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Epidemiological Characteristics and Antibiotic Resistance of *Acinetobacter baumannii* Isolated from Burn Patients

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Abstract

Acinetobacter baumannii is recognized as an important cause of nosocomial infections, although it can be associated with multi-drug resistance. This study was conducted to determine the incidence of *Acinetobacter baumannii* infections among burn patients at the Sulaimani Plastic and Burn Hospital, Kurdistan Region, Iraq. Out of 2244 patients admitted to in the burns center during the study, 42% were males and 58% were females. The median age was 18 (IQR 6, 27). The majority of burns were caused by flame (60.9%). The median of total percentage of burned surface area was 26 (IQR 17, 40). The median hospital stay was 17 days (IQR 9, 31). Antibiotic sensitivity, resistance and intermediate were undertaken. Overall, Colistine seems to be the most effective antibiotic, while Gentamicin is the least effective with 18.2% resistance. Amikacin was also high at 17.3%. There was significant difference between gender in terms of antibiotic resistance for Meropenem, Tobramycin and Gentamicin ($P < 0.05$). In terms of age, there was a significant association between age and antibiotic resistance for SAM, Imipenem, Gentamicin and Ciprofloxacin. There was a significant difference in gender in terms of infection (6.8% of males and 12.9% of females had infection). The patients coming from outside the city and from other provinces were more likely to be infected ($P < 0.01$). Also, the patients with TBSA burnt of 25 - 50% were more likely to have infection compared to patients with TBSA burnt < 25% (12.3% vs. 5.1%, $P < 0.001$). Infection was also associated with a longer hospital stay ($P < 0.001$). In conclusion, Increasing *Acinetobacter baumannii* infection and antibiotic resistance of most of the isolates recovered during extended hospitalization and impact of %TBSA. The microbiological surveillance of patterns and susceptibility profiles of this pathogen in our burn center should be performed regularly in order to prevent the antibiotic resistant pathogens in burn infection patients.

Keywords: *Antibiotic Resistant, Acinetobacter baumannii, TBSA, Burn Patients, Nosocomial Infection*

Abbreviations

TBSA: Total Body Surface Area; IQR: Inter Quartile Range; SAM: Ampicillin/Sulbactam; MDR: Multi Drug Resistant; BICU: Burn Intensive Care Unit; MRSA: Methicillin-Resistant Staphylococcus aureus; API: Analytical Profile Index

Introduction

Bacterial infections continue to be a major cause of mortality among burn patients despite a good prophylaxis and treatment. Often treatment is complicated by the emergence of antimicrobial resistance bacteria. Despite considerable advancements in burn wound care and infection control practices, infection remains the leading cause of death in this group of patients [1-3].

Burn patients are highly susceptible to become infected. Multi-resistant *Acinetobacter baumannii* strains are becoming highly important in nosocomial infections [4]. Antibiotic-resistant *Acinetobacter* nosocomial infection is a leading problem. It acts as an oppor-

tunistic pathogen to cause a wide spectrum of infection including nosocomial pneumonia, meningitis, endocarditis, skin and soft tissue infections, urinary tract infection, conjunctivitis, burn wound infection and bacteremia [5,6]. *Acinetobacter baumannii* is one of the most important pathogens in hospital-acquired infections, especially in burn intensive care unit (BICU) [7]. This opportunistic pathogen can be easily isolated from water, soil, and hospital facilities. Also, it resist to a wide range of antibiotics [7,8]. While *Acinetobacter baumannii* can be associated with multidrug resistant (MDR), its impact on mortality in burn patients has not been fully elucidated [9]. *Acinetobacter baumannii* is a gram-negative *coccobacillus* and resist to multiple antibiotics, management of clusters of *Acinetobacter baumannii* is useful as a model in eradication of multidrug resistant (MDR) infections [10]. The marked increase in the number of multidrug resistant (MDR) *Acinetobacter baumannii* strains highlights the need for a more rational use of broad spectrum antibiotics. However, surveillance of infection control guides were important to minimize the spread of *Acinetobacter baumannii* pathogen [11]. Total burn surface area, burn depth, age, presence of inhalation injury were determined to be the significant risk factors for acquisition of infection. Determining the nosocomial infection profile at a certain burn unites can help the medical staff apply the appropriate treatment regimen and decrease the drug resistance rates [12].

