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Full Length Research Paper

Acinetobacter baumannii complex and its antibiotics susceptibility in selected hospital's intensive care units at Sana'a city-Yemen

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Background and Aim: The main challenge with *Acinetobacter baumannii* complex (Abc) is its ability to rapidly cause antimicrobial-resistance and ineffective antibiotic therapy. This study is therefore designed to investigate the prevalence rates, infection risk factors and antibiotic susceptibility patterns of Abc in ICUs. Methods: This Cross-Sectional study was carried out for duration of one year in the intensive care units (ICU) units of three hospitals in Sana`a city, Yemen with 280 ICU patients as participants and 80 environmental samples. Results: The prevalence rate of Abc of 7.1% was determined for 280 individual patients admitted in ICU, with 12.9 % in respiratory specimens and 1.4% in blood specimens. All isolated Abc`s became multi-drug resistant over time. Resistance rates were found to be 95% for Piperacillin-Tazobactam, 90% for Imipenem, Cefepime and Ticracillin, 80 % for Amikacin and Doxcycyclin, and 60% for Levofloxacin. However, no resistant isolates were found for Colistin and polymyxin B. The prevalence of Abc in ICUs environment was 8.8% and these isolates were indistinguishable from the clinical isolates using biotyping and antibiotics susceptibilities. Conclusions: Based on its characteristic multi-drug resistance and potential risk to ICU patients, suggestions were offered to prevent nosocomial infections by Abc.

Keywords: *Acinetobacter baumannii,* Intensive Care Units, antibiotics susceptibility*, Yemen.*

INTRODUCTION

Acinetobacter baumannii complex (Abc) is an important nosocomial pathogen responsible for the prevalence of several kinds of bacterial diseases among admitted hospital patients (Founier et al., 2006). Clinically, Abc`s is the most important species of *Acinetobacter* genus because it is accountable for more than 90% of

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Acinetobacter related infections that include septicemia, ventilator-associated pneumonia, and surgical site infections (Nemec et al., 2011). Nosocomial infections play a critical part in the rising rates of diseases and mortality in hospitals especially in the intensive care units (ICUs) (Vincent., 2003). ICUs are harbors for critically ill patients who are particularly susceptible to infections, thus providing a haven for invasive microorganisms (Dijkshoorn et al., 2007). Abc has garnered attention as a microorganism significantly involved in numerous nosocomial infections and a number of hospital pandemics particularly in the ICUs (Munoz-Price and Weinstein. 2008). However, such outbreaks are dependent on the severity of causal diseases, the rate of invasive mediations and the recurrent practice of wide-spectrum antibiotics medication.

In addition, the widespread practice of antimicrobial chemotherapy in hospitals has largely contributed to the advent and rise in the number of Abc strains that are resistant to a broad array of antibiotics, comprising broad-spectrum β-lactams, aminoglycosides and fluoroquinolones (Cetin et al., 2009). The indiscriminate use of this chemotherapy enhances Abc high adaptability to the selective pressure from wide-array antimicrobial agents resulting in its resistance to multiple antimicrobial agents (Jawad et al., 1996). In addition, complexities involved in controlling Abc nosocomial infections and epidemics is also credited to the ability of these bacteria to persist in hospital surroundings (Founier et al., 2006). Moreover, Abc has the ability to cultivate at different temperatures and pH conditions and it could be found in humid or dry conditions. These properties support the transmission of Abc between patients, either through human reservoirs or inorganic materials (Zarrilli et al., 2007).

Several researches reported the incidence of diverse species of *Acinetobacter* in hospitals environments relying on the epidemiological setting and hygiene levels (Rafei et al., 2014). For example, the role of the immediate bed surroundings in the propagation of *Acinetobacter* spp. was evident in a large outbreak entailing 63 of 103 patients admitted to a burns unit over a duration of 21-month (Sherertz and Sullivan., 1985). The incidence was attributed to mattresses infection through openings in plastic covers that allowed water permeation and persistence of the organism in the damp mattresses. Pillows filled with feathers were also found to be infected with substantial amounts of *Acinetobacter* in an epidemic in the Netherlands (Weernink et al., 1995).

