimal inhibitory concentration.

Table 2 - Bivariate analysis of factors associated with in-hospital mortality and mortality during treatment in patients with carbapenem-resistant *Acinetobacter* spp. infections treated with ampicillin-sulbactam. Hospital das Clínicas, University of São Paulo, Brazil.

	In-hospital mortality				Mortality during treatment			
	Non-survivors (n = 12)	Survivors (n = 10)	RR (95% CI)	*p*	Death during treatment (n = 7)	Survivors until the end of treatment (n = 15)	RR (95% CI)	*p*
Age (years)								
Mean (SD)	56.3 (21.6)	44.2 (21)		0.11	48.9 (23.7)	51.7 (21.6)		0.97
Median (range)	61 (7-84)	45 (16-81)			58 (7-67)	55 (16-84)		
Male gender (%)	10	4	2.86 (0.82-9.92)	0.04	6	8	3.42 (0.49-23.6)	0.15
APACHE II score (points)				0.44				0.64
Mean (SD)	15.9 (5.3)	14.2 (4.0)			16(5.7)	14.7 (4.4)		
Median (range)	15 (9-23)	14.5 (7-19)			16 (9-22)	15 (7-23)		
Admission to ICU (%)	10	4	2.86 (0.82-9.92)	0.04	5	9	1.43(0.35- 5.74)	0.61
Acinetobacter infection site (n)				0.50				
Bloodstream infection	10	8			7	11		0.32
Pneumonia	2	1			0	3		
Surgical site infection	0	1			0	1		
Time between isolation and beginning of treatment (days)				0.10				0.06
Mean (SD)	3.5 (1.5)	5.1 (3.2)			3.0 (1.0)	4.8 (2.9)		
Median (range)	3 (1-7)	5 (0-11)			3 (1-4)	5 (0-11)		
Daily Dose (grams)					6.8 (3.9)	9.6 (3.7)		0.21
Mean (SD)	6.7 (3.9)	9.0 (3.7)		0.21				
Median (range)	6 (1.5-12)	10.5 (3-12)			6 (1.5-12)	10.5 (3-12)		
Simultaneous use of carbapenem(n)	6	2	1.75(0.85-3.61)	0.15	3	5	1.31(0.39-4.44)	0.66
MIC (mg/L)				0.19				0.24
≤ 4	5	2			1	6		
8	6	4			5	5		
≥16	1	4			1	4		

SD: standard deviation, MIC: minimum inhibitory concentration.

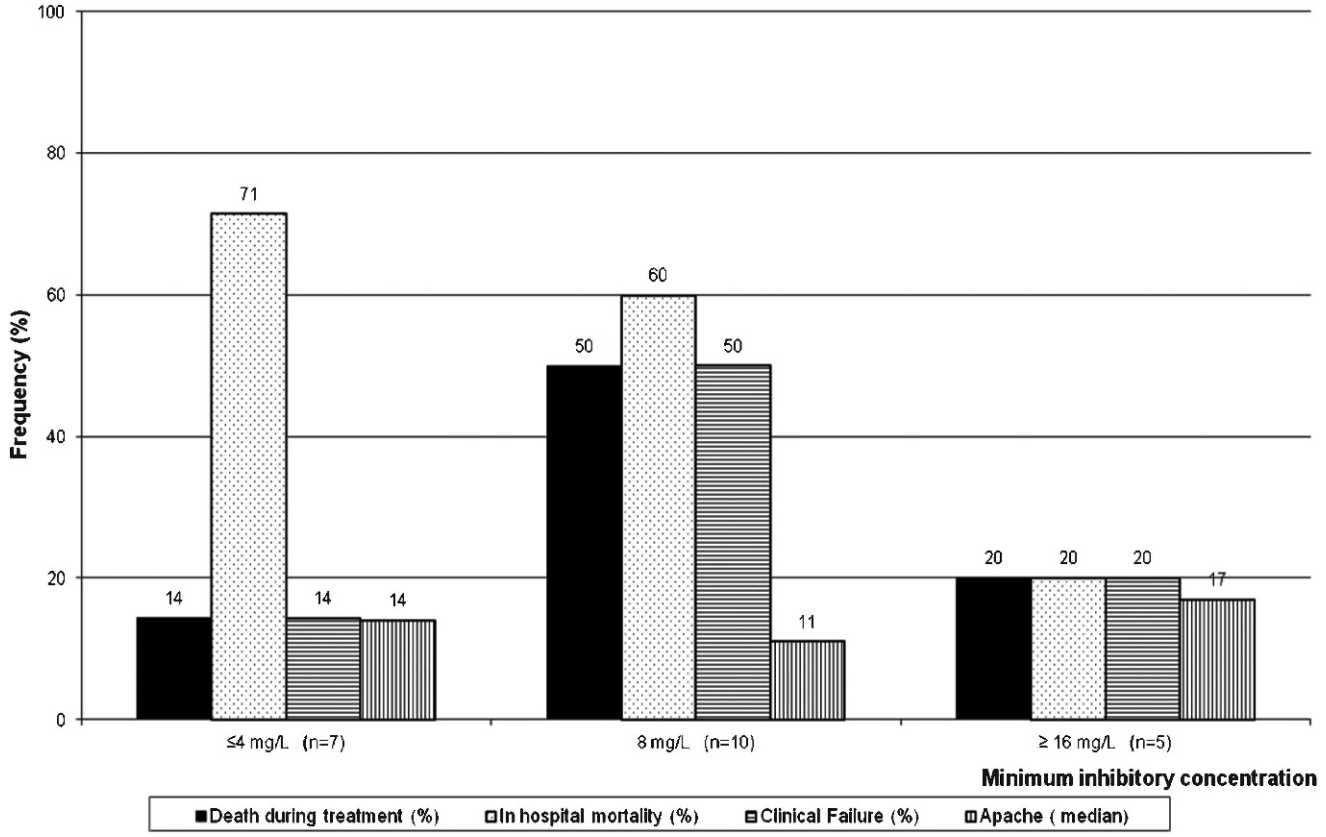

Figure 1 - Clinical outcome, mortality during treatment, in-hospital mortality and median APACHE II score of patients with infections caused by *Acinetobacter* spp. stratified by the minimum inhibitory concentration (MIC) ampicillin/sulbactam. Hospital das Clínicas, University of São Paulo, Brazil.

with the inhibitor at a fixed ratio resulted in more reliable results compared to a fixed concentration (11).

Our study was limited by the small number of patients and its retrospective design. To balance these limitations, we used strict diagnostic criteria for infections, included only blood isolates and used mortality as the main endpoint. Unfortunately, we did not have data concerning catheter removal.

In summary, in this cases series, the MIC was not associated with patient outcome, as 4 of 5 (80%) patients with a MIC≥16 μg/mL (considered resistant), 5 of 10 (50%) patients with a MIC of 8 μg/mL (considered intermediate), and 1 of 7 (29%) patients with a MIC≤4 μg/mL (considered susceptible) survived hospitalization. Although we sought to determine alternative sulbactam breakpoints for *Acinetobacter* infections, this was not possible, which highlights the need for additional studies to improve the correlation between in vitro susceptibility tests and clinical outcome.

AUTHOR CONTRIBUTIONS

Oliveira MS reviewed the medical records, helped writing the manuscript and performed the data analysis. Costa SF performed the data analysis, supervised the experiments and helped writing the manuscript. De Pedri EH performed the experiments. van der Heijden IM performed the experiments and helped writing the manuscript. Levin AS analyzed the data, contributed to the study design and helped writing the manuscript.

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