The overall objective of the current work is to study the risk factors associated with *Acinetobacter baumannii* infection and its antimicrobial resistance pattern in the Plastic and Burn Hospital in Sulaimani, Kurdistan Region, Iraq.

Materials and Methods

Data management and statistical analysis

This retrospective analysis was conducted in the Sulaimani Plastic and Burn Hospital. The epidemiological analysis is based on data collected from the medical records of 2244 burn patients, hospitalized between January 2008 and November 2012. Data collected included age, sex, total body surface area burnt (TBSA), residence, season of the injury, mechanism of injury, and outcome (Table 1). The clinical samples were taken for microbiological tests from various body sources (blood, urine, burn wounds) at different time intervals during the patients' stay in hospital. Culture and sensitivity tests were undertaken at the center's microbiology laboratory inside the hospital. Analysis was undertaken using Stata version 9 (Statacorp 2006). Descriptive analysis was performed; numeric data were summarized as means or medians depending on normality. Associations between categorical variables were tested by chi-squared test and p values equal to or smaller than 0.05 were reported a significant.

Microbiological assessment

Bacterial isolation and identification

Culture and sensitivity tests were undertaken at the microbiology laboratory department inside the hospital. Clinical samples were collected through surface swabs, tissue, blood, and urine. The swabs were dipped in Stuarts transport medium, and then inoculated onto a selective and differential medium (Manitol salt agar), as well as an enriched medium (Blood agar). The isolates were identified using conventional identification techniques after incubation for 18 - 48 hours at 37°C. Positive cultures were sub cultured on blood agar and MacConkey agar, as per routine bacteriologic guidelines. API 20E system was used to identify the isolated gram negative bacteria [13,14].

Antibiotic sensitivity test (Kirby–Bauer method)

All isolated *Acinetobacter baumannii* strains were tested against ten commonly used antibiotics (Table 2). Inoculum from the tested bacterium was prepared depending on Kirby-Bauer antibiotic testing [15]. Inhibition zones were expressed in (mm) as the diameters of clear zones around the discs (Performance Standards for Antimicrobial Susceptibility). The most common antibiotic disc according of the (Bio-Rad, Bioanalyse and Himedia Labs p,t Lid, India) are Cefepime, Ciprofloxacin, Imipenem, Meropenem, Ceftazidime, Amikacin, Gentamicin, Tobramycin, Colistine and Ampicillin-Sulbactum. The international standard concentration of these antibiotics in (µg /disc) are 30, 5, 75/10, 10, 10, 30, 10, 10, 50 and 10/10 respectively.

Results and Discussion

Characteristics of the patients

A total of 2244 burn patients were studied, of which 944 (42%) patients were males and 1300 (58%) were females. The median age of these patients was 18 (IQR 6 - 27) ranging from under one to 86 years, 39% of patients being children below 15 and 40% being aged 15 - 29 years. Most of patients coms from outside city followed by other provinces and inside city 975 (43.4%), 641 (28.6%), 628 (28%) respectively. The majority of the burns was caused by flame (60.9%) followed by scalds (28.9%). The median of total body surface area was equal to 26 with (IQR 17, 40). The median hospital stay was not normally distributed and ranged from 0 - 86 days with a median of 17 days (IQR 9, 31) (Table1).

Characteristics	Number of admitted patients	Percentage of burn (%)
Total	2244	100
Years		
2008	189	8.7
2009	561	24.9
2010	527	23.4
2011	546	24.3
2012	421	18.7
Gender		
Male	944	42
Female	1300	58
Age		
0 - 5 years	559	24.8
6 - 14 years	319	14.2
15 - 29 years	898	40.0
30 - 59 years	416	18.5
60 and over	52	2.3
Residency		
In side Sulaimani city	628	28
Outside Sulaimani city	975	43.4
Other provinces	641	28.6
Mechanism of injury		
Flame	1367	60.9
Scald	648	28.9
Other	229	10.2
Median age in years (IQR)	18 (6, 27)	
Median TBSA% burnt (IQR)	26 (17, 40)	
Median hospital stay in days (IQR)	7 (9, 31)	

Table 1: *Demographic information of the patients.*

Antibiotic sensitivity testing

Overall, 442 positive cultures of Acinetobacter baumannii were retrieved and tested for their antibiotic sensitivity pattern. As shown in Table 2, Colistine seems to be the most effective antibiotic with no cultures showing resistance while Gentamicin is the least effective

drug with 18.2% resistance. SAM was the second most effective antibiotic with 2.4% resistance while resistance to Amikacin was also high at 17.3% (Table 2).