Furthermore, it has been detailed that Abc can continue to exist on dry surfaces for durations exceeding that reported for *Staphylococcus aureus, Escherichia coli* and *Pseudomonas* spp (Musa et al., 1990). In addition, environmental contagion during an outbreak in a pediatric ICU was demonstrated on a range of equipment and numerous surfaces such as telephone handles, door push plates, patient charts and tabletops, all of which were most likely contaminated by the staff hands (Getchell-White et al., 1989).

Therefore, this is the first study in Yemen intended to assess the pervasiveness of Abc in ICU's clinical and environmental samples, and to resolve the antibiotics susceptibility patterns of isolates, in addition to analyzing the risk factors which contributed to nosocomial infections.

MATERIALS AND METHODS

Study design

This descriptive analytical cross sectional study involved ICUs in three hospitals, two public hospitals (Al-Thawra and Al-Kuwait) and one private hospital (University of Science and Technology Hospital) in Sana'a city, Yemen. This study was carried out from October, 2012 to September, 2013. All kinds of ICUs in the selected hospitals were included in this study with the exception of the neonatal ICUs. This study was endorsed by the ethics committee at Sana`a University, Yemen and informed consent was obtained from participants prior to being enrolled in this study.

The sample size

The sample size was calculated in Epi info version 6 taking into account the anticipated frequency of the Abc among ICUs patients which was reported to be 9% in previous studies (Corbella *et al*., 2000 and Falagas *et al*., 2008). Sample size of 280 individuals was selected with confidence level of 99.9%. On the other hand, the expected frequency of the Abc in the ICUs environment was 20% (Choi *et al*., 2010). Therefore, the sample size was 80 and the entire samples enrolled in this study were 360.

Data collection

Data was acquired from each subject and collated in a predesigned questionnaire which contains demographic information and risk factors relevant to the Abc infection.

Specimen types and sampling

A. Sample from human subjects

Only patients who were admitted for duration exceeding 48 hours in the ICUs were admitted in this study based on the National Nosocomial Infection Surveillance System (NNIS) criteria. The respiratory and blood specimens were obtained under aseptic technique and the respiratory aspirates were taken after physiotherapy and prior to bronchial washing. The blood specimens were collected in

commercial aerobic blood culture bottles (bio Merieux, France).

B. Environmental samples

Sterile moist swabs were collected from pillows, ventilators, tracheal tubes and central line catheters.

C. Type of used media

Different types of media were used in this study to isolate and identify *Acinetobacter* species. These media comprise Leeds *Acinetobacter* medium, Nutrient broth, MacConkey Agar, and Muller Hinton Agar (Hi Media, India). Leeds *Acinetobacter* agar base, which is suggested for isolation of *Acinetobacter* species, is composed of 15.00 g/L casein acid hydrolysate, 5.0 g/L soya peptone, 5.0 g/L sodium chloride, fructose 5.0 g/L, sucrose 5.0 g/L, 5.0 g/L mannitol, 1.0 g/L phenylalanine, 0.4 g/L ferric ammonium citrate, 0.02 g/L phenol red, 12.0 g/L agar.

D. Identification

All collected specimens were directly inoculated on Leeds *Acinetobacter* medium and MacConkey agar, and afterward incubated overnight (Memmert, German) under aerobic condition at 37 °C. A pure colony of pure culture were smeared thinly onto a glass slide, fixed over a gentle flame and stained in line with the manufacturer's and standard procedures. The smear on the glass slide was then analyzed microscopically. The colony was identified using Gram stain and biochemical tests that include oxidase test, catalase test, kligler's iron agar (KIA), sulfide indole motility (SIM), citrate agar test and urea test (Eliopoulos et al., 2008 and Peleg et al., 2008) .

The Gram stain was supplied from Techno pharma chem, India. KIA and SIM reagents for citrate, urease and oxidase and catalase tests were supplied by (Hi Media, India). Supplies of API-20NE test kit for Non-*Enterobacteriaceae* bacteria and its reagents were acquired from BioMerieux, France. The API-20NE system was used for the ultimate biochemical detection of all clinical and environmental isolates. The antibiotic discs used in antimicrobial susceptibility test were purchased from Hi Media, India.

