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Carbapenem-resistant *Acinetobacter baumannii* Recovered from Burn Patients

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ABSTRACT - Purpose. Emergence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) and their prolonged presence in burn units increases the risk of acquisition of CRAB. **Methods.** From November 2012 to September 2013, 1474 burn patients were screened for CRAB isolates through testing susceptibility to imipenem and its comparators meropenem, and doripenem. Furthermore, the *in vitro* activity of other antibiotics against CRAB was investigated. **Results.** Three patients were infected with carbapenem-susceptible *A. baumannii* (CSAB) and 168 were infected with CRAB. Approximately one-fifth (n=32) of CRAB isolates were obtained from patients hospitalized in Burn Intensive Care Unit (BICU). Most of CRABs were isolated from wound. The mean length of stay (LOS) in hospital prior to *A. baumannii* isolation was significantly higher for CRAB compared to CSAB cases (P=0.04). Amongst the independent variables, percentage of total burn surface area (TBSA) significantly increased the mortality rate using multivariate logistic regression (P=0.001, OR= 16.5; 95% CI: 4.72-57.7). The majority of tested isolates were resistant to imipenem (94.8%), and to its comparators, doripenem (97.7%), and meropenem (97.7%). The susceptibility of CRAB isolates was less than 10% to all tested antibiotics except for colistin (100%), doxycycline (61.9%), gentamicin (18.5%), and tigecycline (11.9%). **Conclusion.** Resistance to carbapenem reduces the number of effective antibiotics. The coordinated and intensive efforts of healthcare personnel are required to meet the challenge of dissemination of CRAB. **Keywords.** Carbapenem-susceptible *A. baumannii*, burn, imipenem

INTRODUCTION

Acinetobacter baumannii which was initially described as an opportunistic bacterium of low pathogenicity, has evolved to a major pathogen especially in critical care settings during the last decades (1). Emergence of resistant *A. baumannii* has attracted the attention of clinicians to the strains capable of escaping the biocidal action of antibiotics and to their potential risks (2).

Carbapenems such as imipenem, meropenem, doripenem but not ertapenem are the agents of choice for treatment of infections caused by *A. baumannii*, when other antibiotics are not effective (3). Carbapenems have been used as the last-line drugs for the treatment of *A. baumannii* infections until 1991 when the first carbapenem-resistant *Acinetobacter baumannii* (CRAB) was recognized (4, 5). Over the years, the increasing number of CRAB strains have been documented worldwide in hospital- and community acquired infections (6). Recent studies revealed that carbapenem-resistant *Acinetobacter* spp. are more predominant in the Latin America, Middle East, and Asia-pacific than in North America or Europe (7).

A. baumannii strains are becoming major pathogen in healthcare-associated infections,

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mainly in critically ill patients (8). Burn patients are extremely susceptible individuals to infections during hospitalization and are also at risk of infection through their own microbiota. Factors predisposing burn patients to infections include the changes in the physical barriers such as loss of skin as the first line defense followed by creation of favorable environment for bacteria multiplication, increased rate of antibiotic use in burn units, and use of invasive devices (9). Burn wounds are sterile in early hours after injury but the wounds are frequently colonized with the circulating bacteria in healthcare environment (10, 11).

A. baumannii has emerged as predominant bacteria in burn patients with most of its strains previously eradicated by carbapenems (12). However, the emergence of CRAB and their presence for prolonged periods in burn units has increased the risk of acquisition of CRAB strains which consequently impede the successful treatment of affected burn patients. In this study, the burn patients were screened for CRAB strains through testing susceptibility to imipenem and its comparators meropenem, and doripenem. Furthermore, the *in vitro* activity of other antibiotics against CRAB was investigated.

METHODS

Materials

The microbiologic media Blood agar base, MacConkey's agar, Hugh and Leifson's medium, Mueller-Hinton agar, and Mueller-Hinton Broth were purchased from Merck (Darmstadt, Germany). Oxidase disk and all antibiotics disks were purchased from Mast Group Ltd (Bootle, UK). API 20NE system was obtained from bioMerieux (Marcyl'Etoile, France). The antibiotic salts or base were procured from Sigma-Aldrich Co. (St Louis, MO, USA).

