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Epidemiology Features of *Acinetobacter baumannii* Colonizing Respiratory Tracts of ICU Patients

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Abstract

Background: *A. baumannii* is becoming a common opportunistic and nosocomial pathogen in ICUs worldwide. Few studies have investigated the epidemiology of *A. baumannii* isolates colonizing the respiratory tract of hospitalized patients in ICUs.

Methods: A total of 93 patients admitted to various intensive care unites (ICUs) over a period of 10-month at the Jordan University Hospital (JUH) were investigated for colonizing their respiratory tract with *A. baumannii*. Risk factors for respiratory colonization with *A. baumannii* were determined. All *A. baumannii* isolates were examined for their antimicrobial susceptibility pattern, presence of specific β-lactamase genes and common genotypes using enterobacterial repetitive intergenic consensus sequences (ERICs) and PCR.

Results: Two third of hospitalized patients (63/93; 67.7 %) have their respiratory tract colonized with *A. baumannii*, and significant risk factors associated with colonization were longer ICU stay, use of mechanical ventilators and antibiotic treatment with carbapenems. All *A. baumannii* isolates were multidrug resistant (MRAB) and possessed *bla*OXA-51-like gene; in addition, 73% and 19% of the isolates harbored *bla*OXA-23-like and *bla*OXA-24-like genes, respectively. All isolates were negative for MBL-encoding gene (*bla*IMP-1) and only two isolates (3%) harbored (*bla*VIM-2) gene. The genetic similarity of *A. baumannii* isolates using ERICs-PCR and constructed dendrogram showed 2 major genotype clusters of genetically related isolates.

Conclusion: This study shows that few genotypes of MRAB are often colonizing the respiratory tract of hospitalized patients in ICUs, and colonization was rarely associated with blood sepsis and mortality.

Introduction

Acinetobacter baumannii has emerged as an important nosocomial opportunistic pathogen in intensive care units (ICUs) worldwide (1-4). The organism's ability to survive for extended periods on inanimate surfaces and by the presence of humidity conditions allows its existence as a potential pathogen in the hospital environment and its numerous spread among hospitalized patients (4-5).

A. baumannii causes generally ventilator-associated pneumonia, septicemia, and surgical site infection in hospitalized patients (3). It has been also reported that extensive usage of broad-spectrum antibiotics in hospitals was associated with increased incidence of multidrug resistance *A. baumannii* (MRAB) rates, specifically to carbapenems (4).

Additional risk factors for the acquisition of MRAB infection or colonization included longer hospitalization stay, admis-

sion to ICUs, use of mechanical ventilation, wound irrigation, blood sepsis and increased mortality (2,6). The potential of MRAB strains to colonize the respiratory tract seems depend on the expression of specific virulence factors, especially production of biofilm and adherence to epithelial cell surfaces (4,7).

This prospective study investigated the incidence rate, risk factors, antimicrobial resistance profiles and common genotypes of *A. baumannii* colonizing respiratory tract of ICU patients over a 10-month period.

Methods

Patients

A total of 142 respiratory samples (bronchoalveolar lavage, tracheal aspirate and sputum) were consecutively collected from 93 patients admitted to intensive care units (ICUs), Jordan University Hospital (JUH) over a 10-month period (May 2009-Febreuary 2010). For all patients; the name, age, sex, clinical diagnosis, type of antibiotics administered, mechanical ventilation, length of ICU stay, presence of tracheotomy, developing blood sepsis and mortality were recorded. This study was proved by Independent Research Ethics Review (IRB) no. L29/2009, Jordan University Hospital.

Sample processing

All collected samples were cultured on blood agar, CLED agar, and minimal-salt agar supplemented with 1% acetate (MSA) for detection of colonization with *Acinetobacter spp*. All suspected *Acinetobacter* growing colonies were first identified by conventional techniques and confirmed later as *A. baumannii* by Rapid NF plus system, Remel Kit (USA). Only one positive culture was included for each patient, and all isolates were stored in brain-heart infusion agar plus 15% glycerol at -70 °C until tested.

Antimicrobial susceptibility testing

A. baumannii isolates were tested against 11 antimicrobial agents (**Table 2**) using disc diffusion method according to the guidelines of CLSI, 2009 (8). Minimum inhibitory concentrations (MICs) were determined by E-test (AB Biodisk, Solna, Sweden) for imipenem, amikacin, tigecycline, and colistin. *Pseudomonas aeruginosa* ATCC 27853 was used as quality control strain.

