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Major article

Major biologic characteristics of *Acinetobacter baumannii* isolates from hospital environmental and patients' respiratory tract sources

Nathir Obeidat MD [a], Fatin Jawdat MSc [b], Amal G. Al-Bakri PhD [c], Asem A. Shehabi DSc [b,*]

[a] Department of Internal Medicine, Faculty of Medicine-Jordan, The University Hospital, Amman, Jordan
[b] Department of Pathology and Microbiology, Faculty of Medicine, The University of Jordan, Amman, Jordan
[c] Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, The University of Jordan, Amman, Jordan

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Background: This prospective study investigated major biologic characteristics of *Acinetobacter baumannii* isolates from hospital environment and respiratory tract samples of patients admitted to adult intensive care units (ICUs) at the Jordan University Hospital.

Methods: *A baumannii* isolates from both sources were examined for antimicrobial susceptibility and for presence of specific metallo-β-lactamase genes (VIM-2, IMP-1) and OXA-type β-lactamase genes (OXA-type) using polymerase chain reaction and biofilm formation and surviving under various temperatures and pH conditions.

Results: The majority of *A baumannii* isolates from environmental and patients sources was multidrug resistant (MDR), except for colistin and tigecycline. All *A baumannii* examined carried a *bla*OXA_{51}-like gene, 58% has a *bla*OXA_{23}-like gene, and 38.8% has a *bla*OXA_{24}-like gene. Representative MDR *A baumannii* isolates from both sources were capable to form biofilm. *A baumannii* environmental isolates were capable to survive for a longer time in tap, normal saline, and distilled water than respiratory tract isolates with pH range of 4.5 to 8 and temperature between 18°C to 37°C.

Conclusions: This study demonstrates that *A baumannii* isolates from the patients' respiratory tract and hospital environment carried much similar multidrug resistance patterns and biologic characteristics. In conclusion, this study shows that all MDR *A baumannii* strains survived well in the hospital environment, especially in water and moist environment and produced biofilm, which might be responsible for high colonization in the respiratory tract of patients in ICU.

Acinetobacter baumannii has emerged in recent years as an important infectious agent in hospitalized patients worldwide and especially in developing countries.[1-4] The ability of *A baumannii* to survive in the environment under unfavorable conditions for prolonged periods of time may have contributed to the endemic and epidemic behavior of this opportunistic pathogen.[5] There are few major factors identified to contribute to the survival of *A baumannii* in the hospital environment including their resistance to antimicrobial drugs, disinfectants, desiccation, and nutritional starvation in the moist envirnment.[6,7] Previous studies demonstrated that adhesion and biofilm phenotypes of clinical *A baumannii* isolates seem to be associated with antibiotic resistance.[5,8-10]

The high capacity of multidrug-resistant (MDR) clinical isolates of *A baumannii* to form biofilms and to adhere to respiratory epithelial cells make them virulent and enhance their potential to colonize human skin and respiratory tract mucosa.[10] Bacterial colonization of the respiratory tract frequently precedes the onset of serious invasive infection. Investigators have reported that biofilm producing bacteria can be up to 1,000-fold more resistant to antibiotic treatment than the same organism grown in planktonic stage.[11-13] Therefore, one major reason for persistence of *A baumannii* in environment seems to be related to its capacity to grow within biofilms, which protect them against adverse environmental factors. This study compared the survival potential, biofilm production, and antimicrobial resistance in *A baumannii* isolates from both hospital environment and respiratory tract of hospitalized patients.

* Address correspondence to Asem A. Shehabi, DSc, The University of Jordan, Faculty of Medicine, Department of Pathology and Microbiology, Amman 11942, Jordon.
E-mail address: ashehabi@ju.edu.jo (A.A. Shehabi).

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MATERIALS AND METHODS

Bacterial isolates

Over the period of this investigation (May 2009-February 2010), a total of 74 (49.7%) *A baumannii* isolates was recovered by culturing 149 environmental samples collected from 3 adult intensive care units (ICUs) of the Jordan University Hospital (JUH). The majority of these samples was collected randomly from pillows, bed linens, and ventilation masks used by patients as well as from the floor and sinks using wetted swabs with physiologic saline. At the same period, a total of 64 (45.1%) of *A baumannii* isolates was recovered from 142 patients' respiratory samples of bronchoalveolar lavage, endotracheal aspirate, and sputum, which were consecutively collected from of 93 patients in the first 48 hours after admission to ICUs at JUH. All collected samples were cultured within 1 to 2 hours on blood agar, cysteine-lactose-electrolyte-deficient agar (cysteine-lactose-electrolyte-deficient medium; Oxoid, Cambridge, England), and minimal salt agar supplemented with 1% acetate for detection of colonization with *Acinetobacter* spp. All suspected *Acinetobacter* growing colonies were first identified by conventional bacteriologic techniques and confirmed later as *A baumannii* by Rapid NF plus system, Remel Kit (Lenexa, KS). Only 1 positive culture of *A baumannii* isolate was included for each environmental sample or patient. All cultures were kept frozen at −70°C in brain-heart agar with 15% glycerol (Difco, Sparks, MD) until used. *Pseudomonas aeruginosa* (ATCC 29852) was used for quality control of antimicrobial susceptibility test and biofilm formation. All the used media were obtained from Oxoid. This study has been approved by the Postgraduate Committee of the Faculty of Medicine and the Ethics Committee of JUH, a 550-bed, tertiary care, teaching hospital with 24 ICU beds and 3 adult ICUs.

