European Review for Medical and Pharmacological Sciences 2012; 16: 1834-1839

Epidemiological investigation of nosocomial infection with multidrug-resistant *Acinetobacter baumannii*

S.M. EL-AGEERY, M.A. ABO-SHADI[1], A.A. ALGHAITHY, M.A. AHMAD[2], N.H. ALSHARIF[2], S.A. ALHARBI[2]

Medical Laboratories Technology Department, Faculty of Applied Medical Sciences, Taibah University, Saudi Arabia
[1]Biology Department, Faculty of Science, Taibah University, Saudi Arabia
[2]Medical Bacteriology and Molecular Biology Laboratory, King Fahd Hospital, Al-Madinah, Saudi Arabia

Abstract. – BACKGROUND: Multidrug resistant *Acinetobacter baumannii*, (MRAB) is an important cause of hospital acquired infection.

AIM: To document the emergence of MRAB in an Intensive Care Unit (ICU); and to characterize its hospital-wide outbreak by investigating antibiotypes and genotypes.

MATERIALS AND METHODS: A six-month prospective study for the presence of MRAB infection or colonization on inpatients, health care workers and environmental sites was done at an ICU in Fahd Hospital, Saudi Arabia. For all the collected specimens, microbiological analysis and antimicrobial susceptibility testing using an automated system (Phoenix, Becton Dick inson, USA) were performed. Pulsed-Field Gel Electrophoresis (PFGE) analysis was done to determine the clonal relationship between isolates.

RESULTS: A total 18 MRAB were isolated from 12 patients and 3 environmental samples. The risk factors for the acquisition of infection were age less than 60 years, mechanical ventilation, surgical interference and co-morbidity. Five PFGE profiles; pulsotype A to E, were identified. Pulsotype C isolates were further separated into 5 subtypes with predominance of subtype C3.

CONCLUSIONS: The study revealed a causal link between the contaminated ventilator and the subsequent MRAB. A correct antibiotic strategy should be addressed; and strict compliance with basic and potential control measures for the containment of infection should be achieved.

Key Words:
Multidrug resistant Acinetobacter baumannii, Pulsed-field gel electrophoresis, Epidemiology, Nosocomial infection, Antibiotypes.

Introduction

Acinetobacter baumannii (AB) has emerged as a leading nosocomial pathogen worldwide. *Acinetobacter* infections pose a challenge because nosocomial isolates exhibit a remarkable ability to develop antibiotic resistance rapidly, which leads to multidrug resistance within a few decades[1].

The rapid spread of MRAB (Multidrug Resistant *Acinetobacter baumannii*) in hospitals has been documented in several studies[2,3]. Clonal spread of MR *Acinetobacter spp*. has occurred in hospitals[4], whereas the inter-hospital spread of MRAB clones among different wards and hospitals has been observed in several countries[5].

A. baumannii infections have become increasingly common among critically-ill patients in intensive care units (ICUs) worldwide[6].

This organism, like *Pseudomonas aeruginosa*, is intrinsically resistant to a number of antimicrobial agents and the emergence of multidrug-resistant (MDR) clinical isolates has been widely reported from hospitals in Europe, North America, Argentina, Brazil, China, Taiwan, Hong Kong, Japan, and Korea[7]. Evidence of pan-drug resistance among *A baumannii* isolates (i.e., resistance to all available antimicrobial agents, including polymyxins) has been reported[8].

The aim of our study was to document the emergence of MRAB at an ICU of King Fahd Hospital in Al-Madinah, Saudi Arabia; and to characterize its spread by investigating antibiotypes and genotypes.

Materials and Methods

Setting and Study Design

A six-month prospective study on in-patients, HCWs (HealthCare Workers) and environmental sites was done at an ICU of King Fahd Hospital in Al-Madinah, Saudi Arabia. We tested for the presence of MRAB infection or colonization. Surveil-

Corresponding Author: Maha A. Abo-Shadi, Ph.D.; e-mail: m_a_shadi@hotmail.com

lance cultures were performed on the HCWs including the nursing personnel and the physicians. In addition, environmental cultures were also performed within the unit at the same time.