Detection of β-lactamase genes

The genomic DNA was extracted from overnight cultures of *A. baumannii* isolates in brain heart infusion broth using the Wizard Genomic DNA purification kit (Promega, USA). A multiplex was used to identify metalo-beta-Lactamases (VIM-2, IMP-1) and OXA type β-lactamase genes (OXA-58, OXA-51, OXA-24 ,OXA-23) as described by Sung *et al.* (9).

Enterobacterial repetitive intergenic consensus (ERIC)-PCR typing

DNA fingerprinting was performed using ERIC-PCR method with primers ERIC1 and ERIC2 as previously described by Versalovic *et al.*(10). Amplification conditions were 95°C for 5 min; 30 cycles of 50 sec at 92°C, 55 sec at 52 °C and 7 min at 70 °C with a final step at 70 °C for 10 min. PCR products were detected in 2% agarose gel.

Statistical analysis

All data were analyzed using the computerized statistical analysis (SPSS, version 16). p value <0.05 was considered statistically significant.

Results

A total of 63/93 (67.7 %) of ICU patients has their respiratory tract colonized by *A. baumannii*. Only one patient died following developing blood sepsis due to infection / colonization with *A. baumannii* during the study period. Demographic data of patients show the common risk factors associated with *A. baumannii* colonization/infection (**Table 1**). The antimicrobial susceptibility pattern of 64 *A. baumannii* isolates including one blood isolates from the same patient is shown in **Table 2**. The susceptibility results for 11 tested agents ranged between 63% and 100%, and 58/64 (89%) of the isolates were multidrug resistant to at least for 6 tested antimicrobial agents. There was no resistance observed to both tigecycline and colistin. The MIC_{90} and MIC range to amikacin, imipenem, colistin and tigecycline are shown in **Table 2**. The distribution of β-lactamases genes among 64 *A. baumannii* isolates is shown in **Table 3**. All isolates possessed the encoding gene for an intrinsic OXA-51 carbapenemase, 73% were positive for *bla*OXA-23-like gene, and 19% were positive for *bla*OXA-24-like gene. None of the isolates harbored *bla*OXA-58-like or MBL-encoding gene (*bla*IMP-1) and Only two isolates(3%) harbored (*bla*VIM-2) gene.Two major clus-

Table 1. Major demographic characteristics of 93 adult patients admitted to ICUs and examined for respiratory tract colonization with *A .baumannii.*

Variables	No. (%) colonized patients	No. (%) not-colonized patients	P-value
Total of patients*	63 (67.7)	30 (32.3)	<0.001
Gender			
Male	36 (72.0)	14 (28.0)	0.344
Female	27 (62.8)	16 (37.2)	
Mean age	60.17 ±18.993	58.1±19.971	0.629
Length of stay in ICU	7.51 ± 1.595	2.73 ± 2.37	< 0.001
1-3 days	1 (3.7)	26 (96.3)	
4-8 days	56 (94.9)	3 (5.1)	< 0.001
> 8 days	6 (85.7)	1 (14.3)	
Antibiotics treatment** treatment			
Yes	53 (81.5)	12 (18.5)	< 0.001
No	10 (35.7)	18 (64.3)	
Mechanical ventilation			
Yes	60 (84.5)	11 (15.5)	<0.001
No	3 (13.6)	19 (86.4)	
Length of ventilation (mean±std)	5.02 ± 1.17	1.36 ± 0.67	<0.001
Tracheostomy			
Yes	11 (78.6)	3 (21.4)	0.347
No	52 (65.8)	27 (34.2)	

* All underlying diseases in patients were not significant for respiratory tract colonization with *A.baumanni*, and only one male patient was colonized and developed fatal blood sepsis with the same strain.

** Most patients (77%) were treated only with carbapenems during their stay in ICUs.

Table 2. Antimicrobial resistance patterns of 64 *A. baumannii* isolates from 63 ICU patients.

Antimicrobial	No. (%) Resistant isolates*	MIC90 (mg/L)	MIC-range (mg/L)
Ceftazidime (Caz)	64 (100)	ND **	ND
Ertapenem (Etp)	64 (100)	ND	ND
Meropenem (Mem)	64 (100)	ND	ND
Piperacillin/Tazobactam (Ptz)	63 (98)	ND	ND
Aztreonam (Atm)	63 (98)	ND	ND
Gentamicin (Gm)	60 (94)	ND	ND
Ciprofloxacin	58 (91)	ND	ND
Amikacin (Ak)	47 (73)	13.6	1-16
Imipenem (Imi)	40 (63)	12	0.19-12
Colistin (Co)	0 (0)	1	0.19-1
Tigecycline (Tgc)	0 (0)	2	0.38-4

* ND = Not done.

