ORIGINAL ARTICLE

Isolation and Pattern of Antimicrobial Susceptibility Testing of *Acinetobacter baumannii* Clinical Isolates at Taksin Hospital: A Retrospective Study from 2019 to 2021

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Objective: To determine the number of isolates and drug susceptibility testing of *Acinetobacter baumannii* at Taksin Hospital.

Materials and Methods: A 3-year retrospective descriptive study was conducted between January 2019 and December 2021. Drug susceptibility testing of isolates to 13 drugs, which included amikacin, gentamycin, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, three carbapenem drugs that were imipenem, meropenem, and doripenem, ciprofloxacin, trimethoprim-sulfamethoxazole, colistin, and tigecycline, were evaluated by standard disk diffusion method and automated microdilution method.

Results: One thousand six hundred sixty-five non-duplicate *A. baumannii* isolates from different patients were detected and included 535 in 2019, 500 in 2020, and 630 in 2021. The patients' ages ranged from three days to 100 years. The median age of patients was 70 years with an interquartile range of 56 to 80 years. *A. baumannii* was mostly found in patients aged older than 50 years old at 83.6%. They were isolated from sputum in 72.3%, urine in 9%, pus in 6.3%, tissue in 6.3%, and blood in 5.2%. Overall, *A. baumannii* demonstrated 91.5% and 79.8% susceptibility to colistin and tigecycline, respectively, but less susceptible to other drugs tested, with a range of 0.4% to 32.6%. Drug susceptibility to carbapenem such as imipenem, meropenem, and doripenem were 19.5%, 20%, and 20.9%, respectively. The carbapenem-resistant *A. baumannii* (CRAB) was found at 77.6%, 80%, and 79.1% in 2019, 2020, and 2021, respectively. For total isolates tested, the mean of CRAB was 78.9±1.2%.

Conclusion: The present study results suggested the importance of monitoring *A. baumannii* at a hospital in Bangkok. Colistin and tigecycline had the high percentages of drug susceptibility rates in vitro. The antibiogram of susceptibility helps provide guidelines for clinician to consider empirical treatment.

Keywords: *Acinetobacter baumannii*; Drug resistance; Susceptibility

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Acinetobacter baumannii is an important bacterium responsible for causing many human diseases including hospital-acquired pneumonia, bloodstream, and wound, soft tissue, and urinary tract infections[1,2]. The World Health Organization published a list of bacteria with high rate of drug resistance. They classified these bacteria into three groups as critical, high, and medium, according to the urgent need to develop new drug treatment. Only bacteria with critical priority such as carbapenem-resistant *A. baumannii*, carbapenem-resistant *Pseudomonas aeruginosa*; and carbapenem-resistant Enterobacteriaceae, were described[3]. Carbapenem-resistant *A. baumannii* (CRAB) is a major threat to health care systems and patients in the European Union countries and European Economic Area with 2,363 deaths reported in 2015[4,5]. Multiple drug resistance to *A. baumannii* has previously been reported[6,7]. Patterns of *A. baumannii* antimicrobial susceptibility may vary according to country and geography.

The aim of the present study was to determine the number of isolates and drug resistance of *A. baumannii* isolated from patients at Taksin Hospital, a public tertiary care hospital with 500 in-patient beds in Bangkok operated by the Bangkok Metropolitan

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Administration. The present study determined the antibiogram pattern profiles to provide guidance on the treatment of *A. baumannii* disease to clinicians.

Materials and Methods

The present study was conducted after ethical approval was obtained from the Human Research Committee at Siam University, reference code SIAMPY-IRB 2023/007.

Bacterial isolates and identification procedure

One thousand six hundred sixty-five *A. baumannii* isolates were collected from different patients, to prevent duplicates of the same antibiogram profile, at Taksin Hospital, Bangkok over a three-year period between January 1, 2019 and December 31, 2021. All *A. baumannii* consecutive isolates between 2019 and 2021 were used for antimicrobial susceptibility testing. There was no randomization process. If there were multiple clinical isolates from a patient, data from the first isolate only was included in the analysis. *A. baumannii* was isolated from clinical specimens using standard microbiological methods. The sputum specimen, which contained more than 25 polymorphonuclear cells and less than 25 squamous epithelial cells per low-power field at a 10×10 microscope magnification, was accepted for culture. *A. baumannii* was identified on typical gram stained as a gram-negative coccobacillus, non-motile, non-fermentative, colonial morphology with non-hemolytic on 5% sheep blood agar, and confirmed by various biochemical tests such as grow at 44°C, glucose oxidizing, oxidase test, or sugar fermentation tests according to standard microbiological methods[2,8]. *A. baumannii* is the only bacterium in the genus *Acinetobacter* that can grow at 44°C[8].

