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ORIGINAL ARTICLE

Clonal spread of multidrug-resistant *Acinetobacter baumannii* in eastern Taiwan

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Background and Purpose: This study was conducted to investigate the molecular epidemiology and antimicrobial susceptibility of multidrug-resistant (MDR) *Acinetobacter baumannii* to three types of antibiotics.
Methods: One hundred and thirty-four specimens of MDR *A baumannii* were collected from three branches (Taipei, Dalin, and Hualien branches) of Buddhist Tzu Chi Hospital, which are located in northern, southern, and eastern Taiwan, during 2007. Genotyping was performed by pulsed-field gel electrophoresis. Antibiotic susceptibilities to colistin, rifampicin, and tigecycline were determined. The synergistic effects of rifampin and colistin were also evaluated.
Results: Antibiotic susceptibility testing showed that 10.4%, 47.8% and 45.5% of the MDR *A baumannii* isolates are resistant to colistin, rifampicin, and tigecycline, respectively. A majority of the rifampicin-resistant isolates (62.7%) were found in the Haulien branch, whereas 62.2% of tigecycline-resistant isolates were found in the Taipei branch. The combination of colistin and rifampicin had a synergistic effect on all of the isolates. Genotyping by pulsed-field gel

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electrophoresis identified 17, 23, and 11 pulsotypes in the Taipei, Dalin, and Haulien branches, respectively. Furthermore, 74.5% of isolates in the Haulien branch were identified as one of three pulsotypes. Among 37 rifampicin-resistant and 22 tigecycline-resistant MDR *A baumannii* isolates found in the Haulien branch, 51.3% (19/37) and 50% (11/22) of the isolates belonged to the same clone, respectively.

Conclusion: This study confirms the high prevalence of resistance to rifampicin and tigecycline in MDR *A baumannii* in the three hospitals that were studied, and the high proportion of identical strains that exist in eastern Taiwan.

Introduction

Acinetobacter baumannii has emerged as an important multidrug-resistant (MDR) pathogen that is responsible for hospital-acquired infections.[1,2] Common infections include ventilator-associated pneumonia, bacteremia, and infections in and around burn wounds and the urinary tract.[3] The epidemic potential of *A baumannii* is primarily related to this organism's ability to develop resistance to a variety of antimicrobial agents, including broad-spectrum cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides.[4]

Carbapenem, imipenem, and meropenem, remain the drugs of choice for the treatment of *A baumannii*.[5] However, the efficacy of these drugs can be compromised by the spread of carbapenemases,[6,7] a situation which urgently requires other antibiotic options. Several studies have shown that three antibiotics—tigecycline, rifampicin and colistin—are effective against carbapenem-resistant strains of *A baumannii*.[8–10] However, the reduced susceptibility to these three drugs in *A baumannii* has recently been reported in several countries.[11,12] Nevertheless, several studies have shown that tigecycline- or rifampicin-based regimens are more effective for treating severe infections caused by MDR *A baumannii*.[13,14] To date, no studies are available on the efficacy of rifampicin or combination rifampicin/colistin against *A baumannii* isolates in Taiwan, although rifampicin has been used for the long-term treatment of infections caused by *Mycobacterium tuberculosis*. Furthermore, the spread of MDR *A baumannii* with resistance to tigecycline, rifampicin, and colistin in hospitals has never been reported in Taiwan.

The goal of this study is to compare the susceptibility of MDR *A baumannii* to colistin, rifampicin, tigecycline, and combination colistin/rifampicin at three hospitals in northern, southern, and eastern Taiwan. The molecular epidemiologies of each of these isolates were also further investigated in each hospital.

Methods

Definition

A baumannii is defined as multidrug-resistant when the organism is resistant to piperacillin, piperacillin-tazobactam, ampicillin/sulbactam, imipenem, ceftazidime, gentamicin, amikacin, tetracycline, chloramphenicol, ciprofloxacin, and cotrimoxazole.[15]

Hospital settings and bacterial isolates

This study analyzed MDR *Acinetobacter* spp. Samples were nonrepetitively collected from three branches of Buddhist Tzu Chi General Hospital in Taiwan. Hualien Tzu Chi Medical Center (Haulien branch) is a 966-bed university hospital located in eastern Taiwan. The Taipei branch is an 890-bed regional hospital in northern Taiwan. The Dalin branch is an 896-bed regional hospital in southern Taiwan. The infection control policies regarding MDR *Acinetobacter* spp., including contact precautions, were the same at each hospital. All clinical MDR *Acinetobacter* spp. isolates collected during 2007 were stored at −80 °C in trypticase soy broth (Difco Laboratories, Detroit, MI, USA) that was supplemented with 20% glycerol before testing. All isolates were transported to the clinical microbiology laboratory at Tzu Chi University for further study.