Setting

The study proposal was approved by the local ethical committee of Tehran University of Medical Sciences. Informed consent was obtained prior to sample collection which was performed according to standard protocols. Motahari Brun Center is a tertiary referral center located in Tehran, Iran, that has intensive care facilities and provides comprehensive care for children and adults suffering from burn injuries. This center houses burn patients who have minor to life-threatening injuries. Initially, all patients are admitted to Emergency Unit (EU) before, depending on the age, gender, and extent/severity of burn, they are directed to different units including Burn Intensive Care Unit (BICU), Pediatrics, Burn 1, and Burn2. In addition, this center has operating rooms designed for burn surgery. When all available beds are occupied, the admitted patients have to stay in the EU. BICU is restricted to critically ill patients suffering from complications of severe burns. Pediatrics unit is designated to children younger than 12 years of age, Burn1 to adult women and Burn2 to adult men.

Motahari Bun Center team members are strictly adhered to infection control and cross-transmission prevention measures and are experienced in the management of burn wounds. For all patients, first aids begin immediately upon admission and patients benefit from initial treatment followed by daily treatment, advanced treatment of complex wounds, and rehabilitation therapy. In this center, the core measures are resuscitation with balanced electrolyte solutions, cleansing and debridement of wound, regular dressing of wound, prescribing of topical mupirocin as well as silver sulfadiazine as prophylactic agents, and showering hydrotherapy.

A total of 1474 burn cases referred to Motahari Brun Center from November 2012 to September 2013 were screened for CRAB. Cases with CRAB were patients from whom a CRAB isolate was recovered at least once; cases with CSAB were patients with carbapenem-susceptible *A. baumannii* (CSAB), the other cases were negative for *A. baumannii*. Demographic characteristics and medical histories were collected from the information recorded in medical files. The obtained data were: age, gender, unit of admission, isolation site, length of stay (LOS) in hospital prior to *A. baumannii* isolation, type of burn injury, percentage of total burn surface area (%TBSA), depth of burn injury, use of invasive devices, and mortality rate during hospitalization.

Isolates and Identification

All clinical samples submitted for microbiological examination were plated onto blood agar and MacConkey's agar and incubated at 37° C. After the growth, colonies suspected for *A. baumannii* were identified through a combination of colony

morphology**,** growth at 44°C, and biochemical tests such as oxidase, dextrose oxidation in Hugh and Leifson's medium, and API 20NE system (bioMerieux, France). Confirmatory identification of the isolates was performed by PCR amplification of *bla*$_{\text{OXA-51-like}}$ gene using specific primers (13).

Antimicrobial Susceptibility Testing

Disk Diffusion

For each clinical isolate of *A. baumannii*, susceptibility to imipenem (10 µg), meropenem (10 µg), and doripenem (10 µg) was determined by disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (14). For all the antibiotic-impregnated paper disks, the concentrations of antibiotics were based on base antibiotic. Imipenem and meropenem susceptibility was determined according to breakpoints defined by CLSI. However, in the case of doripenem, the US Food and Drug Administration (FDA) breakpoints were used (15). CRAB and CSAB were defined as resistance and susceptibility, respectively, to both imipenem and meropenem; strains with intermediate susceptibility were considered as resistant/non-susceptible. The susceptibility of CRAB and CSAB was assessed against other antibiotics include colistin sulphate (25 µg), tigecycline (15 µg), trimethoprim-sulfamethoxazole (25 µg), ceftazidime (30µg), cefepime (30 µg), aztreonam (30 µg), gentamicin (10 µg), piperacillin/tazobactam (110 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), doxycycline (30 µg), gatifloxacin (5 µg), ceftriaxone (30 µg), amikacin (30 µg), tetracycline (30 µg), and cefotaxime (30 µg). Because no tigecycline breakpoints for *A. baumannii* were recommended by the CLSI, the disk diffusion breakpoints of ≥16 and ≤12 mm were considered as susceptible and resistant, respectively (16). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 strains were used as quality control for the susceptibility tests.