Antimicrobial susceptibility testing

A. baumannii isolates were tested for antimicrobial susceptibility to 30 µg amikacin, 10 µg gentamycin, 100 µg piperacillin/10 µg tazobactam, 30 µg cefotaxime, 30 µg ceftazidime, 30 µg cefepime, 10 µg imipenem, 10 µg meropenem, 10 µg doripenem, 5 µg ciprofloxacin, and 1.25 µg trimethoprim/23.75 µg sulfamethoxazole by the disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI)[9]. The bacterial inoculum was prepared using the *A. baumannii* colony suspension method in which colonies from an overnight culture of between 20 and 24 hours, on sheep blood agar were used. The turbidity of inoculum was adjusted to the 0.5 McFarland standard and spread on Mueller Hinton agar (MHA) (Oxoid, UK). The MHA was incubated at 35°C for 20 to 24 hours. The criteria for interpretation were susceptible, intermediate, and resistant according to CLSI recommendation[9]. For quality control group of standard disk diffusion method, American Typing Culture Collection isolates of *P. aeruginosa* ATCC 27853 was used. In addition, *Escherichia coli* ATCC 25922 was needed as the quality control strain for tetracyclines and trimethoprim-sulfamethoxazole[9]. Bacterial susceptibility to colistin and tigecycline were evaluated for minimal inhibitory concentration (MIC) in µg/mL by automated microdilution method (Microscan WalkAway: Siemens, USA) according to the manufacturer's guidelines. The automated microdilution method (Microscan WalkAway) was validated before being applied to routine testing by using the following reference strains for internal quality control: *Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, and *Enterococcus faecalis* ATCC 29212. Colistin MICs of 2 or less and 4 mg/L or more were considered susceptible and resistant, respectively, according to a recent study in King Chulalongkorn Memorial Hospital[10] and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guideline[11]. Tigecycline MIC breakpoints were found in the Tygacil® package insert, such as 2 mg/L or less for susceptibility and 8 mg/L or more for resistance[10,12].

Data analysis

Data were entered and analyzed using IBM SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY, USA) for descriptive analyses. Categorical variables, such as ages of patients and source of clinical specimens were expressed as frequency and percentages. The ages of patients were also expressed as range and median (interquartile range, IQR).

Results

In the present study, there were 535, 500, and 630 isolates in 2019, 2020, and 2021, respectively (Table 1). The age of patients ranged from three days to 100 years. The median age of patients was 70, with an IQR of 56 to 80. *A. baumannii* was mostly found in patients aged older than 50 in 83.6% (Table 1). The age group with the lowest number of patients was children aged less than 1 to 10 with 0.9%. For the sources of clinical specimens, the five most common clinical specimens were sputum at 72.3%,