Identification and antimicrobial susceptibilities

The clinical strains of Acinetobacter spp. collected at the Haulien branch were isolated and identified using the Phoenix system (Becton Dickinson Diagnostic Systems, Sparks, MD, USA); isolates from the Taipei and Dalin branches were identified using the Vitek system (Biomerieux Vitek, Inc., Hazelwood, MO, USA). Characterization of these isolates as A baumannii or non-A baumannii spp. was performed using one-tube multiplex PCR, based on the method described by Chen et al.[16] Susceptibilities to tigecycline, colistin, and rifampicin were determined using the broth-dilution method, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[17] In vitro interaction of colistin with rifampicin was assessed by the checkerboard method, as previously described.[8] Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as the reference strains for susceptibility testing. The breakpoints for Enterobacteriaceae, which are approved for use by the U.S. Food and Drug Administration, were applied in order to define tigecycline susceptibility (susceptibility, $\leq$2 mg/L; resistance, $\geq$8 mg/L).[18] The breakpoints for colistin were those recommended by the CLSI guidelines (susceptibility, $\leq$2 mg/L; resistance, $\geq$4 mg/L).[19] Breakpoints for rifampicin were those recommended by CLSI for staphylococci (susceptibility, $\leq$1 mg/L; resistance, $\geq$4 mg/L).[20] The effects of synergism, indifference, and antagonism due to combination colistin/rifampicin were previously defined.[8]

Strain typing

Pulsed-field gel electrophoresis (PFGE) was performed using genomic DNA from all of the multidrug-resistant *Acinetobacter* isolates, as previously described.[21] Chromosomal DNA plugs were incubated with *Apa*I endonuclease. Restriction fragments were separated by PFGE in 1% Seakem Gold (SKG) agarose and run in a 0.5× Tris-borate-EDTA buffer on a CHEF-DRIII system (Bio-Rad Laboratories, Hercules, CA). The initial switch time was 5 seconds, the final switch time was 20 seconds, and the run time was 21.5 hours at 6 V/cm. The gel was stained with ethidium bromide, washed in distilled water, and photographed under ultraviolet (UV) light. The PFGE banding patterns were interpreted according to previously described criteria.[22] Saved.tiff files of the photographed gels were further analyzed by Molecular Analyst 1.6 software (Bio-Rad). The percentage similarity was identified using the dendrogram-derived unweighted pair-group method using arithmetic means based on Jaccard coefficients. Both band position tolerance and optimization were calibrated at 1.0%. Isolates with PFGE band similarity >80% were classified as the same type.

Results

Antimicrobial susceptibility testing

A total of 134 MDR *Acinetobacter* spp. samples, including 37, 38, and 59 isolates from the Taipei, Dalin, and Hualien Branches of Buddhist Tzu Chi General Hospital, respectively, were collected in 2007. All of the isolates were identified as *A baumannii* according to the presence of an internal 208-bp fragment from the Intergenic spacer (ITS) region that had been previously described by Chen et al.[16] The antimicrobial susceptibility test showed that 10.4%, 47.8% and 45.5% of the MDR *A baumannii* isolates were resistant to colistin, rifampicin, and tigecycline, respectively (Table 1). Resistance to the three antimicrobials varied at each of the three hospitals. All of the MDR *A baumannii* isolates taken from the Dalin branch were susceptible to colistin. A majority of the rifampicin-resistant isolates (62.7%) were found in the Hualien branch, whereas 62.2% of isolates found at the Taipei branch were resistant to tigecycline. Furthermore, colistin displayed a synergistic effect with rifampicin in all of the isolates tested.