Broth Microdilution

Minimum inhibitory concentration (MICs) for imipenem, ceftazidime, gentamicin, and ciprofloxacin were determined by broth microdilution method using Cation-Adjusted Mueller-Hinton Broth (CAMHB). An initial bacterial suspension was prepared by suspending 3-5 single colonies in 0.9% saline equivalent to 0.5 MacFarland standard. The suspension was diluted 1:100 in CAMHB and 100 µl of this suspension was inoculated to each well of microplates. The final concentration of inoculums was 5×10^5 CFU/ml. The inoculated microplates were incubated at 35 ±2°C in ambient air for 18–24 hours. The following antibiotics with two-fold dilutions were tested: imipenem monohydrate (0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 µg/ml), ceftazidime pentahydrate (4, 8, 16, 32, 64, 128, 256, and 512 µg/ml), gentamicin sulphate (4, 8, 16, 32, 64, 128, 256, and 512 µg/ml), and ciprofloxacin base (0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 µg/ml) (14).

Statistical Analysis

Data analysis was performed using SPSS version 18.0 (SPSS Inc., USA). Descriptive results were shown as frequencies, mean, mode, and quantiles. To evaluate the 95% Confidence Interval (CI) of quantiles, Bootstrap method with 5000 samples was used. For comparison of the categorical variables, Chi-square and Fisher's exact tests of non-parametric data were used. For continuous variables, the Mann-Whitney test was used. In CRAB cases, the impact of percentage of TBSA on mortality was estimated in terms of odds ratio (ORs) with 95% CI using a Backward Wald elimination of variables in multivariate logistic regression analysis, in which both factors of age and gender were included. Multiple comparisons between groups were conducted using the Benjamini and Hochberg false discovery rate (FDR) procedure (17). P values of less than 0.05 were considered as significant.

RESULTS

Cases

During the study period, 172 of 1474 burn patients had at least a single positive culture for *A. baumannii*. Isolates were identified as *A. baumannii* by oxidase and nitrate negative but dextrose oxidation positive reactions and growth at 44°C. All of the isolates yielded a 353 base pair amplicon in PCR of *bla*$_{\text{OXA-51-like}}$ gene, confirming the identity of *A. baumannii*. Out of 172 *A. baumannii* infected patients, one case harboring imipenem susceptible, meropenem non-susceptible isolate was excluded from the study. The frequency of burn patients with

CRAB was 168 (98.2%) and only 3 (1.8%) patients infected with CSAB. The demographic characteristics and medical histories of CRAB and CSAB cases are illustrated in Table 1. There was no statistically significant difference in age, gender, unit of admission, isolation site, type of burn injury, percentage of TBSA, and mortality rate between two groups. Most of the CRAB isolates were obtained from patients hospitalized in Burn 2 (n=73, 43.5%), followed by Burn1 (n=35, 20.8%), BICU (n=32, 19%), Pediatrics (n=23, 13.7%), and Emergency (n=5, 3%) units. However, when the CRAB isolates were normalized to the overall screened patients in each unit (Figure 1), the Burn1

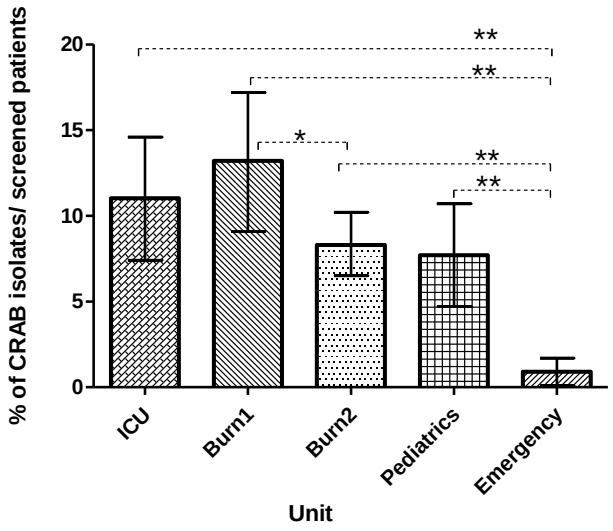

Figure 1. Frequency of carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates in relation to total screened patients of each unit (* $P \leq 0.05$ and ** $P \leq 0.001$). The error bars stand for 95% confidence interval (CIs).