Table 1. Isolation and source of specimens of *A. baumannii*

	No. of patients; n (%)			
	2019	2020	2021	Total
Age groups (years)				
≤1 to 10	8 (1.5)	4 (0.8)	3 (0.5)	15 (0.9)
11 to 20	6 (1.1)	8 (1.6)	8 (1.3)	22 (1.3)
21 to 30	13 (2.4)	11 (2.2)	18 (2.9)	42 (2.5)
31 to 40	25 (4.7)	16 (3.2)	27 (4.3)	68 (4.1)
41 to 50	49 (9.2)	35 (7.0)	43 (6.8)	127 (7.6)
51 to 60	79 (14.8)	63 (12.6)	99 (15.7)	241 (14.5)
61 to 70	99 (18.5)	106 (21.2)	143 (22.7)	348 (20.9)
71 to 80	127 (23.7)	118 (23.6)	161 (25.6)	406 (24.4)
≥80	129 (24.1)	139 (27.8)	128 (20.2)	396 (23.8)
Total	535 (100)	500 (100)	630 (100)	1,665 (100)
Specimens				
Sputum	392 (73.3)	365 (73.0)	447 (71.0)	1204 (72.3)
Urine	55 (10.3)	48 (9.6)	47 (7.5)	150 (9.0)
Pus	32 (6.0)	32 (6.4)	41 (6.5)	105 (6.3)
Blood	27 (5.0)	20 (4.0)	39 (6.2)	86 (5.2)
Tissue*	13 (2.4)	22 (4.4)	34 (5.4)	105 (6.3)
Other body fluid**	16 (3.0)	13 (2.6)	22 (3.4)	51 (3.1)
Total	535 (100)	500 (100)	630 (100)	1,665 (100)

* Buttock, coccyx, cervical swab, vaginal swab; ** Ascitic fluid, bile, bronchial wash, pleural fluid

urine at 9.0%, pus at 6.3%, tissue at 6.3%, and blood at 5.2%.

Antimicrobial susceptibility testing

A. baumannii was susceptible to amikacin in 32.6%, gentamycin in 28.6%, piperacillin/tazobactam in 16.1%, cefotaxime in 0.4%, ceftazidime in 20%, cefepime in 19.8%, imipenem in 19.5%, meropenem in 20%, doripenem in 20.9%, ciprofloxacin in 19.1%, trimethoprim-sulfamethoxazole in 31.3%, colistin in 91.5%, and tigecycline in 79.8% (Table 2).

The data on colistin and tigecycline susceptibility testing for MIC were in ug/mL. Colistin MIC range, MIC_{50} and MIC_{90} value were 2 or less to 4 or more, 2 or less and 4 or more, respectively. Tigecycline MICrange, MIC_{50} and MIC_{90} value were 2 or less to 8 or more, 2 or less and 8 or more, respectively (Table 3).

The carbapenem resistant *A. baumannii* was found at 77.6% (415 out of 535 cases), 80% (400 out of 500 cases), and 79.1% (498 out of 630 cases) in 2019, 2020, and 2021, respectively (Table 4). For total isolates tested, the mean of CRAB was 78.9±1.2%.

The authors compared the antimicrobial susceptibility patterns of *A. baumannii* from blood, pus, urine, and sputum isolates, which may be useful for clinicians as shown in Table 5.

Table 2. Antimicrobial susceptibility of *A. baumannii* by the disk diffusion method

Antimicrobial agents	No. of isolates; n (%)		
	Susceptible	Intermediate	Resistant
Amikacin (n=1,628)	531 (32.6)	10 (0.6)	1,087 (66.8)
Gentamycin (n=1,486)	425 (28.6)	37 (2.5)	1,024 (68.9)
Cefoperazone/sulbactam (n=1,640)	367 (22.4)	150 (9.1)	1,123 (68.5)
Piperacillin/tazobactam (n=1,638)	264 (16.1)	69 (4.2)	1,305 (79.7)
Cefotaxime (n=1,639)	7 (0.4)	312 (19.0)	1,320 (80.5)
Ceftazidime (n=1,629)	325 (20.0)	26 (1.6)	1,278 (78.4)
Cefepime (n=1,351)	267 (19.8)	9 (0.7)	1,075 (79.5)
Imipenem (n=1,612)	315 (19.5)	7 (0.5)	1,290 (80.0)
Meropenem (n=1,636)	328 (20.0)	10 (0.6)	1,298 (79.4)
Doripenem (n=925)	193 (20.9)	10 (1.1)	722 (78.0)
Ciprofloxacin (n=818)	160 (19.1)	1 (0.1)	657 (80.8)
SXT (n=1,587)	496 (31.3)	144 (9.1)	947 (59.6)
Colistin (MIC)* (n=862)	789 (91.5)	-	73 (8.5)
Tigecycline (MIC)* (n=1,141)	911 (79.8)	8 (0.7)	222 (19.5)

SXT=trimethoprim-sulfamethoxazole; MIC=minimal inhibitory concentration

* Antimicrobial susceptibility for MIC in µg/mL by automated micro-dilution method

