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ORIGINAL ARTICLE

Difference in imipenem, meropenem, sulbactam, and colistin nonsusceptibility trends among three phenotypically undifferentiated *Acinetobacter baumannii* complex in a medical center in Taiwan, 1997—2007☆

Chen Liang-Yu [a], Shu-Chen Kuo [a], Chun-Yu Liu [b,c], Bin-Syun Luo [d], Ling-Ju Huang [a,c], Yi-Tzu Lee [c,e], Chien-Pei Chen [a], Te-Li Chen [a,c,d,*], Chang-Phone Fung [a,c,d]

[a] *Division of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan*
[b] *Division of Hematology and Oncology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan*
[c] *School of Medicine, National Yang-Ming University, Taiwan*
[d] *Institute of Clinical Medicine, National Yang-Ming University, Taiwan*
[e] *Division of Internal Medicine, Chutung Veterans General Hospital, Taiwan*

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Background: To determine whether the susceptibilities and the trends of nonsusceptibility of imipenem, meropenem, sulbactam, and colistin differed among *Acinetobacter baumannii*, *Acinetobacter* genomic species 3 (AGS 3), and *Acinetobacter* genomic species 13TU (AGS 13TU) over 11 years.
Methods: A total of 1,039 nonduplicate blood isolates of *A baumannii* complex from bacteremic patients between 1997 and 2007 were collected at Taipei Veterans General Hospital and were identified to the species level using a multiplex polymerase chain reaction method and sequence analysis of 16S—23S intergenic spacer. The minimal inhibitory concentrations of antibiotics were determined by the agar dilution method.
Results: The nonsusceptibility rates of carbepenems and sulbactam were highest in *A baumannii*, which also showed a trend toward increasing rate of carbapenems nonsusceptibility over

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* Corresponding author. Division of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital, No. 201, Sec. 2, Shih-Pai Rd, Taipei 112 Taiwan.
E-mail address: tlchen@vghtpe.gov.tw (T.-L. Chen).

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the 11-year period of the study. AGS 13TU had the highest nonsusceptible rate to colistin, comparably increasing trend of carbapenem nonsusceptiblity as that of *A baumannii*, and is the only species with increasing sulbactam nonsusceptibility. AGS 3 had the lowest rate of nonsusceptibility to all four antimicrobial agents.

Conclusion: Although *A baumannii* had the highest nonsusceptibility rate to imipenem, meropenem, and sulbactam over the years, the higher rate of colistin nonsusceptibility and the emergence of nonsusceptibility of carbapenems and sulbactam in AGS 13TU suggested that this species might cause a great problem in the near future.

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Introduction

The nosocomial infection because of *Acinetobacter baumannii* complex is associated with a high-mortality rate in debilitated patients.[1,2] These bacteria pose a great threat to health care systems and infection control because they develop antibiotic resistance by means of multiple mechanisms.[3] Carbapenems including imipenem and meropenem are the antibiotics of last resort for the treatment of multidrug-resistant *A baumannii* complex.[4] However, strains resistant to carbapenems have been evolving worldwide, which has limited the choice of treatment and mandated the use of other therapeutic agents such as tigecycline, colistin, or sulbactam.[3,4]

The genus *Acinetobacter* contains more than 32 species, but *A baumannii*, *Acinetobacter* genomic species 3 (AGS 3), and *Acinetobacter* genomic species 13TU (AGS 13TU) are the three most clinically relevant pathogens. These three species are phenotypically similar and cannot be differentiated by commercially available identification methods.[1] They are grouped into the so-called *A baumannii* complex and sometimes their properties are studied and described collectively,[1,5] including their antibiotic susceptibilities.[6] However, many studies have reported differences in epidemiology, resistance patterns, and resistance mechanisms among *A baumannii*, AGS 3, and AGS 13TU.[7–10] *A baumannii* isolates are often more resistant to different classes of antimicrobials than are AGS 3 and AGS 13TU.[7] Furthermore, the carbapenemases harbored by *A baumannii* are different from those harbored by AGS 3 and 13TU. For example, carbapenem-hydrolyzing class D beta-lactamases are often identified in *A baumannii*,[11–13] whereas metallo-beta-lactamases are more frequently detected in AGS 3 or AGS 13TU.[8,10] Hence, it is crucial to investigate the three species separately. To date, it is unknown whether antimicrobial resistance trends differ among the three *Acinetobacter* spp. This study aimed to identify the trends in the development of antibiotic nonsusceptibility by *A baumannii*, AGS 3, and AGS 13TU in a medical center in Taiwan. A better understanding of the antimicrobial nonsusceptibility patterns of these pathogens and trends may improve hospital management and control of *A baumannii* complex infection.