unit had significantly higher proportion of patients with CRAB (13.2%, 95%CI: 9.1-17.2) in comparison to Burn 2 (8.3%; P=0.038, 95%CI: 6.5 - 10.2), Pediatrics (7.7%; P=0.053, 95%CI: 4.7 - 10.7), and Emergency (0.9%; P=0.001, 95%CI: 0.1 to 1.7) units. The number of CRAB cases ranged from 9 to 27 per month during the study period; one and a half time increase in the number of CRAB cases was seen in February 2013 compared to January 2013. The predominant anatomical site of isolation of bacteria in both CRAB and CSAB cases was wound. The mean LOS in hospital prior to isolation of *A. baumannii* was significantly higher for CRAB compared to CSAB cases (*P*=0.04). All three CSAB isolates were recovered from patients hospitalized less than 72 hours but 115 (68.5%) of CRAB isolates were recovered from burn patients hospitalized for longer than 72 hours and this difference was statistically significant (*P*=0.034). Among the patients who were categorized as CRAB cases, 34 died during stay in hospital, of whom 16 were hospitalized in BICU (Figure 2). Thirty one (91.2%) of died patients had more than 30% TBSA whereas 3 (8.8%) had less than 30% TBSA; this difference reached the statistical significance (*P*=0.001). As illustrated in Figure 2, the mean of percentage of TBSA in patients admitted to BICU (58.13 ±20.96) was higher than that of Burn 2 (28.64 ±16.8), Burn 1 (30.06 ±15.88), pediatrics (23.57 ±15.7), and emergency (25.6 ±22.07) units. Multivariate logistic regression showed that amongst the independent variables, the percentage of TBSA significantly affected the mortality rate (P=0.001, OR= 16.5; 95% CI: 4.72-57.7).

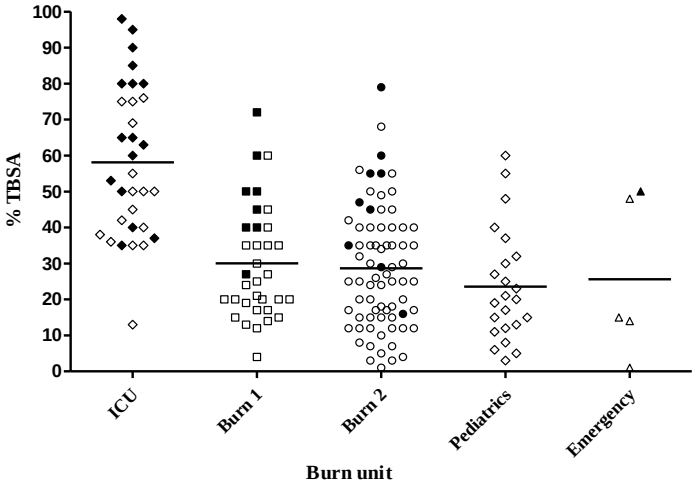

Figure 2. Percentage of total burn surface area (TBSA) in patients with CRAB admitted to Burn Intensive Care Unit (BICU) and non-BICU. The horizontal lines indicate mean values of %TBSA. Filled and unfilled shapes show died and survived patients during stay in hospital, respectively.

Table 1. Age, gender, unit of admission, site of infection, length of stay in hospital (LOS), type of burn injury, percentage of total burn surface area (TBSA), depth of injury, devices or catheters, and mortality rate in burn patients infected by carabapenem-resistant *Acinetobacter baumannii* (CRAB) or carbapenem-susceptible *Acinetobacter baumannii* (CSAB).

Characteristic	**CRAB n=168**	**CSAB n=3**	***P***
Age (years: mean±SD, range)	31.5±18.01, 1-88	18±14.42, 2-30	0.13
Gender (n: male/female)	108/60	3/0	0.55
Burn unit (n: BICU[a]/non-BICU)	32/136	1/2	0.47
Anatomical site or lines (n:wound/blood/ urine/CVP[b]/unknown)	157/7/1/2/1	3/0/0/0/0	-
Hospital length of stay (LOS) prior to isolation of *A. baumannii* (days:mean±SD)	7.2±6.4	2±1	0.04
Type of burn injury –n (%)			
Electrical	15 (8.9)	0	
Thermal	152 (89.9)	3	1
(flash/flame/scalds/contact)	79/37/35/0	1/0/1/1	
% Total burn surface area (TBSA) (mean ± SD/range)	28.3±19.6 (3-80)	10	0.33
Children (n=30)	34.93±21 (1-98)	57±4.2 (54-60)	
Adults (n=141)			
Depth of burn injury			
Second degree	47(28)	1 (33.3)	
Third degree	108 (64.3)	2 (66.7)	-
Fourth degree	12 (7.1)	0	
Unknown	1 (0.6)		
Devices			
Urinary catheter/sond/vein catheter/ ventilator/other	6/113/2/31/3	0/1/0/1/0	-
Mortality, n (%)	34 (20.2)	1 (33.3)	0.37

[a] Burn intensive care unit, [b]Central venous pressure (CVP) line.