Table 3. Minimal inhibitory concentration (MIC) in µg/mL of *A. baumannii*

Antimicrobial agents	MIC range	MIC_{50}	MIC_{90}
Colistin	≤2 to ≥4	≤2	≥4
Tigecycline	≤2 to ≥8	≤2	≥8

MIC_{50}, MIC_{90} are required to inhibit the growth of 50% and 90% of *A. baumannii*, respectively

Table 4. Prevalence of carbapenem resistant *A. baumannii* (CRAB)

Year	CRAB	%
2019	415/535	77.6
2020	400/500	80.0
2021	498/630	79.1
Total	1,313/1,665	78.9

Discussion

In general, *A. baumannii* is one of the major organisms causing nosocomial infection and is resistant to multiple classes of antimicrobial agents. It was considered a low-category pathogen in the past, but now emerged as a frequent cause of pneumonia and septicemia in immunocompromised patients. It exhibits high level of resistance to many antimicrobial agents and can persist for a long time in harsh environments such as walls, surfaces, and medical devices, in the hospital[8]. In addition, *A. baumannii* can tolerate desiccation and survive on inanimate dry surfaces for several months[13]. The burden of

Table 5. Comparison of drug susceptibility patterns of *A. baumannii* according to types of clinical specimens

Drug	Blood; n (%)				Pus; n (%)				Urine; n (%)				Sputum; n (%)			
	n	% S	% I	% R	n	% S	% I	% R	n	% S	% I	% R	n	% S	% I	% R
AK	86	25 (29.1)	3 (3.5)	58 (67.4)	105	35 (33.3)	0 (0.0)	70 (66.7)	150	58 (38.7)	0 (0.0)	92 (61.3)	1,204	413 (34.3)	7 (0.6)	784 (65.1)
GM	77	30 (39.0)	2 (2.6)	45 (58.4)	93	27 (29.0)	2 (2.2)	64 (68.8)	150	52 (34.7)	4 (2.7)	94 (62.6)	1,166	316 (27.1)	29 (2.5)	821 (70.4)
TZP	86	13 (15.1)	8 (9.3)	65 (75.6)	100	12 (12.0)	6 (6.0)	82 (82.0)	150	25 (16.7)	10 (6.7)	115 (76.6)	1,302	214 (16.4)	45 (3.5)	1,043 (80.1)
CTX	83	3 (3.6)	14 (16.9)	66 (79.5)	100	2 (2.0)	14 (14.0)	84 (84.0)	150	0 (0.0)	20 (13.3)	130 (86.7)	1,306	2 (0.2)	264 (20.2)	1,040 (79.6)
CAZ	83	18 (21.7)	4 (4.8)	61 (73.5)	100	17 (17.0)	1 (1.0)	82 (82.0)	150	25 (16.7)	0 (0.0)	125 (83.3)	1,297	265 (20.4)	22 (1.7)	1,010 (77.9)
FEP	57	14 (24.6)	0 (0.0)	43 (75.4)	90	13 (14.4)	1 (1.1)	76 (84.5)	120	19 (15.8)	0 (0.0)	101 (84.2)	1,084	221 (20.4)	8 (0.7)	855 (78.9)
IPM	81	19 (23.5)	0 (0.0)	62 (76.5)	105	38 (36.2)	0 (0.0)	67 (63.8)	120	28 (23.3)	0 (0.0)	92 (76.7)	1,306	230 (17.6)	7 (0.5)	1,069 (81.9)
MEM	83	18 (21.7)	0 (0.0)	65 (78.3)	105	40 (38.1)	0 (0.0)	65 (61.9)	120	30 (25.0)	0 (0.0)	90 (75.0)	1,328	240 (18.1)	10 (0.7)	1,078 (81.2)
DOR	48	14 (29.2)	0 (0.0)	34 (70.8)	90	25 (27.8)	0 (0.0)	65 (72.2)	105	26 (24.8)	0 (0.0)	79 (75.2)	682	128 (18.8)	10 (1.5)	544 (79.7)
CIP	74	18 (24.3)	0 (0.0)	56 (75.7)	90	22 (24.4)	0 (0.0)	68 (75.6)	120	33 (27.5)	0 (0.0)	87 (72.5)	534	87 (16.3)	1 (0.2)	446 (83.5)
SXT	79	33 (41.8)	7 (8.9)	39 (49.3)	90	25 (27.8)	4 (4.4)	61 (67.8)	120	30 (25.0)	5 (4.2)	85 (70.8)	1,298	408 (31.4)	128 (9.9)	762 (58.7)
CL	74	70 (94.6)	0 (0.0)	4 (5.4)	95	88 (92.6)	0 (0.0)	7 (7.4)	150	134 (89.3)	0 (0.0)	16 (10.7)	543	497 (91.5)	0 (0.0)	46 (8.5)
TGC	48	46 (95.8)	2 (4.2)	0 (0.0)	90	73 (81.1)	1 (1.1)	16 (17.8)	105	83 (79.1)	2 (1.9)	20 (19.0)	898	709 (79.0)	3 (0.3)	186 (20.7)