Materials and methods

Bacterial isolates

This study was conducted at Taipei Veterans General Hospital, a 2,900-bed tertiary care medical center in Taiwan. Nonduplicate blood isolates from bacteremic patients were consecutively collected from year 1997 to 2007 if they were phenotypically identified as *A baumannii* complex by the 32GN system (bioMérieux, Marcy l'Etoile, France). The bacteria were stored at −70°C in trypticase cystine agar (Difco Laboratories, Le Pont de Claix, France) supplemented with 15% glycerol before use.

Genomic species identification

Multiplex polymerase chain reaction described previously[14] was used to identify *A baumannii*. The nonbaumannii *Acinetobacter* spp were identified by amplification and sequence analysis of 16S−23S intergenic spacer using universal primers as previously described.[15]

Antimicrobial susceptibilities

The minimum inhibitory concentrations (MICs) of imipenem, meropenem, sulbactam, and colistin were determined by agar dilution methods according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[16] *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *E coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control. For imipenem and meropenem, a MIC of $\leq$4 μg/mL was considered to be susceptible, and those with MIC $>$4 μg/mL to be nonsusceptible; whereas for colistin, a MIC of $\leq$2 μg/mL was considered to be susceptible, and those with MIC $>$2 μg/mL to be nonsusceptible.[16] Because there were no breakpoints existed for sulbactam in the CLSI guidelines, we adapted that for ampicillin/sulbactam as interpretation reference. A MIC for sulbactam of $\leq$4 μg/mL was considered to be susceptible for sulbactam, and those with MIC $>$4 μg/mL to be nonsusceptible. The nonsusceptibility rate was defined as the number of nonsusceptible isolates divided by the total number of isolates.

Statistical methods

Each of the MICs of the four antibiotics for the three *Acinetobacter* genospecies during the study period was recorded. Geometric means of the MICs of a single antibiotic for a single species within 1 year were calculated. Trends of geometric means of MICs over time of each of the four antimicrobial agents for a single genomic species were assessed by simple linear regression analyses. A $p < 0.05$ denoted a significant linear association between the mean MIC of a single antibiotic for a single species and time period.

Table 1 The number and percentage of isolates identified as *Acinetobacter baumannii complex* from blood samples over 11 yr

Year	*Acinetobacter* spp			Total of isolates
	A baumannii	AGS 13TU	AGS 3	
1997	25 (37.31%)	32 (47.76%)	10 (14.93%)	67
1998	47 (54.65%)	31 (36.05%)	8 (9.30%)	86
1999	38 (47.50%)	31 (38.75%)	11 (13.75%)	80
2000	36 (41.86%)	44 (51.16%)	6 (6.98%)	86
2001	45 (33.83%)	69 (51.88%)	19 (14.29%)	133
2002	25 (32.47%)	41 (53.25%)	11 (14.29%)	77
2003	27 (43.55%)	25 (40.32%)	10 (16.13%)	62
2004	43 (38.74%)	61 (54.95%)	7 (6.31%)	111
2005	43 (43.43%)	42 (42.42%)	14 (14.14%)	99
2006	47 (41.59%)	43 (38.05%)	23 (20.35%)	113
2007	63 (50.40%)	48 (38.40%)	14 (11.20%)	125

AGS = *Acinetobacter* genomic species.