Susceptibility of Isolates

The susceptibility of isolates to imipenem, meropenem, and doripenem and antibiotype profiles are indicated in Table 2. The majority of tested isolates were resistant to imipenem (94.8%) and its comparators doripenem (97.7%) and meropenem (97.7%). The most frequent antibiotype profile was RRR (94.2%) which represent the resistance to imipenem, meropenem, and doripenem, respectively. As indicated in Table 3, susceptibility of CRAB isolates was less than 10% to all tested antibiotics except for colistin (100%), doxycycline (61.9%), gentamicin (18.5%), and tigecycline (11.9%). One hundred sixty five (98.8%) of CRAB isolates were resistant to both ciprofloxacin and levofloxacin, and only 2 (1.2%) of ciprofloxacin-resistant isolates showed intermediate susceptibility to levofloxacin. Among 94 tetracycline-resistant CRAB isolates, 11 (11.7%) and 36 (38.3%) were susceptible to tigecycline and doxycycline, respectively. In addition, 52 out of 82 (63.4%) tigecycline resistant CRAB isolates were susceptible to doxycycline.

The MIC ranges, MIC_{50}, and MIC_{90} of imipenem, ceftazidime, gentamicin, and ciprofloxacin against CRAB isolates are shown in Table 4.

Table 2. Susceptibility and antibiotype profile of 172 non-repetitive isolates of *Acinetobacter baumannii* to imipenem, meropenem, and doripenem using disk diffusion method based on agency breakpoint

Antibiotic	Susceptible n (%)		Intermediate n (%)		Resistant n (%)		Antibiotype profile[a] / n (%)
	CLSI[b]	FDA[c]	CLSI	FDA	CLSI	FDA	RRR/ 162 (94.2)
Doripenem[b]	-	4 (2.3)	-	-	-	168 (97.7)	SSS/3 (1.7) IRR/ 4 (2.3)
Imipenem	4 (2.3)		5 (2.9)		163 (94.8)		IRS / 1 (0.6)
Meropenem	3 (1.7)		1 (0.6)		168 (97.7)		RIR / 1 (0.6) SRR/ 1(0.6)

[a] The antibiotype profile is the susceptibility results for imipenem, meropenem , and doripenem, respectively. R, I, and S indicate resistant, intermediate, and susceptible respectively. [b] CLSI : Clinical and Laboratory Standards Institute. [c] FDA: The US Food and Drug Administration.

DISCUSSION

Infection, especially with resistant bacteria is a major challenge in the patients suffering from burn. The bacteria significantly contributed to morbidity and mortality of burn patients, *Acinetobacter baumannii* is an emerging opportunistic pathogen which is rapidly developing resistance to a wide range of antibiotics (18). Carbapenems were used as drug of choice for treating of *Acinetobacter*-associated infections but their therapeutic effect is compromised by the emergence of CRAB (19). CRABs are considered as an emerging threat in healthcare setting particularly in acute and long-term care facilities. As described by the International Network for the Study and Prevention of Emerging Antimicrobial Resistance, the emergence of carbapenem resistance in *Acinetobacter* is a global sentry event that demands microbiological and epidemiological interventions (20). Active surveillance of multi-resistant microorganisms such as CRAB has been recommended in high risk populations including patients of burn units (21).

To screen CRABs in burn patients admitted to the referral burn center, we conducted a cross-sectional study in all burn units, comprising BICU and non-BICU. Culture of specimens for CRAB showed that one tenth of burn patients harboring CRAB. The high rate of CRAB may be related in part to the extensive use of antibiotics in Iran and to the selection of resistant variants by antibiotic pressure. In addition, we found that only very small number of *A. baumannii* was susceptible to both imipenem and meropenem; categorized as CSAB. This result is consistent with a study that reported 98% carbapenem resistance in *A. baumannii* isolated from 43 burn patients in Tehran (22). However, higher rate of resistance to imipenem were obtained in our study compared to a report in 23 burn patients showing 48.9% of non-susceptibility to imipenem (23). This variation may be attributed to small number of isolates in the latter study.