Antibiotics	Resistant Number (%)	Sensitive Number (%)	Intermediate Number (%)
SAM Ampicillin/sulbactam	54 (2.4)	283 (12.6)	105 (4.6)
Cefepime	320 (14.2)	68 (3)	54 (2.4)
Imipenem	120 (5.3)	232 (10.3)	90 (4.0)
Meropenem	253 (11.2)	123 (5.5)	66 (2.9)
Cetazidime	323 (14.4)	76 (3.4)	43(1.9)
Amikacin	390 (17.3)	42 (1.9)	10 (0.4)
Gentamicin	410 (18.2)	25 (1.1)	7 (0.3)
Tobramycin	388 (17.2)	42 (1.9)	12 (0.5)
Ciprofloxacin	297 (13.2)	72 (3.2)	73 (3.2)
Colistine	0	442 (19.6)	0

Table 2: *Antibiotic resistance and sensitivity in 442 positive Acenitobacter boumanni cultures.*

Antibiotic resistance according to sex and age of patients

Resistance to antibiotics was also compared according to gender and age of patients. In terms of antibiotic resistance, there was significant differences between males and females for Meropenem, Tobramycin and Gentamicin ($p < 0.05$). In terms of age, there was a significant association between age and antibiotic resistance for four antibiotics SAM, Imipenem, Gentamicin and Ciprofloxacin. Resistance to antibiotics increased with advancing age except for age group 60 years and over. For example, resistance to SAM was 0.5% in children aged 0 - 5 while this was increased in age groups 15 - 29 years to 6.3% and then decreased to 0.5% in patients aged 60 and over. Likewise, resistance to Gentamicin was 12.2% in children and 46.6% in the adult persons and then decreased to 2.3% in older persons. See Table 3 for more information.

Sex and Age groups	Antibiotics									
	SAM	Cefepime	Imipenem	Meropenem	Cetazidime	Amikacin	Gentamicin	Tobramycin	Ciprofloxacin	Colistine
Sex										
Male	4.5*	23.5*	8.1*	16.5†	25.8*	30.1*	32.8†	32.8†	23.1*	0
Female	7.7	48.9	19.0	40.7	47.3	58.1	60.0	55.0	44.1	0
Age groups										
0 - 5 years	0.5†	9.7*	1.6†	6.3*	9.5*	11.8*	12.2†	11.8*	6.6†	0
6 - 14 years	1.6	6.6	4.1	4.8	6.3	8.6	39.9	9.0	6.8	0
15 - 29 years	6.3	34.4	14.9	29.9	35.5	45.2	46.6	43.0	35.7	0
30 - 59 years	3.4	19.5	5.9	14.0	19.7	20.4	22.6	21.9	16.1	0
60 and over	0.5	2.3	0.7	2.3	2.0	2.3	2.3	2.0	2.0	0

Table 3: *Percentage of Acinetobacter baumannii samples resistance to antibiotic according to sex and age.*

(†) is mean significant
(*) is mean non significant

Factors associated with *Acinetobacter baumannii* infections

Detailed data for patient characteristics of those with and without Acinetobacter baumannii infection were available for 2244 patients admitted during 2008 until 2012 which enabled a comparison of the two groups. There was significant difference in gender in terms of Acinetobacter baumannii infection (6.8% of males and 12.9% of females had infection, $p < 0.001$). There was significant association between age and infection. Age group 15 - 29 was more associated with infection than age 60 and over (9.9% vs. 0.5%, $p < 0.001$). Mechanism of burn was also associated with infection, patients caused by flame burn marked in a high proportion of infection (13.9%) followed by scald (3.4%) and others was (2.4%) $p < 0.001$. Patients coming from Sulaimani city were significantly less likely to be infected compared to patients coming from outside the city and from other provinces (4.3%, 8.6% and 6.7% respectively, $P < 0.01$). Patients with total body surface area (%TBSA) burnt of 25 - 50% were more likely to have infection compared to patients with %TBSA < 25% (12.3% vs. 5.1%, $P < 0.001$). Infection was also associated with a longer hospital stay. While 11.8% of patients staying over 3 weeks had infection, only 2.5% of those staying up to 7 days had infection ($P < 0.001$) (Table 4).