E. Confirmation of Acinetobacter baumannii complex

The Analytical Profile Index (API20-NE) and heat tolerance were used to verify the isolates as Abc. Temperature tolerance involved inoculating the isolates on Leeds Acinetobacter agar and incubate at (44 ^{o}C) aerobically overnight in a static incubator.

F. Antibiotics susceptibility test

Disc diffusion testing was carried out on the isolates according to Clinical Laboratory Standard Institute (CLSI, M100 2007) guidelines. Mueller-Hinton (MH) agar was employed as the test medium as suggested by CLSI. A single pure colony from an overnight culture was inoculated into 3 mL of nutrient broth (Hi Medi, India) and mixed thoroughly. The inoculum size was standardized by comparing the turbidity of the bacterial suspension to that of a 0.5 McFarland Standard. A sterile cotton-tipped swab dipped into the standardized suspension was used to spread the bacterial suspension over the entire agar plate surface. The inoculum was allowed to dry before the appropriate antibiotic discs were placed on the agar. The plate was subjected to incubation at 37°C overnight under aerobic conditions. The zone diameter of inhibition growth was measured and the susceptibility interpreted as susceptible, intermediate or resistant using the CLSI guidelines. The analyzed antibiotics comprise Imipenem, Cefipime, Piperacillin/tazobactam, Amikacin, Doxycycline, Polymyxin B, Levofloxacin, Ticracillin and Colistin which were obtained from (Hi Media, India).

Statistical analysis

The collated data were analyzed using Statistical Package for Social Sciences (SPSS) version 20 and chi-square (χ^2) test was used for comparative analysis between two variables in order to determine the *p* value and odds ratio (OR) was used with 95% confidence interval. *P* values <0.05 was considered statistically significant.

RESULTS

Isolates characterization

In this study, out of the 360 samples, 280 samples were obtained from patients, while 80 samples were acquired from the ICUs environments of a selection of hospitals in order to study the prevalence of Abc in ICUs patients and environments.

The 280 patient's samples were classified according to sex (160 males and 120 females), of whom 24 (approximately 8%) were in age group <19 years old, 78 (28 %) in age group 19-39 years, 140 (50%) in age group 40-69 years and 38 (14%) in age group >70 years. The mean age was calculated to be 47.9 years. The prevalence rate of Abc among patients was 7.1% (8.3% among females and 6.25% among males) (Table 1). As for the age groups, the highest rate was 10.5% in the age group >69 years, followed by 7.7% in age group 19-39 years and

Table 1: Prevalence and associated odds ratio of *Acinetobacter baumannii* complex among ICUs patients in selected hospitals

Characters	Number	+ve culture No.	%	OR	CI	χ^2	*P*-value
Sex							
Males	160	10	6.25	0.73	(0.29-1.8)		
Females	120	10	8.33	1.3	(0.54-3.4)	0.45	0.64
Age groups/years							
<19	24	0	0	0	(0-5.9)	2.02	0.15
19-39	78	6	7.7	1.12	(0.4-3.3)	0.05	0.82
40-69	140	10	7.1	1.00	(0.4-2.7)	0.0	1.00
>69	38	4	10.5	1.7	(0.44-5.7)	0.76	0.38
Type of specimen							
Respiratory	140	18	12.9				
Blood	140	2	1.4				
Total		20	7.1				

OR; Odds ratio> 1(at risk), CI; Confidence intervals 95%, χ^2Chi-square ≥ 3.84 and p< 0.05(significant)

7.1% in age group 40-69 years old. The differences in age and sex groupings were not statistically significance (p= 0.64). Respiratory and blood specimens showed 12.9% and 1.4% prevalence rate, respectively.

Isolation and identification of Acinetobacter

Gram negative coccobacilli were recognized on Leeds *Acinetobacter* medium and MacConkey agar based on the results of conventional and API20 NE biochemical tests comprising oxidase, SIM, citrate, and urease tests. It was noted that the identified bacteria was grown at 37 °C and 44 °C. The results of biochemical tests of isolated Abc on API20 NE are shown in table 2. The prevalence rate of Abc was 44.4% among patients who were admitted in ICUs between 15-30 days posed a significant risk (OR = 16.7) with p<0.001, followed by 10% of patients who stayed for duration of 7-14 days (OR 1.63) at p=0.33. For patients who were admitted for under 7 days, the prevalence rate was determined to be 3% with OR = 0.15 and p<0.001. However, only 4 patients among those admitted for a duration exceeding 30 days ultimately showed no isolates (Table 3).