PFGE typing

Genotyping revealed that a total of 17, 23, and 11 different pulsotypes were identified in the Taipei, Dalin and Haulien branches, respectively (Table 2). The distribution of the pulsotypes among the three branches differed substantially. Strains found in Dalin branch were more heterogeneous, with 23 pulsotypes identified among 38 isolates; more homogeneous strains were found in the Haulien branch, with 11 pulsotypes identified among 59 isolates. The percentages of MDR *Acinetobacter* isolates identified as one pulsotype that contained six or more isolates was 19.8% (7/37) and 74.5% (44/59) in the Taipei and Hualien branches, respectively (Table 2).

The pulsotypes of the rifampicin-resistant MDR *A baumannii* strains identified in the three hospitals were further investigated, and the results are shown in Table 3. Of the 37 rifampicin-resistant MDR *A baumannii* isolates obtained from the Haulien branch, 19 (51.3%) belonged to the same clone, whereas more heterogeneous rifampicin-resistant isolates were found in the other branches.

The pulsotypes of the tigecycline-resistant MDR *A baumannii* strains in the three hospitals were also analyzed (Table 3). Of the 22 tigecycline-resistant MDR *A baumannii* isolates obtained at the Haulien branch, 11 (50%) belonged to the same clone. In contrast, those isolates with more heterogeneous pulsotypes were found in the other two branches.

The pulsotypes of the colistin-resistant MDR *A baumannii* strains found in the Haulien and Taipei branches were examined. All these strains were unrelated to the strains found in the other two hospitals.

Table 1 Antibiotic susceptibilities of MDR *A baumannii* strains isolated from three branches of Buddhist Tzu General Hospital to colistin, rifampicin, and tigecycline

Hospital	Haulien branch[a] (n = 59)	Taipei branch[b] (n = 37)	Dalin branch[c] (n = 38)	Total (n = 134)
Antimicrobials	No. of isolates (%)			
(Resistance breakpoints)	R	R	R	R
Colistin[d] (4)	8 (13.6)	6 (16.2)	0 (0)	14 (10.4)
Rifampicin[d] (4)	37 (62.7)	10 (27.0)	17 (44.7)	64 (47.8)
Tigecycline (8)	22 (37.3)	23 (62.2)	16 (42.1)	61 (45.5)
Colistin + rifampicin	6 (10.2)	0 (0)	0 (0)	6 (4.5)
Colistin + tigecycline	4 (6.8)	2 (5.4)	0 (0)	6 (4.5)
Rifampicin + tigecycline	9 (15.6)	7 (18.9)	12 (31.6)	28 (20.9)
All	4 (6.8)	0 (0)	0 (0)	4 (3)

[a] Eastern Taiwan.
[b] Northern Taiwan.
[c] Southern Taiwan.
[d] The synergistic effects on all of the isolates were tested.

Table 2 Pulsotypes of MDR *A baumannii* obtained from three branches of Buddhist Tzu Chi Hospital

Branch	No. of isolates	No. of isolates in one pulsotype	No. of pulsotypes
Taipei	37		17
		1	10
		2	1
		3	2
		4	3
		7	1
Dalin	38		23
		1	13
		2	7
		3	1
		4	2
Hualian	59		11
		1	5
		2	1
		3	1
		5	1
		9	1
		13	1
		22	1

Discussion

The increasing large number of reports detailing the emergence of MDR *A baumannii* indicates a significant public health concern.[2] Typically, tigecycline and colistin are used to treat MDR *A baumannii* infections as a result of limited antibiotic choices. One study conducted on 19 hospitals in Taiwan during 2006 showed that 6.9% of *A baumannii* strains are resistant to tigecycline.[23] Another report conducted on three medical centers in Taiwan between 2001 and 2005 showed that the resistance rates of imipenem-non-susceptible *A baumannii* to tigecycline and colistin were 18% and 1%, respectively.[24] The present study reveals that 45.5% and 10.4% of MDR *A baumannii* isolates are resistant to tigecycline and colistin, which is much higher than previously reported.[23,24] The high prevalence of tigecycline and colistin resistance in this study might be due to the selection criteria for MDR strains that were used compared with the criteria used by Liu et al.[23] However, the gradual increase of tigecycline and colistin-resistant MDR *A baumannii* in Taiwan is alarming. Strict regulations regarding the use of tigecyclin began in each of the three branches in 2007. However, the rates of resistance to tigecycline in MDR *A baumannii* among the three hospitals in our study are clearly different. In Taiwan, many medical centers are located in the greater Taipei area. Tigecycline began to be used much earlier in the hospitals located in and around Taipei than in hospitals located farther away from Taipei. This may be one of the reasons for the higher rate of resistance to tigecycline found among MDR *A baumannii* isolates obtained from the Taipei branch of Buddhist Tzu Chi Hospital.