Table 3. Prevalence of β-lactamases among 64 *A. baumannii* isolates.

β-lactamases	Type of β-lactamase	Prevalence (%)
Class B	IMP	0 (0)
	VIM	2 (3)
Class D	OXA-51	64 (100)
	OXA-23	47(73)
	OXA-24	12 (19)
	OXA-58	0 (0)

Table 4. Genotyping of 64 clinical *A. baumannii* isolates based on constructed dendrogram.

Major cluster	genotype	Size of common DNA fragments(bp)	No. of isolates
1st	1	500-1500	23
	2	600-2000	16
	3	200-3000	7
2nd	1	700-1500	18

ters of few genotypes were detected among 64 *A. baumannii* isolates The majority of isolates (46/64; 71.9%) showed 4-6 DNA fragments ranged between 500-3000bp (**Table 4** and **Fig. 1**). On the basis of similarity index of constructed dendrogram, 71.9% of the isolates are suggesting to be one clone.

Discussion

This study found that about two third (67.7%) of hospitalized patients in various ICUs at JUH in Amman, have their respiratory tract colonized with *A. baumannii.* Major demographic characters of patients were incorporated for multivariate analysis which revealed that antibiotics treatment, the longer duration of mechanical ventilation and stay in the ICUs ($P<0.05$) were significant independent risk factors for colonization with *A. baumannii.*

In particular, antibiotic treatment with carbapenems has been shown to be mostly associated with respiratory tract colonization. In addition, none of the clinical diseases diagnosed in our patients was a significant risk factor for *A.baumannii* respiratory tract colonization (**Table 1**). Previous studies from different parts of the world have demonstrated that some or most of these risk factors were responsible for developing colonization/infection with *A. baumannii* in ICU patients (4,11-13). However, our study observed that only one fatal case of blood sepsis due to *A. baumannii* might be associated with respiratory colonization, and was initiated by using mechanical ventilation. Since antimicrobial resistance pattern of both *A. baumannii* isolates and their molecular typing by ERIC-PCR generated DNA fragments suggest that both isolates were similar and might be originated from the same clone. Other recent studies have reported higher blood sepsis and mortality rates in association with MDR *A. baumannii* in hospitalized patients (3,6,13-14).

The present study showed high resistance rates of *A. baumannii* isolates to many used antimicrobial agents in Jordan, except to colistin and tigecycline. The majority of *A. baumannii* isolates (89%) in this study were multidrug resistant (resistance to more than 6 tested drugs) as shown in **Table 2**. Recently, numerous studies worldwide reported high incidence of antimicrobial resistance rates among clinical *A. baumannii* isolates, but resistance to colistin was absent or rarely observed as it has been demonstrated in our study (1-2,15-18).

Carbapenems are among the drugs of choice used to treat nosocomial infections caused by MDR *A. baumannii*, and carbapenems were used frequently in treatment of our ICU patients. However, emergence of resistance to carbapenems causes serious concern in hospitalized patients and should be monitored (1,4). The naturally occurring β-lactamases have been identified as a source of carbapenem resistance in *A. baumannii*.

These enzymes belong either to the class B, or to the class D oxacillinase (19). Regardless of their susceptibility to carbapenems, all *A. baumannii* isolate produced the *bla*OXA-51-like gene. This result confirm other studies which have suggested that *bla*OXA-51-like gene is species-specific to *A. baumannii* (16-17,20), and it was proposed that studies of *A.baumannii* epidemiology could involve initial screening of *bla*OXA-51-like alleles to identify isolates belonging to major epidemic clones(21). The findings of this study also indicated that OXA-23 carbapenemase was predominant, whereas OXA-24 carbapenemase was less common. Other studies found also that many of *A. baumannii* isolates have acquired OXA-23 carbapenemase (18-19,22-23). The overall genetic relatedness of 64 *A. baumannii* isolates using ERIC-PCR and a constructed dendrogram showed 2 major clusters (clones); one cluster accounted for 71.9% of all *A. baumannii*