To describe the relationship between the emergence of nonsusceptible strains of each *Acinetobacter* species and the time period, two-dimensional plots with trend lines were used for descriptive analysis. The emergence of nonsusceptible strains as a function of time (year) was tested by logistic regression analysis, using the emergence of nonsusceptible strains as a binary dependent variable (antimicrobial resistance *vs.* sensitivity), and time (continuous variable) as a co-variable, and data were expressed as beta and *p* values. Plus or minus values of beta denoted the directions (increase or decrease) of the resistance, and a $p < 0.05$ in this logistic regression model indicated a significant increase or decrease of resistance rate year after year during this 11-year period.

All statistical analyses were performed using SPSS software (version 13.00, SPSS, Chicago, IL, USA).

Results

Isolates and species identification

A total of 1,039 isolates of *A baumannii* (439 isolates, 42.25%), AGS 13TU (467, 44.95%), and AGS 3 (133, 12.80%) were collected during the study period. The percentage of isolates identified as particular species and sorted by year was listed in Table 1.

The MIC distribution

From year 1997 to 2007, the mean MICs of imipenem and meropenem for *A baumannii* increased from 0.9 to 3.43 mg/L ($p < 0.001$) and 1.25 to 3.74 ($p < 0.001$), respectively (Table 2). The mean MICs of imipenem, meropenem, and sulbactam for AGS 13TU increased from 0.42 to 0.93 ($p = 0.001$), 0.68 to 1.43 ($p = 0.001$), and 2.00 to 3.13 ($p = 0.003$), respectively. The mean MICs of imipenem, meropenem, and sulbactam remained stable for AGS 3. The mean MICs of colistin were unchanged for all three species throughout the survey period.

Resistance rate and trends of resistance

A baumannii and AGS 13TU had higher nonsusceptibility rate of imipenem and meropenem than AGS 3 (Fig.1 A, B). *A baumannii* and AGS 13TU also had significant increase in nonsusceptibility rates of imipenem and meropenem over 11 years (all $p < 0.001$), but AGS 3 did not. The nonsusceptibility rate of imipenem seemed to increase more rapidly in *A baumannii* than AGS 13TU (beta, 0.275 and 0.207, respectively), whereas the emergence of nonsusceptibility for meropenem was faster in AGS 13TU than *A baumannii* (beta, 0.327 and 0.298, respectively). Although the nonsusceptibility rate of sulbactam in *A baumannii* was

Table 2 Geometric mean of MICs of four antimicrobial agents in different *Acinetobacter baumannii* complex from blood samples over 11 yr

Antibiotics	*Acinetobacter* spp	Geometric mean of MICs versus year											*p*
		1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	
Imipenem	*A baumannii*	0.90	1.23	1.67	1.56	1.66	2.43	3.17	3.09	3.19	2.65	3.43	<0.001*
	AGS 13TU	0.42	0.67	0.37	0.44	0.70	0.73	0.80	0.90	1.30	1.08	0.93	0.001*
	AGS 3	0.35	0.42	0.69	2.00	0.50	0.47	0.38	0.34	0.67	0.49	0.32	0.532
Meropenem	*A baumannii*	1.25	1.16	1.47	1.78	1.69	2.11	3.52	2.89	3.19	3.16	3.74	<0.001*
	AGS 13TU	0.68	0.84	0.49	0.85	1.14	0.64	1.18	1.31	1.67	1.60	1.43	0.001*
	AGS 3	0.66	0.50	1.37	2.24	0.90	0.41	0.87	0.74	1.28	1.03	0.86	0.975
Sulbactam	*A baumannii*	8.22	7.54	8.45	9.51	7.07	8.69	9.33	8.25	7.50	11.23	11.25	0.064
	AGS 13TU	2.00	1.96	1.79	1.88	2.28	1.78	3.20	2.27	3.39	3.24	3.13	0.003*
	AGS 3	2.30	1.68	3.31	1.78	2.68	1.88	1.87	1.81	2.32	2.06	1.81	0.396
Colistin	*A baumannii*	1.18	1.06	1.18	0.96	1.11	0.90	0.90	0.86	1.17	1.06	1.10	0.532
	AGS 13TU	2.43	1.71	2.67	1.94	1.79	2.58	2.23	1.52	1.87	1.62	1.86	0.156
	AGS 3	1.62	1.30	1.37	1.59	1.39	1.00	1.15	1.00	1.35	1.31	1.72	0.725

* *p* Value < 0.05.
AGS = *Acinetobacter* genomic species; MIC = minimum inhibitory concentration.