Characteristics	Infection Number (%)	No infection Number (%)	P value
Gender			
Male	153 (6.8)	791 (35.2)	$X^2 = 12.54$, 1df, $p < 0.001$
Female	289 (12.9)	1011 (45.1)	
Age			
0 - 5 years	59 (2.6)	500 (22.3)	$X^2 = 60.77$, 4df, $p < 0.001$
6 - 14 years	43(1.9)	276 (12.3)	
15 - 29 years	223 (9.9)	675 (30.1)	
30 - 59 years	105 (4.7)	311 (13.9)	
60 and over	12 (0.5)	40 (1.8)	
Residency			
Sulaimani city	97 (4.3)	531 (23.7)	$X^2 = 13.23$, 2df, $p < 0.01$
Outside Sulaimani	194 (8.6)	781 (34.8)	
Other provinces	151 (6.7)	490 (21.8)	
Mechanism of injury			
Flame	313 (13.9)	1054 (47.0)	$X^2 = 36.58$, 2df, $p < 0.001$
Scald	76 (3.4)	572 (25.5)	
Other	53 (2.4)	176 (7.8)	
Hospital stay			
0 - 7 days	56 (2.5)	345 (15.4)	$X^2 = 84.46$, 3df, $p < 0.001$
8 - 14 days	54 (2.4)	490 (21.9)	
15 - 21 days	67 (3.0)	291 (13.0)	
22 days and over	265 (11.8)	674 (30.1)	
Median TBSA% burnt	115 (5.1)	923 (41.2)	$X^2 = 96.17$, 3df, $p < 0.001$
0 - 25%	276 (12.3)	777 (34.7)	
25.1 - 50%	46 (2.1)	96 (4.3)	
50.1 - 75%	4 (0.2)	4 (0.2)	
75.1 - 100%			

Table 4: *Association between Acinetobacter baumannii infection and patient characteristics.*

Sites of the positive specimens

The study included 442 instances of positive cultures for *Acinetobacter baumannii* from 2244 patients. Number of positive samples per patient ranged from 1 to 8 samples. The majority of the samples were taken from the lower limbs excluding foot (45%) followed by the upper limbs and hands (38%) and then blood (5%), but the smallest percentage positive sample was taken from earlobe (1%). See Table 5 for more details.

Sample site	Number	(%)
Total	442	100
Lower limb excluding foot	200	45.0
Trunk	19	4.0
Upper limb excluding hand	138	31.0
Hand	29	7.0
Blood	20	5.0
Head and neck excluding earlobe	19	4.0
Foot	12	3.0
Earlobe	5	1.0

Table 5: *Number and percentage site of the positive Acinetobacter baumannii.*

Discussion and Conclusion

A previous study showed that Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) isolated from burn patients samples were sensitive to vancomycin and nitrofurantoin [16]. A marked increase in the number of hospital infections due to *Acinetobacter baumannii* has also been reported in many other countries [12,17,18]. The present study represents the first attempt to determine the incidence of *Acinetobacter baumannii* infections among burn patients at Sulaimani Plastic and Burn Hospital. In this study, we aimed not only to present the frequencies of the bacterium in burn patients, but also to test its antimicrobial resistance profile against most used antibiotics in order to improve the preventive and therapeutic strategies. The majority of burns was caused by flame (60.9%) followed by scalds (28.9%), our result is in accordance with a pervious study which is done by Akther J M., *et al*. in India [19].

As shown in table 2, Antibiotic sensitivity resistance and intermediate were undertaken for Ampicillin/sulbactam (SAM), Cefepime, Imipenem, Meropenem, Cetazidime, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin and Colistine. Overall, 442 positive *Acinetobacter baumannii* cultures, Colistine seems to be the most effective antibiotic with no cultures showing resistance, while Gentamicin is the least effective with 18.2% resistance, these results are closely related to some previous studies [20,21]. SAM was the second most effective with 2.4% resistance while resistance to Amikacin was also high at 17.3%. There was significant differences between males and females in terms of antibiotic resistance for Meropenem, Tobramycin and Gentamicin $P < 0.05$ (Table 3). A similar study done by Lortholary., *et al*. [22] showed same results. These differences are expected as a result of variations in disinfection protocols and antimicrobial therapy protocols which may favor the survival of some pathogens over others. Resistance to antibiotics was also compared according to gender and age of patients.