Ethical Committee of King Fahd Hospital and the Deanship of Scientific Research of Taibah University approved the study.

Microbiological Analysis

Samples from patients were collected according to the site of infection. For instance: wound swab, blood, sputum, abdominal drain fluid and tracheal aspirate. Samples from HCWs were collected from right and left hands, which were obtained by placing their hands into 2 separate blood agar plates (Oxoid, Basingstoke, Hampshire, UK) directly without washing their hands before culture. Environmental samples were collected before the regular daily cleaning by rolling sterile cotton swabs moistened with sterile saline several times over a surface area of approximately 5 × 5 cm^2 and then inoculated into blood agar plates. The environmental sites cultured included the patients' charts, bed rails, faucets, monitors, oxygen adapters, suction switches, and ventilator buttons. MRAB were identified and tested to the species level using an automated system Phoenix (Becton Dickinson, Fkanklin Lakes, NJ, USA).

Antibiotypying

The antimicrobial susceptibility testing was performed using an automated system (Phoenix, BD, USA). The breakpoint minimum inhibitory concentration (MIC) was determined for 20 antimicrobial agents: ceftazidime, cefepime, cephotaxime, cefuroxime, cefoxitin, cephalothin, ampicillin, amoxycillin-clavulanate, piperacillin-tazobactam, aztreonam, imipenem, ertapenem, meropenem, gentamicin, amikacin, levofloxacin, ciprofloxacin, nitrofurantoin, colistin and trimethoprim-sulphamethoxazole. National Committee for Clinical Laboratory Standards[9] were used for the interpretation of susceptibility results and breakpoints. The definition of MRAB varies in the literature, but several authorities consider an isolate to be multidrug resistant if it is resistant to three or more classes of antibiotics[10].

Molecular Typing

Genomic DNA in agarose gel plugs was prepared according to the protocol recommended by Antibiotic Resistance Prevention and Control (http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1194947313339).

In-gel digestion of genomic DNA with *Sma*I (Promega, Madison, WI, USA) overnight at 37ºC according to[11]. Electrophoresis was performed in a 1% SeaKem LE agarose gel (BMA, Rockland, ME, USA) prepared and run in 0.5 × Tris-borate-EDTA buffer on a CHEF-DR III apparatus (Bio-Rad Laboratories, Hercules, CA, USA). The initial switch time was 5 s, the final switch time was 20 s, and the run time was 22 h at 6 V/cm. Gels were stained in ethidium bromide, destained in distilled water, and photographed under ultraviolet (UV) light. PFGE DNA patterns were compared and interpreted according to the criteria of[12]. Isolates with ≥ 80% similarity were considered to belong to the same pulsotype and subtypes were assigned to isolates having ≤ 3 DNA band differences within the same pulsotype[12].

Results

Demographic and Clinical Data of Patients

Demographic, clinical data and type of sample of positive MRAB patients are listed in Table I. MRAB was isolated from sputum of p1, p2, p4, p5, p8 and p9 and tracheal aspirate of p7. MRAB was also isolated from wound of p2, p6, p10 and p11; blood culture of p2, p7 and p12; and abdominal drain fluid of p3.

Antimicrobial Susceptibilities

All MRAB isolates (15 patient samples and 3 environmental samples) presented the same resistance profile to carbapenems, cephalosporines, aztreonam, penicillins, and nitrofurantoin, with different susceptibility to the other tested drugs, as shown in Table II. On the other hand, all isolates were sensitive to colisitin.

PFGE Analysis

PFGE pattern of MRAB is presented in Table III. A total of 5 PFGE profiles, pulsotypes A to E, were identified among 15 MRAB. Pulsotype C isolates were further separated into 5 subtypes, subtypes C1 to C5 (Figure 1). Most of the subtypes among the strain isolates of pulsotype C were subtype C3.

Discussion

The bacteria *A. baumannii* has emerged as an important nosocomial pathogen, and outbreaks

Table I. Demographic and clinical characteristics of patients with positive MRAB patients.