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed for 11 different therapeutically relevant antibiotics by disk diffusion method, and susceptibility of each isolate to the antimicrobial drugs was determined according the guidelines of the Clinical and Laboratory Standard Institute.[15] The tested antibiotics (Mast Group LTD, Merseyside, England) and their concentrations are shown in Table 1. Minimum inhibitory concentrations (MICs) for amikacin, colistin, imipenem, and tigecycline were determined by the E-test method according to the manufacturer's guidelines (AB Biodisk, Solna, Sweden). MDR was defined as resistance to $\geq$ 3 classes of antimicrobial agents, including cephalosporins, aztreonam, carbapenems, aminoglycosides, and fluoroquinolones. The following tests (OXA-51, OXA-23, OXA-24, VIM-2, IMP-1, and OXA-58 genes) were included for each 8 MDR *A baumannii* isolates from hospital environmental and patients' respiratory tract sources as shown in Table 2.

Detection of β-lactamase genes

A polymerase chain reaction multiplex was used to detect metallo-β-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.[16] Genomic DNA was extracted according to the manufacturer's instructions of Wizard Genomic DNA purification kit (Promega, Madison, WI).

Determination of survival at different temperature in vitro

A single colony of each fresh overnight culture was added to 10 mL Mueller Hinton broth. The resultant bacterial suspension was plated onto nutrient agar using standardized (0.001 μmL) loop and incubated at 4°C, laboratory room temperature (range, 18°C-24°C), 37°C, 42°C, 45°C, and 48°C for 24 hours, and each test was performed in duplicate on nutrient agar.

Table 1
Antimicrobial resistant patterns of 74 environmental and 64 *A baumannii* patients' respiratory tract isolates

Antimicrobial agent (disk concentration/μg)	No. (%) resistant environmental isolates*	MIC_{90} (mg/L)	No. (%) resistant patients isolates*	MIC_{90} (mg/L)
Amikacin (Am/30)	47 (63)	14	47 (73)	13.2
Aztreonem (Atm/30)	68 (92)	-	63 (98)	ND
Ceftazidime (Caz/30)	72 (97)	-	64 (100)	ND
Ciprofloxacin (Cip/5)	46 (62)	-	58 (91)	ND
Colistin (Col/10)	Null	0.5	Null	1
Gentamycin (Gm/10)	66 (89)	-	60 (94)	ND
Imipenem (Imi/10)	51 (69)	12	41 (64)	12
Meropenom (Mem/10)	72 (97)	-	64 (100)	-
Piperacillin/tazobactum (Ptz/110)	69 (93)	-	63 (98)	-
Tigecycline (Tag/15)	Null	1.5	Null	2

MIC, minimum inhibitory concentration; *ND*, not done.
*Data from Al-Dabaibah et al.[14]

Table 2
Distribution of β-lactamase genes in representative of each 8 *A baumannii* isolates from hospital environment and patients' respiratory tract samples

	Antibiotic resistance pattern of each examined isolates*	β-Lactamase resistance genes		
		OXA-51	OXA-23	OXA-24
No. *A baumanni* isolates from patients†				
1	Atm, Caz, Mem	+	+	−
2	Atm, Caz, Gm, Cip, Ptz	+	+	+
3	Atm, Caz, Cip, Gm, Mem, Ptz	+	+	−
4	Ak, Atm, Caz, Gm, Imi, Mem, Ptz	+	+	+
5	Ak, Atm, Caz, Cip, Gm, Mem, Ptz	+	+	+
6	Ak, Atm, Caz, Gm, Imi, Mem, Ptz	+	+	+
7	Ak, Atm, Caz, Gm, Imi, Mem, Ptz	+	+	+
8	Ak, Atm, Caz, Cip, Gm, Imi, Mem, Ptz	+	+	+
No. *A baumannii* isolates from environment†				
1	Atm, Caz, Mem	+	+	−
2	Caz, Gm, Imi, Mem, Ptz	+	−	+
3	Ak, Caz, Cip, Gm, Imi, Mem, Ptz	+	+	+
4	Ak, Caz, Cip, Gm, Imi, Mem, Ptz	+	+	+
5	Atm, Caz, Gm, Imi, Mem, Ptz	+	−	+
6	Ak, Atm, Caz, Cip, Mem, Ptz	+	+	−
7	Ak, Atm, Caz, Gm, Imi, Mem, Ptz	+	+	+
8	Ak, Atm, Caz, Imi, Gm, Mem, Ptz	+	+	−

NOTE. Antimicrobial agent definitions for column 1 are found in Table 1.
*All tested *A baumannii* isolates were negative for metallo-β-lactamases (VIM-2, IMP-1 genes) and OXA-58 genes.
†There was no significant difference in biofilm formation between patients and environmental isolates.

Determination of survival at different environmental conditions

The effect of pH on *A baumannii* growth was performed as described by Benjamin and Datta[17] with some slight modification. The effect of temperature on the *A baummanni* was determined by suspending a single colony of 8 fresh overnight cultures in 10 mL Mueller Hinton broth. The bacterial suspension was plated onto nutrient agar using standardized (0.001) loop and incubated at 4°C, 18°C, 24°C, and 37°C for 24 hours. The effects of pH range (4.5-8), type of water (distilled, tap, saline), room temperature (range, 18°C-24°C), and days on the viability of *A baumannii* were presented as major if the colony count was less than or equal to 10 colony-forming units/plate and minor if the count was more than 50 colony-forming units/plate. All tests were performed in duplicate.