S=susceptible; I=intermediate; R=resistant; AK=amikacin; GM=gentamycin; TZP=piperacillin/tazobactam; CTX=cefotaxime; CAZ=ceftazidime; FEP=cefepime; IPM=imipenem; MEM=meropenem; DOR=doripenem; CIP=ciprofloxacin; SXT=trimethoprim/sulfamethoxazole; CL=colistin; TGC=tigecycline; n=number of isolates tested

antimicrobial resistance in developing countries is high and is attributed to the high prevalence of infectious diseases, inadequate hygiene, and weak health systems[14]. There are many mechanisms of resistance to various antimicrobial agents in *A. baumannii* such as spontaneous genetic mutation, acquiring new genes or deleting a gene, down regulation or upregulation of gene expression and efflux pump[15].

In the present study, *A. baumannii* came mainly from patients older than 50 years of age. Ages of patients susceptible to *A. baumannii* infection are unpredictable and vary according to geographical locations but are more common in adults[13,15-17].

Sputum is the lower respiratory specimen. The present study observed the majority of *A. baumannii* isolates was from sputum, similar to the report from Pakistan at 46.5%[18], but skin and soft tissue were the predominant source in a report from Honduras, which is a country in central America[19]. Different health status and geographical features among countries may account for the observations.

ESKAPE pathogens are important for nosocomial infection and can escape the killing action of antimicrobial agents. The ESKAPE pathogens are a group of bacteria such as *E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and Enterobacteriaceae. Noticeably, *A. baumannii* is the most challenging bacterium because of its high drug resistance[14,20,21].

Carbapenems are the drugs of choice for multidrug resistant *A. baumannii*, which is resistant to at least three classes of drugs. However, CRAB has been frequently encountered. One of the major mechanisms of carbapenem resistance in this pathogen is the production of carbapenem hydrolyzing beta-lactamases. Imipenem, meropenem, and doripenem tested in the present study were classified as carbapenems[16,17,20].

The authors defined CRAB as carbapenem-resistant if *A. baumannii* was tested as "resistant" to at least one of the carbapenems. Percent CRAB isolates exhibiting resistance to imipenem, meropenem, and colistin in the present study, which was 78.2, 73.4, and 0.8%, respectively, and was higher than that reported in Iran[20].

There was a high concern from a research review paper that indicated that CRAB was found at 50%, 70%, 85%, 92%, 100%, and ranged 62% to 100% in Singapore, Chile, India, Korea, Portugal, and Pakistan, respectively[8]. However, CRAB isolates were low in Germany, at 3.5% in 2018[16]. Colistin or polymyxin E is considered the last resort antimicrobial agent for the treatment of infections caused by multidrug resistant *A. baumannii* and CRAB[18]. It is bactericidal by disrupting cell membrane of bacteria.

Tigecycline, being the first member of glycylcycline and a novel drug, was approved by the U.S. Food and Drug Administration for treatment of multidrug-resistant gram-negative bacteria[8]. The authors found 79.8% of *A. baumannii* was susceptible to tigecycline (Table 2). A report from South Africa[7] found that it was 100% susceptible. The effectiveness against *A. baumannii* and other species of *Acinetobacter* were shown in studies[8]. However,

varying percentages of resistance to tigecycline were reported worldwide, with Türkiye reported the highest at 81% resistance[8].