Patient no	Sex/age	Underlying condition	Sample
P1	M/31	Head injury, with mechanical ventilation	Sputum
P2	F/57	Road traffic accident, with mechanical ventilation	Wound swab Blood Sputum
P3	F/65	Post laparotomy	Abdominal drain fluid
P4	M/56	Diabetic ketoacidosis	Sputum
P5	F/19	Systemic lupus erythematosius (SLE)	Sputum
P6	M/48	Head injury	Wound swab
P7	F/37	Septic shock	Blood Tracheal aspirate
P8	F/69	Chronic obstructive pulmonary disease with mechanical ventilation	Sputum
P9	M/62	Hepatoencephalopathy	Sputum
P10	M/16	Road traffic accident	Wound swab
P11	M/13	Post appendecectomy wound	Wound swab
P12	M/57	Upper GIT bleeding	Blood

P = patient, M = male, F = female.

due to multiply-resistant strains have been difficult to control, especially in ICUs[13]. We have documented an outbreak of MRAB in ICU King Fahd Hospital during a six-month period, affecting 12 patients. The risk factors for the acquisition of infection were age < 60 years in p3, p8, p9, mechanical ventilation in p1, p2, surgical interference in p3, p11, co-morbidity, especially neurologic impairment in p1, p6, SLE in p5, uncontrolled diabetes in p4, hepatoencephalopathy in p9, upper GIT bleeding in p12, chronic obstructive pulmonary disease in p8, and major trauma in p2, p10. These findings are consistent with other reports[10,14-16].

The neurologically impaired patients in our ICU had a high prevalence of chronic wounds.

Table II. Antibiotic resistance patterns of MRAB clinical isolates.

Antibiotics	MIC µg/ml	Sensitivity patterns					
		Sensitive		Intermediate		Resistant	
		no	%	no	%	no	%
Amikacin.	< 32	2	11	1	0	16	89
Gentamicin.	< 8	1	5.5	0	6	17	94.5
Ertapenem,	< 4	0	0	0	0	18	100
Imipenem,	< 8	0	0	0	0	18	100
Meropenem	< 8	0	0	0	0	18	100
Cephalothin,	< 16	0	0	0	0	18	100
Cefuroxime	< 16	0	0	0	0	18	100
Cefoxitin	< 16	0	0	0	0	18	100
Ceftazidime	< 16	0	0	0	0	18	100
Cephotaxime	< 32	0	0	0	0	18	100
Cefepime	< 16	0	0	0	0	18	100
Aztreonam	< 16	0	0	0	0	18	100
Ampicillin	< 16	0	0	0	0	18	100
Amoxycillin-clavulinate	< 16/8	0	0	0	0	18	100
Piperacillin-tazobactam	< 64/4	0	0	0	0	18	100
Colistin	≥ 1	18	100	0	0	0	0
Trimethoprim-sulfamethoxazole.	< 4/76	3	16	0	0	15	83.3
Nitrofurantoin	< 64	0	0	0	0	18	100
Ciprofloxacin	< 2	1	5.5	0	0	17	94.5
Levofloxacin	< 4	1	5.5	0	0	17	94.5

Table III. PFGE pattern of MRAB.

Isolate no	Sample	PFGE pattern
P1	Sputum	B
P2	Wound swab	C3
	Blood	C3
	Sputum	C3
P3	Abdominal drain fluid	A
P4	Sputum	C1
P5	Sputum	C2
P6	Wound swab	E
P7	Blood	A
	Tracheal aspirate	A
P8	Sputum	D
P9	Sputum	C2
P10	Wound swab	C5
P11	Wound swab	C4
P12	Blood	A
V1	Suction switch	C1
V2	Ventilator button	B
V3	Ventilator button	C3

P = patients, V = environmental sample

This most likely explains the increased risk of resistant *Acinetobacter* among them. Neurologic injury has been shown to be associated with the development of resistant Acinetobacter[17].