In terms of age, there was a significant association between age and antibiotic resistance for four antibiotics SAM, Imipenem, Gentamicin and Ciprofloxacin. Resistance to antibiotics increased with advancing age except for age group 60 years and over. For example, resistance to SAM was 0.5% in children aged 0 - 5 while this was increased in age groups 15 - 29 years to 6.3% and then decreased to 0.5% in patients aged 60 and over. Likewise, resistance to Gentamicin was 12.2% in children and 46.6% in the adult persons and then decreased to 2.3% in older persons (Table 3). This antibiotic variation resistance is probably because of only occasional use of some antibiotics versus prolonged and widespread use of other antibiotics without culture and sensitivity tests which has led to emergence of more resistant bacterial strains, as shown in the previous studies [23]. Furthermore, there was significant difference in gender in terms of *Acinetobacter*

baumannii infection 6.8% of males and 12.9% of females had infection (Table 4), our result accordance to the previous study [24]. This could be due to the higher number of admitted female patients in comparison to the male patients.

Mechanism of burn was also associated with infection, patients caused by flame (13.9%) more likely to be infected with *Acinetobacter baumannii* bacteria than patients caused by scald (3.4%) and others was (2.4%) and there was significantly significant $p < 0.001$. This variation may be due to reducing mechanical barrier (skin and mucosa) led to reduction of flow of blood to burn area and the pathogen invade tissues and cause infection (Table 4), previous study nearly accordance to this study raised factors to nosocomial infection and elevated mortality rates [25]. Patients coming from Sulaimani city were significantly less likely to be infected compared to patients coming from outside the city and from other provinces (4.3%, 8.6% and 6.7% respectively, $P < 0.01$). This is because our burn center is located in Sulaimani and so far from the other provinces thus the infection may occurred in the interval times (Table 4) [19]. Patients with total body surface area (%TBSA) burnt of 25 - 50% were more likely to have infection compared to patients with %TBSA < 25% (12.3% vs. 5.1%, $P < 0.001$). Infection was also associated with a longer hospital stay. These variations are due to the larger size of the wounds. This, in combination with their loss of physical defenses and need for invasive devices. These patients also represent a significant risk for contamination of their surrounding environment with organisms, which may then be spread to other patients on the unit. For these reasons, it is recommended that patients with larger burn injuries be isolated in private rooms or other enclosed bed spaces to ensure physical separation from other patients on the unit. While 11.8% of patients staying over 3 weeks had infection, only 2.5% of those staying up to 7 days had infection ($P < 0.001$), the probability of emerging infection increases, since the patients spend more time in hospital and more frequent with dressing and using materials (Table 4), there was nearly in accordance to other previous studies [19,26,27]. The majority of the samples were taken from the lower limbs excluding foot (45%) followed by the upper limbs and hands (38%) and then blood (5%), A few of the positive cultures taken from ear lobe may be due to locations on the body it is separated and smaller part rather than others (Table 5). This finding is in agreement to the previous study [28]. Finally, there was significant association between age and infection as, age group 15-29 was more associated with infection than age 60 and over (9.9% vs. 0.5%, $p < 0.001$) (Table 4). This could be due to the higher number of these ages admitted in comparison to the other ages.

Thus, the microbiological surveillance of patterns and susceptibility profiles of this pathogen in our burn center should be performed regularly in order to prevent the antibiotic resistant pathogens in burn infection patients.

Infection in the burn patients has been the major cause of morbidity. The present study has given us the knowledge regarding incidence of *Acinetobacter baumannii* colonization of burn patients and factors in relation to *Acinetobacter baumannii* infection. Increasing antibiotic resistance patterns of most of the *Acinetobacter baumannii* and factors in relation to isolates recovered during extended hospitalization, impact of % TBSA, sex, age, and mechanism of injury may affect empirical antimicrobial therapy and patient management decisions during treatment. Thus, the microbiological surveillance of patterns and susceptibility profiles of this pathogen in our burn center should be performed regularly in order to prevent the antibiotic resistant pathogens to infect our patients in the burn unit.

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Conflict of Interest

The authors declare that there is conflict of interests regarding the publication of this paper.

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