This study provided evidence that infection with CRAB continuously occurred during an eleven-month period but two rises in rates of CRAB were seen in February and April 2013, followed by a decreasing trend in summer months. The point raises in the number of CRAB may be due to the failure in infection control measures and cross-transmission between patients. We found that burn wound infection is the main complication in burn patients with CRAB and CSAB. Wounds are more prone to infections and are favorable sites for colonization of bacteria until they are closed. Therefore, routine wound cultures must be done as early as patients admitted to burn units. The mean LOS in the hospital prior to isolation of *A. baumannii* was significantly higher for CRAB compared to CSAB cases. This finding should be interpreted with caution due to small number of

Table 3. Susceptibility of isolates which categorized to carbapenem-resistant *Acinetobacter baumannii* (CRAB) and carbapenem-susceptible *Acinetobacter baumannii* (CSAB) against various classes of antibiotics.

Antibiotics	**CRAB n (%)**	**CSAB n (%)**
Aminoglycosides		
amikacin	1 (0.6)	3 (100)
gentamicin	31 (18.5)	3 (100)
Cephems		
cefepime	1 (0.6)	3 (100)
cefotaxime	0 (0)	0 (0)
ceftazidime	1 (0.6)	2 (66.7)
ceftriaxone	0 (0)	0 (0)
Monobactams		
aztreonam	0 (0)	0 (0)
β-Lactam/β-lactamase inhibitor combinations		
piperacillin/tazobactam	0 (0)	2 (66.7)
Tetracyclines		
tetracycline	12 (7.1)	3 (100)
doxycycline	104 (61.9)	3 (100)
tigecycline	20 (11.9)	3 (100)
Fluoroquinolones		
ciprofloxacin	0 (0)	3 (100)
levofloxacin	1 (0.6)	3 (100)
gatifloxacin	2 (1.2)	3 (100)
Folate pathway inhibitors		
trimethoprim-sulfamethoxazole	0 (0)	2 (66.7)
Lipopeptides		
colistin	168 (100)	3 (100)

Table 4. Minimum inhibitory concentration (MICs) ranges, MIC_{50}, MIC_{90}, and mode of tested antibiotics against Carbapenem-Resistant *Acinetobacter baumannii* (CRAB) isolates (n=168) using broth microdilution method

Antibiotic	Solvent	Diluent	MIC_{50} (95% CI[a])	MIC_{90} (95% CI)	Mode	Range [b]
Imipenem monohydrate	Phosphate buffer, pH 7.2, 0.01 mol/L	Phosphate buffer, pH 7.2, 0.01 mol/L	64 (8 to 64)	64 (8 to 64)	64	8-64
Ceftazidime pentahydrate	Sodium carbonated	Water	512 (512 to 512)	512 (512 to 512)	512	4-512
Gentamicin sulphate	Water	Water	256 (16 to 512)	512 (512 to 512)	512	4-512
Ciprofloxacin base	Water	Water	128 (128 to 256)	512 (512 to 512)	512	2-512

[a] CI: confidence interval. [b] Range indicates the minimum and maximum observed value of MIC among the 168 isolates which defined as CRAB.

CSAB cases and case control studies with larger sample size need to be designed to understand the effect of LOS on acquisition of CRAB. All three CSABs were recovered from patients hospitalized less than 72 hours whereas more than half of CRAB were isolated from patients hospitalized more than 72 hours; showing the probability of acquiring the infection in burn units of hospital. The results showed that the number of died cases increased with increasing the percentage of TBSA. The majority of died patients were suffering from TBSA of more than 30% and half of the deaths occurred in BICU. This death rate seems to be related to the status of patients admitted to such unit; mainly to the percentage of TBSA. While TBSA was more than 30%, the risk of mortality increased to more than 16-fold; indicating that the percentage of TBSA is a highly significant determinant which affects the mortality rate. The severity of burn and the requirement for long term care facilities may explain the high mortality rate in patients infected with CRAB. However, the associations between harboring CRAB and mortality rate is difficult to establish owing to other factors affecting the mortality rate such as percentage of TBSA. Infection with CRAB and having TBSA greater than 30% may represent a significant risk factor for transmission of CRAB to care facilities and to other patients.