Chronic obstructive pulmonary disease and diabetes showed a trend toward significant associations with the development of MRAB. It is well accepted that patients with chronic lung disease are at increased risk of airway colonization and pneumonia, especially when they require intubation. Additionally, intubated patients with chronic pulmonary disease are often treated with prophylactic antibiotics which increase the risk of resistance[18].

Resistant *A. baumannii* is a significant problem as seen in this study where all the isolates were considered multidrug resistant and all were resistant to imipenem, piperacillin-tazobactam, and 89% were resistant to amikacin, formerly very effective antibiotics. Also carbapenem resistance is a sentinel event for emerging antimicrobial resistance[19]. Moreover, all isolates were resistant to all commonly used antibiotics including cephalosporines, aztreonam, penicillins, and nitrofurantoin. So, this finding confers high resistance and therapeutic challenges. The mechanisms of acquiring resistance of *A. baumannii* to cephalosporins and carbapenems include altered penicillin-binding proteins, the presence of metallo beta lactamases, and the loss of porins[11].

Genotyping by PFGE has been shown to be a powerful tool for a better understanding of the epidemiology of nosocomial infection[20-22]. In this setting, it was observed that pulsotypes B, C1 and C3 were detected in the sputum of p1, p4 and p2 who were on mechanical ventilator from which the same pulsotypes were isolated, suggesting that the hospital field was the original source of infection.

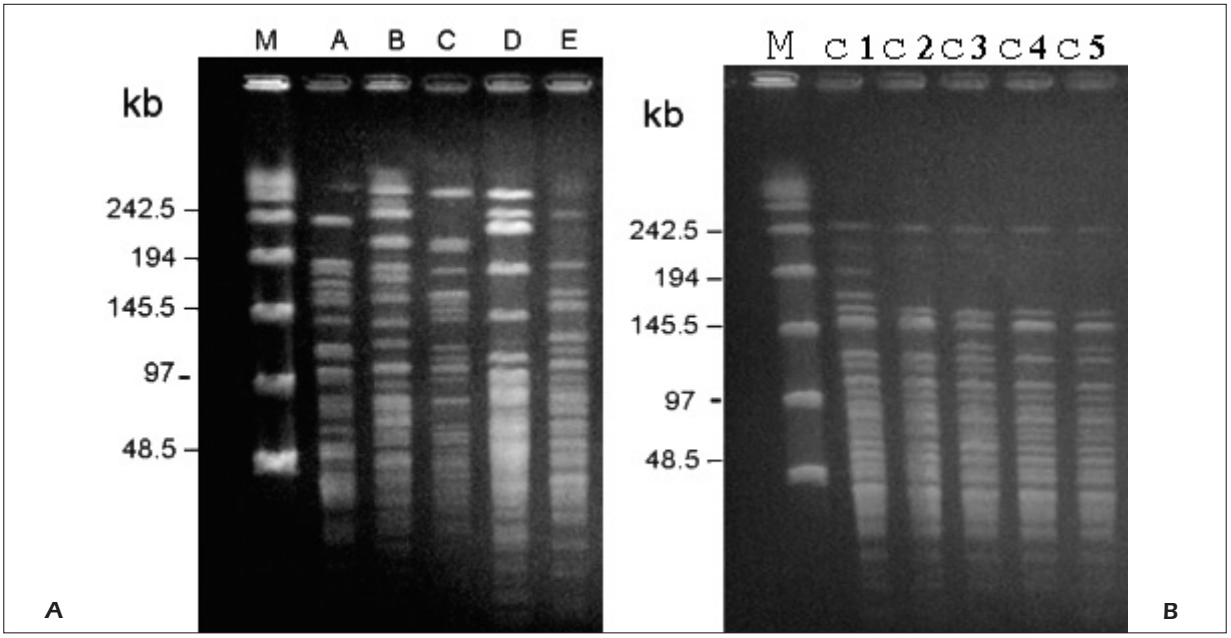

Figure 1. MRAB different biotypes obtained by PFGE after digestion with SmaI. ***A,*** Five pulsotypes in lanes A to E and Lane M, molecular size lambda marker. ***B,*** The five C subtypes, C1 to C5 of MRAB.