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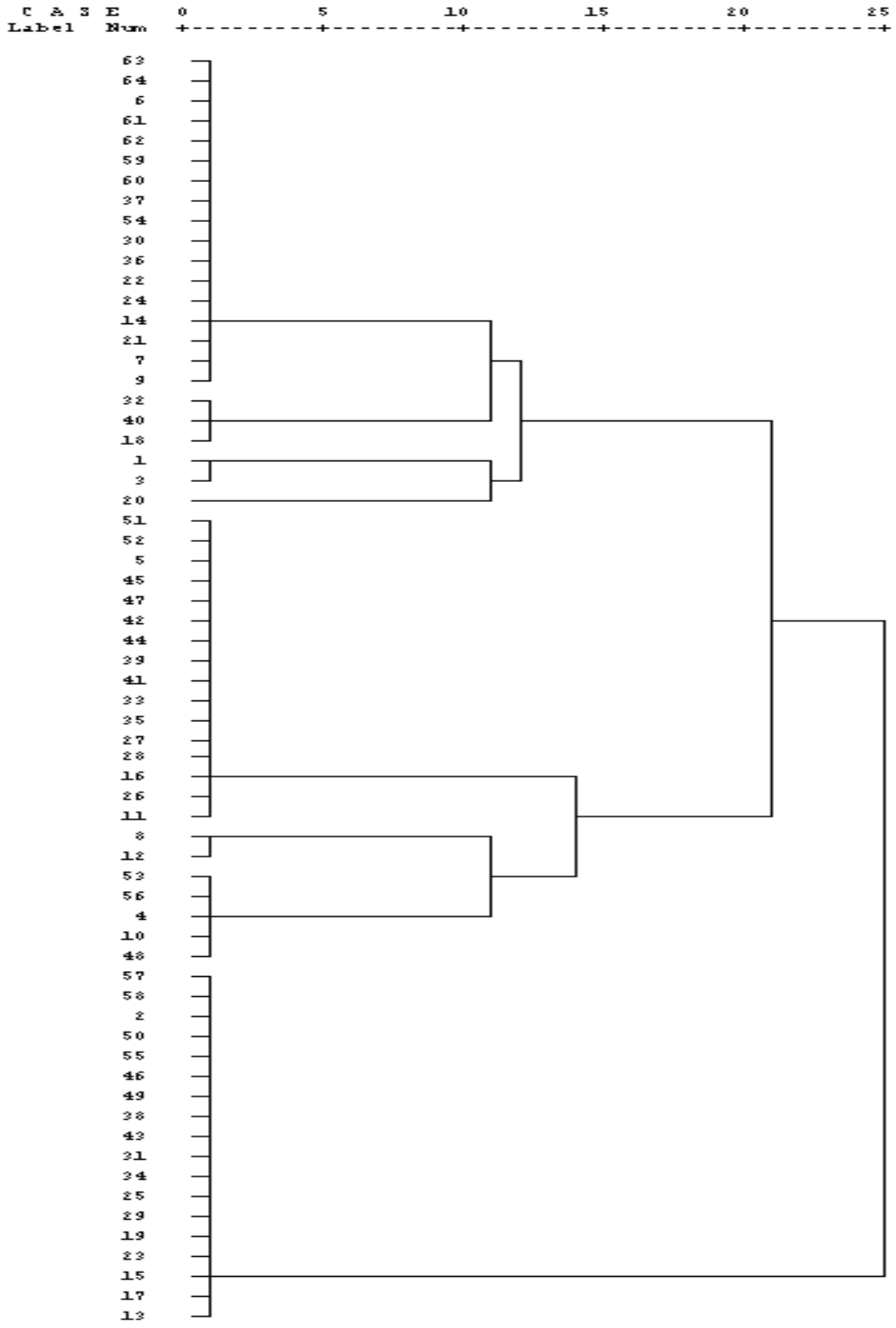

Figure 1. Dendrogram of 64 *A. baumannii* isolates.

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isolates (**Figure 1**). This cluster was divided into 3 common genotypes assigned as genotype 1, 2 and 3 (**Table 4**). These findings suggest that *A. baumannii* strains originated from two clones have been successfully circulated between ICU patients at the JUH during the study period, and may indicate cross transmission within the ICU setting. Various studies have demonstrated that ERIC-PCR method proved to be a useful to detect clones with acceptable reproducibility and discrimination index among certain Gram-negative bacterial strains including *A. baumannii* (9,224-25).

In conclusion, this study demonstrates that patients in ICUs are frequently colonized with few predominant genotypes of multidrug resistance *A. baumannii*.

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