Data from the National Antimicrobial Resistant Surveillance Center, Thailand (NARST) for *Acinetobacter* spp. from all specimens in 83 hospitals, between January 2019 and December 2021 showed percentages of susceptibility to amikacin at 45.6%, piperacillin-tazobactam at 27.3%, cefepime at 28.0%, imipenem at 25.7%, and ciprofloxacin at 27.5%[22]. In the present study, 32.6% of *A. baumannii* was susceptible to amikacin, 16.1% susceptible to piperacillin-tazobactam, 19.8% susceptible to cefepime, 19.5% susceptible to imipenem, and 19.1% susceptible to ciprofloxacin (Table 2). For imipenem and meropenem, which are commonly used carbapenems in Thailand, the percentages of susceptibility of *Acinetobacter* spp. in NARST report were 25.7% and 25.8%, respectively[22]. In the present study, 19.5% of *A. baumannii* was susceptible to imipenem, and 20% susceptible to meropenem. Therefore, *A. baumannii* in the present study had lower susceptibility to all drugs tested when compared to NARST data. There was no NARST report on susceptibility to colistin and tigecycline for comparison with the present study.

Noticeably, the CLSI[9] susceptibility interpretive criteria were used for most antimicrobial agents. However, CLSI has no susceptibility interpretive criteria for colistin and tigecycline. Therefore, colistin MIC breakpoints were considered susceptible and resistant, respectively. There was no intermediate interpretation.

There were limitations in the present study. First, data on *A. baumannii* isolates were from a single study site. Second, the authors did not collect clinical data of patients, but from microbiological database. Therefore, clinical data related to colistin and tigecycline resistance were lacking. Due to the lack of clinical information in the present study, the authors did not correlate the result of the susceptibility test in vitro and clinical outcome in the same individual. Therefore, there is a big missing link of the result of susceptibility test and clinical outcome. Further research is needed to assess the effects. Third, clinical settings, such as community and hospital-associated settings of *A. baumannii* isolates, should be added in a future study. Fourth, a larger sample size of *A. baumannii* isolates would have increased the generalizability of the study findings. Fifth, a wider range of antimicrobial agents will be of interest for further study to provide a comprehensive overview of antibiotic resistance patterns in *A. baumannii*. Sixth, it will be useful to assay these drug-resistant genes in *A. baumannii* isolates from patients at Taksin Hospital to elucidate the precise molecular characterization and clonal spread. In addition, it is important that the environment of the patients with *A. baumannii* infection/colonization are risk factors for dissemination of this bacterium among other patients.

Conclusion

The results from the present study suggest that colistin and tigecycline could be effective drugs in the treatment of infections caused by *A. baumannii*, carbapenem resistant. The authors' data should support ongoing studies evaluating current trends and help improve surveillance of drug resistance so it would be minimized. Furthermore, antibiogram profiles are necessary to avoid ineffective empirical drug treatments.

What is already known on this topic?

A. baumannii is a gram-negative coccobacillus, non-fermentative, and opportunistic bacterium that is now recognized as a pathogen responsible for various diseases, including ventilator-associated pneumonia, bloodstream, wound, and urinary tract infections. According to the previous studies, drug resistance is a global problem. Therefore, further studies of *A. baumannii* prevalence and drug resistance should be conducted, particularly in Thai patients.

What this study adds?

A. baumannii found in 1,665 patients was isolated at Taksin Hospital between January 2019 and December 2021. The five most common sites of *A. baumannii* infections included sputum in 72.3%, urine in 9.0%, pus in 6.3%, tissue in 6.3%, and blood in 5.2%. *A. baumannii* demonstrated susceptibility to amikacin, gentamycin, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, imipenem, meropenem, doripenem, ciprofloxacin, and trimethoprim-sulfamethoxazole in the range of 0.4% to 32.6%, colistin at 91.5% and tigecycline at 79.8%. This study demonstrates how antimicrobial susceptibility results can provide guidance on treatment and prevention of *A. baumannii* diseases to clinicians.

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Conflicts of interest

All authors declare no personal or professional conflicts of interest, and no financial support.

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