Rifampicin has been used solely for the treatment of *M. tuberculosis* infections for more than three decades in Taiwan. However, because MDR *A baumannii* infections are associated with an increase in attributed mortality and due to the limited treatment options available for this disease, rifampicin-based regimens have been proposed as an alternative treatment.[9] One report on the development of rifampicin resistance in MDR *A baumannii* showed that rifampicin-resistant mutants are found after 48—72 hours of in vitro and in vivo exposure to rifampicin.[25] In spite of the lack studies on rifampicin exposure, up to 47.7% of MDR *A baumannii* isolates identified in this study are resistant to rifampicin. In addition, this study is the first report the high prevalence of rifampicin-resistant MDR *A baumannii* in Taiwan. The high resistance rate of MDR

Table 3 Distribution of MDR *A baumannii* with rifampin and/or tigecycline resistance in each branch of Buddhist Tzu Chi Hospital

Branch	Rifampicin resistance			Tigecycline resistance		
	No. of isolates	No. of isolates in one pulsotype	No. of pulsotypes	No. of isolates	No. of isolates in one pulsotype	No. of pulsotypes
Taipei	10		5	23		11
		1	3		1	5
		3	1		2	2
		4	1		3	3
					5	1
Dalin	17		13	16		11
		1	9		1	7
		2	4		2	3
					3	1
Hualien	37		11	22		8
		1	6		2	4
		2	2		1	5
		3	1		3	2
		5	1		11	1
		19	1			

A baumannii to rifampicin was also observed by Giamarellos-Bourboulis et al.[26] The reason for choosing combination colistin/rifampicin instead of tigecycline in combination with colistin or rifampicin is based on the findings of a previous report that showed a lower percentages of synergistic combinations with tigecycline were observed when in combination with colistin (11%) or rifampicin (11%).[8] Meanwhile, Bassetti et al[27] showed that combination colistin/rifampicin is effective against MDR *A baumannii*, which is consistent with the findings presented here.

The intrahospital dissemination of MDR *A baumannii* has been reported by several studies conducted in southern, central, and northern Taiwan.[28,29] Nevertheless, the localized spread of MDR *A baumannii* strains in eastern Taiwan remains unclear. This study shows that 74.5% of the isolates found in the Haulien branch belong to three pulsotypes, even if no evidence of outbreak was noted. This suggests that an outbreak or cross-transmission between patients in this hospital could have occurred. Thus, it is urgent to monitor and control the spread of MDR *A baumannii* in the hospitals of eastern Taiwan through timely antimicrobial resistance surveillance and strict infection-control strategies.

The spread of MDR *A baumannii* with resistance to tigecycline, colistin, and rifampicin is an important issue that hospitals must control. Such epidemiological surveillance is still lacking in Taiwan, despite the alarming emergence of drug-resistant strains of *A baumannii*, particularly among those isolates that are not susceptible to tigecycline or colistin.[28] This study shows that the spread of rifampicin- and tigecycline-resistant MDR *A baumannii* isolates varies between the three hospitals. More heterogeneous MDR *A baumannii* isolates were obtained from the Taipei and Dalin branches. These two hospitals receive more patients that are transferred from nearby, smaller hospitals. Such transfers could result in the interhospital transmission of strains. In contrast, the intrahospital dissemination of MDR *A baumannii* might account for the clonal spread of rifampicin- and tigecycline-resistant isolates with different pulsotypes in the Hualien branch. This phenomenon emphasizes the importance of implementing effective infection-control strategies that could reduce clonal spread within hospitals.

In conclusion, the results of this study provide the first direct evidence of the clonal spread of tigecycline- and rifampicin-resistant MDR *A baumannii* within hospitals in eastern Taiwan. Alarmingly high rates of resistance to tigecycline and rifampicin in MDR *A baumannii* isolates were found in three hospitals located in different areas of Taiwan. The spread of tigecyline- and rifampicin-resistant MDR *A baumannii* strains via intra- and interhospital transmission warrants further investigations.

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