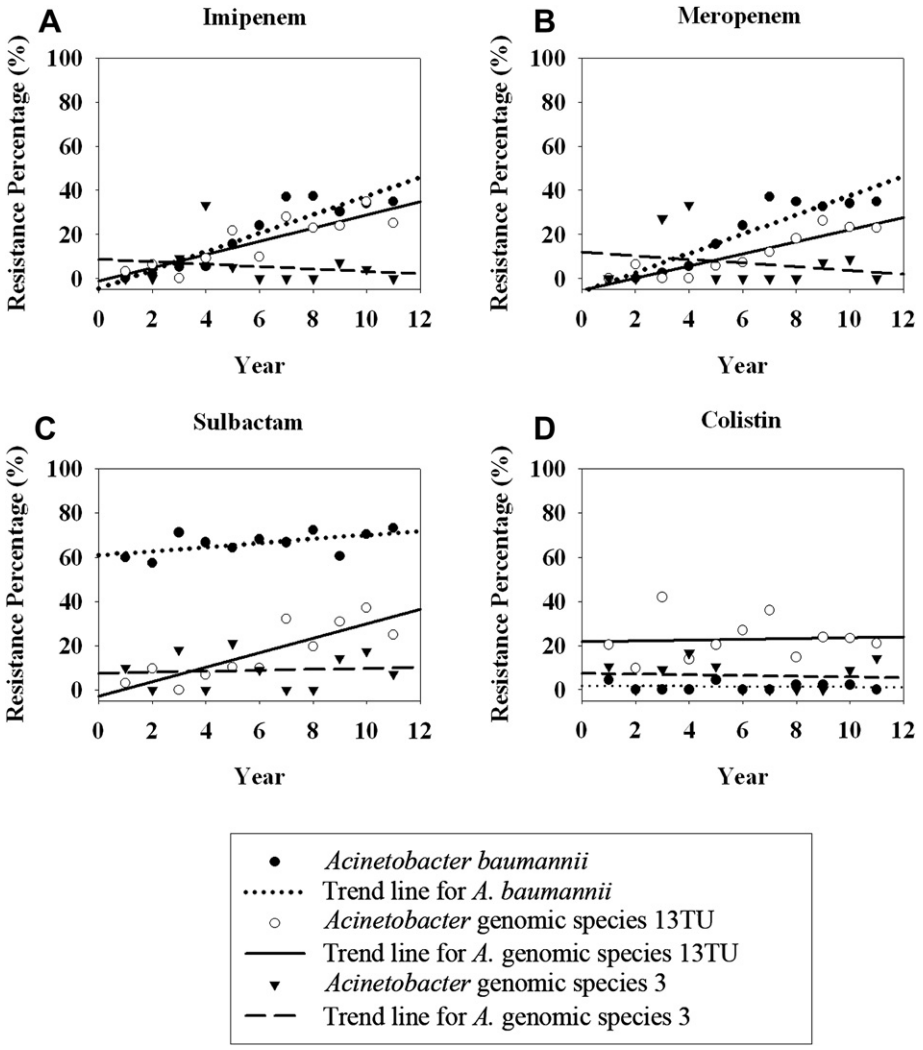

Figure 1. Two-dimensional plots with trend lines of nonsusceptibility percentage to (A) imipenem, (B) meropenem, (C) sulbactam, and (D) colistin in each *Acinetobacter* spp versus time period (from 1997 to 2007, 11-year period).

constantly higher than AGS 13TU (Fig. 1 C), AGS 13TU was the only species with increasing nonsusceptibility rate of sulbactam ($p < 0.001$). The nonsusceptibility rate of sulbactam in AGS 3 was the lowest among the three species and did not change significantly over 11 years. AGS 13TU had higher rate of nonsusceptibility to colistin than did *A baumannii* and AGS3 (Fig. 1 D). The susceptibility of these three species to colistin remained unchanged over 11 years.