So, this study definitively establish a causal link between the contaminated ventilator and the subsequent MRAB ventilator-associated pneumonia. If the hospital field was contaminated, we would have detected multiple environmental samples to have been positive for *A. baumannii*. *A. baumannii* is a ubiquitous micro-organism that colonizes inanimate surfaces and medical equipment. Our patients may have just acquired the MRAB through microaspiration or even hematogenous spread from an infected IV site. Once acquired, the ventilator may have been secondarily contaminated as suggested by Cox et al[23] and Dealer[24].

Chang et al[25] identified 17, 23, and 11 pulsotypes in the three hospital branches located in northern, southern, and eastern Taiwan by PFGE. Furthermore, Kansakar et al[26] reported that 73% of MRAB were clustered into three predominant PFGE types: 6, 7, and 36 by PFGE.

Genotyping by PFGE has been shown other three pulsotypes in the ICU during the study period. Surveillance made during six-month period demonstrated that these pulsotypes were not isolated from environmental sources or the hands of health care workers present in the unit. This may be explained by lapses in infection-control measures, resulting in an increase in cross-transmission between patients or the non-restrictive antibiotic use at the field hospitals.

Conclusions

We report a nosocomial outbreak due to MRAB in ICU. To confront the imminent threat of untreatable infection caused by this organism, a correct antibiotic strategy should be addressed, and strict compliance with basic and potential control measures for the containment of infection should be achieved.

Acknowledgements

This work was supported by project No. 76/432 from Deanship of Scientific Research of Taibah University, Al-Madinah, Saudi Arabia.

References

1) Lin MF, Chang KC, Lan CY, Chou J, Kuo JW, Chang CK, Liou ML. Molecular epidemiology and antimicrobial resistance determinants of multidrug-resistant *Acinetobacter baumannii* in five proximal hospitals in Taiwan. Jpn J Infect Dis 2011; 64: 222-227.

2) Qi C, Malczynski M, Parker M, Scheetz MH. Characterization of genetic diversity of carbapenem-resistant *Acinetobacter baumannii* clinical strains collected from 2004 to 2007. J Clin Microbiol 2008; 46: 1106-1109.

3) Jean SS, Hsueh PR, Lee WS, Chang HT, Chou MY, Chen IS, Wang JH, Lin CF, Shy JM, Ko WC, Wu JJ, Liu YC, Huang WK, Teng LJ, Liu CY. Nationwide surveillance of antimicrobial resistance among non-fermentative Gram-negative bacteria in intensive care units in Taiwan, SMART programme data 2005. Int J Antimicrob Agent 2009; 33: 266-271.

4) Chen CM, Liu PY, Ke SC, Wu HJ, Wu LT. Investigation of carbapenem-resistant *Acinetobacter baumannii* isolates in a district hospital in Taiwan. Diag Microbiol Infect Dis 2009; 63: 394-397.

5) Mendes RE, Bell JM, Turnidge JD, Castanheira M, Jones RN. Emergence and widespread dissemination of OXA-23, -24/40 and -58 carbapenemases among *Acinetobacter* spp. in Asia-Pacific nations: report from the SENTRY Surveillance Programs. J Antimicrob Chemother 2009; 63: 55-59.

6) Navon-Venezia S, Leavitt A, Carmeli Y. High tigecycline resistance in multidrug-resistance *Acinetobacter baumanii*. J Antimicrob Chemother 2007; 59: 772-774.

7) Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2007; 51: 3471-3484.

8) Al Johani SM, Akhte J, Balkhy H, Younan AE, Memish Z. Prevalence of antimicrobial resistance among Gram-negative isolates in an adult intensive care unit at a tertiary care center in Saudi Arabia. Ann Saudi Med 2010; 30: 364-369.

9) National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial testing. NCCLS 2001; Suppl. M100-S11.