The effect of three carbapenems was comparatively studied against *A. baumannii* isolates and all three carbapenems showed weak activity against the isolates. Doripenem and meropenem were equally active against isolates but imipenem showed a very slightly greater activity than its comparators. This study showed that CRAB isolates were resistant to all tested cephems as well as fluoroquinolones, monobactams, and folate pathway inhibitors. Amongst the fluoroquinolones, ciprofloxacin is available in Iran and used for treatment of a wide variety of infections, whereas levofloxacin and gatifloxacin are not available as easy as ciprofloxacin and rarely administered. The high resistance rate to levofloxacin and gatifloxacin may be associated with overuse of ciprofloxacin and cross-resistance. In addition, piperacillin combined with tazobactam was not effective against CRAB. This finding highlights the low *in vitro* activity of tazobactam as a β-lactamase inhibitor against CRAB. Colistin retained the most activity against CRAB isolates followed by doxcycline, gentamicin, and tigecycline. The excellent *in vitro* activity of colistin has been demonstrated previously by several studies but its systemic use is limited due to the adverse effects. The most common adverse effects of colistin therapy are nephrotoxicity, neuromuscular blockade, and neurotoxicity and the less common effects are hypersensitivity reactions, skin rash, urticaria, generalized itching, fever, and mild gastrointestinal disorders (24-27). Given the high resistance rate of CRAB to almost all classic antibiotics, colistin remains as the last resort antibiotic for treatment of CRAB associated infections (28). To avoid the systemic side effects, colistin might be used topically as a cream that is evenly distributed over the burn area. Furthermore, colistin should be used in combination with other antibiotics to reduce the probability of selection of resistant variants during treatment (29). Use of colistin alone or in combination need to be guided by the results of clinical trials and animal models studies. Previous studies showed that tigecycline is an effective antibiotic against Gram-negative bacteria such as tetracycline-resistant strains (30, 31). For *A. baumannii*, no standard susceptibility breakpoint of tigecycline is recommended by the CLSI but based on the study of Jones *et al.* (16), we have found that while most of the CRAB isolates were resistant to tigecycline, they remained susceptible to doxycycline. These data indicate that emerging resistance to tigecycline has been occurred in these isolates, despite that tigecycline is not routinely applied for treatment of infections in our community. On the other hand, doxycycline was found to be active against CRAB, suggesting a potentially valuable antibiotic for treatment of infections caused by CRAB.

In the present study, the MIC_{50} and MIC_{90} values for imipenem and ceftazidime were similar while the MICs of ciprofloxacin and gentamicin that inhibited the growth of 90% (MIC_{90}) of isolates was higher than their MIC_{50}. Considering the breakpoints recommended by the CLSI (2012), an isolate with the MIC value $\geq$ 16 μg/mL was categorized as imipenem resistant. More than 90% of isolates tested here, had MIC of 64 μg/mL. A significantly higher proportion of the isolates were inhibited at MIC values of 64 μg/ ml of imipenem. The considerably high MICs for imipenem are of prime importance, as the imipenem or its comparators are the last-line antibiotics for the

treatment of *A. baumannii*-associated infections. The MIC values of imipenem and ciprofloxacin against the isolates in this study was also similar to those previously reported (32-35). These results indicate that the MIC values of tested antibiotics are high, thereby, indicating that most of CRAB might be also multidrug resistant.

CONCLUSION AND RECOMMENDATION

The current study depicts the presence of high number of carbapenem-resistant *A. baumannii* in burn units. The resistance to carbapenem reduces the number of effective antibiotics. CRAB associated infection is a great concern in burn units particularly in patients with higher percentage total burn surface area and a challenge for physicians in the treatment and control of such infections. For patients suffering from infections caused by CRAB strains, the available therapeutic options are limited. The eradication of CRAB isolates may not be feasible in burn units. Hence, coordinated and intensive efforts of healthcare personnel are required for management of CRAB. Early detection of CRAB at the admission time, monitoring for CRAB during hospitalization, and testing the susceptibility of CRAB to other antibiotics need to be routinely performed . These surveillance based information provide a guide for clinical therapy decisions and may help clinicians to restrict the use of carbapenems in high risk patients harboring CRAB, to cautiously prescribe the antibiotics, and to adopt infection control measures in order to lessen the transmission of CRAB in burn units.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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