Discussion

A baumannii has long been considered as the most common pathogen in the *A baumannii* complex.[1] A study from southern Taiwan showed that 75.9%, 23.0%, and 0.7% of 291 *A baumannii* complex isolates recovered from bacteremic patients over a 5.5-year period were *A baumannii*, AGS 3, and AGS 13TU, respectively.[7] Unexpectedly, our study demonstrated that the number of AGS 13TU isolates was equal to, or even higher than that of *A baumannii* in some years. The potential for bias was reduced by following trends over 11 years and consecutive collection of blood samples. It is worth to study in the future that whether the increase of AGS 13TU is caused by clonal spreading.

The carbapenem nonsusceptibility rate of *A baumannii* has risen in recent years especially in countries of the Asia-Pacific rim.[3] Our study revealed that among species of the *A baumannii* complex, *A baumannii* had the highest rate of resistance to carbapenem and sulbactam. Although carbapenem nonsusceptibility tended to increase, sulbactam nonsusceptibility did not change significantly. However, the changes of nonsusceptibility rates had more clinical implication than that of MICs did. Therefore, we examined the antimicrobial nonsusceptibility rates of each *Acinetobacter* spp during the 11-period (Fig. 1), and analyzed the trends of antimicrobial nonsusceptibility among them.

The increase in antimicrobial nonsusceptibility rates might be related to spreading of resistant determinants, or a clonal outbreak within this hospital. Nosocomial infection is common and there were reports of nosocomial outbreak of carbapenemase-resistant *A baumannii* in the intensive care units.[17–19] Interhospital dissemination of imipenem-resistant

Acinetobacter spp with carbapenemase gene was also reported by Kuo et al.[12] recently in Taiwan. Inorganic materials such as computer keyboards in the nursing station might play a role as potential reservoirs of these resistant pathogens.[20] Plasmid transfer of carbapenemase gene had also been reported.[21]

The most worrisome finding was the emergence of nonsusceptibility in AGS 13TU. The nonsusceptibility of AGS 13TU has gradually gained attention, but the power of these studies was hampered by small number of isolates or diversity of samples.[8,9] Our study revealed that nonsusceptibility to carbapenems (imipenem and meropenem) in AGS 13TU was accelerating, suggesting that AGS 13TU may be developing similar carbapenem nonsusceptibility rate equal to that of *A baumannii* in the near future. In this study, sulbactam nonsusceptibility rate was higher in *A baumannii* than in AGS 13TU, but it has been increasing in AGS 13TU at a rate approaching that of *A baumannii* during the years. The finding of a higher rate of nonsusceptibility to colistin in AGS 13TU than *A baumannii* is consistent with previous findings.[9,22]

In contrast to the evolving of nonsusceptibility rate in *A baumannii* and AGS 13TU, AGS 3 was constantly more susceptible to carbapenems and sulbactam in our study. The nonsusceptibility to these antimicrobials or colistin did not increase over years, which may imply a difference among AGS3, *A baumannii*, and AGS 13TU in ability to acquire nonsusceptibility.[7–10]

Although we did not evaluate the patients outcome, the increased geometric mean MIC and MIC90 through 1997–2007 would probably related to higher rate of treatment failure and worse prognosis in the clinical situation. Higher mortality rate related to inadequate treatment on *A baumannii* complex infection had been reported.[23]

We did not include tigecycline in this study because currently there is no tigecycline breakpoint for *Acinetobacter* spp according to CLSI guidelines[16] and the use of tigecycline in the treatment of bacteremic patients is generally not recommended because of low-plasma concentration.

In conclusion, our study of a large collection of isolates over a prolonged period revealed a difference in antimicrobial resistance and emergence of resistance among *A baumannii*, AGS 13TU, and AGS 3. *A baumannii* had the highest rate of nonsusceptibility to carbapenems and sulbactam in recent years, but the higher resistance rate to colistin and rapid emergence of carbapenems and sulbactam nonsusceptibility in AGS 13TU indicated that therapeutic options for the treatment of patients with AGS 13TU bacteremia might become limited in the near future.

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