10) Falagas ME, Kopterides P. Risk factors for the isolation of multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: a systemic review of the literature. J Hosp Infect 2006; 64: 7-15.

11) Hsueh PR, Teng LJ, Chen CY, Chen WH, Ho SW, Luh KT. Pandrug-resistant *Acinetobacter baumannii* causing nosocomial infections in a University Hospital, Taiwan. Emerg Infect Dis 2002; 8: 827-832.

12) Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995; 33: 2233-2239.

13) Levin AS, Levy CE, Manrique AEI, Medeiros EAS, Costa SF. Severe infections with imipenem-resistant *Acinetobacter baumannii* treated with ampicillin/sulbactam. Int J Antimicrob Agents 2003; 21: 58-62.

14) Seifert H, Strate A, Pulverer G. Nosocomial bacteremia due to *Acinetobacter baumannii*: Clinical features, epidemiology, and predictors of mortality. Medicine (Baltimore) 1995; 74: 340-349.

15) Mahgoub S, Ahmed J, Glatt AE. Underlying characteristics of patients harboring highly resistant *Acinetobacter baumannii.* Am J Infect Control 2002; 30: 386-390.

16) Baran G, Erbay A, Bodur H, Öngürü P, Akinci E, Balaban N, Cevik MA. Risk factors for nosocomial imipenem resistant Acinetobacter baumannii infections. Int J Infect Dis 2008; 12: 16-21.

17) Maragakis LL, Tucker MG, Miller RG, Carroll KC, Perl TM. Incidence and prevalence of multidrug-resistant Acinetobacter using targeted active surveillance cultures. JAMA 2008; 299: 2513-2514.

18) Nseir S, Di Pompeo C, Cavestri B, Jozefowicz E, Nyunga M, Soubrier S, Roussel-Delvallez M, Saulnier F, Mathieu D, Durocher A. Multiple-drug-resistant bacteria in patients with severe acute exacerbation of chronic obstructive pulmonary disease: Prevalence, risk factors, and outcome. Crit Care Med 2006; 34: 2959-2966.

19) Kluytmans-Vandenbergh M, Kluytmans J, Voss A. Dutch guideline for preventing nosocomial transmission of highly resistant microorganisms (HRMO). Infection 2005; 33: 309-313.

20) Melamed R, Greenberg D, Porat N, Karplus M, Zmora E, Golan A, Yagupsky P, Dagan R. Successful control of an *Acinetobacter baumannii* outbreak in a neonatal intensive care unit. J Hosp Infect 2003; 53: 31-38.

21) Wang SH, Sheng WH, Chang YY, Wang LH, Lin HC, Chen ML, Pan HJ, Ko WJ, Chang SC, Lin FY. Healthcare associated outbreak due to pan-drug resistant Acinetobacter baumannii in a surgical intensive care unit. J Infect 2003; 53: 97-102.

22) Al-Sweih NA, Al-Hubal M, Rotimi VO. Three distinct clones of carbapenem-resistant *Acinetobacter baumannii* with high diversity of carbapenemases isolated from patients in two hospitals in Kuwait. J Infect Public Health 2012; 5: 102-108.

23) Cox TR, Roland WE, Dolan ME. Ventilator-related *Acinetobacter* outbreak in an intensive care unit. Mil Med 1998; 163: 389-391.

24) Dealler S. Nosocomial outbreak of multi-resistant *Acinetobacter* sp. on an intensive care unit: possible association with ventilation equipment. J Hosp Infect 1998; 38: 147-148.

25) Chang KCh, Lin MF, Lin NT, Wu WJ, Kuo HY, Lin TY, Yang TL, Chen YC, Liou ML. Clonal spread of multidrug-resistant Acinetobacter baumannii in eastern Taiwan. J Microbiol Immunol Infect 2012; 45: 37-42.

26) Kansakar P, Dorji D, Chongtrakool P, Mingmongkolchai S, Mokmake B, Dubbs P. Local dissemination of multidrug-resistant *Acinetobacter baumannii* clones in a Thai hospital. Microbial Drug Resist 2011; 17: 109-119.