The highest rate of association between the management procedures in ICUs and contracting of *A. baumannii* was found to be 15.2% among patients who had tracheal tubes (p<0.001), and 9% among patients who were subjected to mechanical ventilation (p<0.01). Rates of 10.3% and 8.3% were detected among patients who had central venous catheter and nasogastric tube, respectively. The estimated risk in these two rates attained double fold of other rates as shown in table 4. In addition, the prevalence rate of Abc was 13.1% for patients who had undergone three invasive procedures (p<0.05), and 6.0% and 4.3% in cases of patients subjected to one and two invasive procedures, respectively, although with no statistical significance (Table 5).

The prevalence rates of Abc in patients affected with underlying malignancy, pulmonary or central nervous system diseases were 25%, 16.7% and 13.3%, with significant risks of 5.2, 3.7 and 3.5, respectively (p< 0.01). On the other hand, patients suffering from diabetes mellitus or renal diseases exhibited prevalence rates of 15.4% and 11.1% with estimated risks of 2.7 and 1.8, respectively. The prevalence rates of 7.9% and 6.9% were determined for patients with cardiac diseases and surgery, although with no significant risks and statistically significance. Finally, there was no isolate in patients who had GIT diseases (Table 6). Mortality rate of 17.9% was determined for patients with Abc with a significant risk of 4.7 (p<0.001) (Table 7).

Antibiotic susceptibility

The antibiotic susceptibility rates of 20 isolates of *A. baumannii* were 100% to Polymyxin B and colistin, approximately 20% to Levofloxacin, Amikacin and Doxycyclin, 10% to Imepenem and Cefipime and 5% to Pipracillin-Tazobactam. Finally, about 20% of the isolates were moderately sensitive to Levofloxacin and 10% to Ticracillin. One the other hand, 95% of the isolates were resistant to Pipracillin-Tazobactam; 90% to Ticracillin, Imepenem and Cefipime; 80% to Amikacin and Doxcycyclin, and finally 60% to Levofloxacin (Table 8). The prevalence rate of Abc in 80 analyzed samples was 8.8%. The isolation rates were 15% for pillows, 10% for tracheal tubes, 5% for ventilator and 5% for central venous catheter samples.

Table 2: Biochemical characteristics of isolated *Acinetobacter baumannii complex* on API20 NE

Tests	Active ingredients	Reaction/Enzyme	Isolates Number (%)
NO_3	potassium nitrate	Reduction of nitrates	27 (100%) Negative
TRP	L-tryptophane	Indole production	27 (100%)Negative
GLU	D-glucose	Fermentation	27 (100%) Negative
ADH	L-arginine	Arginine DiHydrolase	27 (100%) Negative
URE	Urea	UREase	27 (100%) Negative
ESC	Esculin ferric citrate	Hydrolysis(β-glucosidase)	27 (100%) Negative
GEL	Gelatin (bovine origin)	hydrolysis (protease)	27 (100%) Negative
PNPG	4-nitrophenyl-β-D galactopyranoside	β-galactosidase	27 (100%) Negative
GLU	D-glucose	Assimilation	17 (63%) Positive
ARA	L-arabinose	Assimilation	24 (89%) Positive
MNE	D-mannose	Assimilation	22 (81%) Negative
MAN	D-mannitol	Assimilation	27 (100%) Negative
NAG	N-acetyl-glucosamine	Assimilation	27 (100%) Negative
MAL	D-maltose	Assimilation	27 (100%) Negative
GNT	potassium gluconate	Assimilation	16 (59%) Positive
CAP	capric acid	Assimilation	27 (100%) Negative
ADI	adipic acid	Assimilation	27 (100%) Negative
MLT	malic acid	Assimilation	27 (100%) Negative
CIT	trisodium citrate	Assimilation	24 (89%) Positive
PAC	phenylacetic acid	Assimilation	27 (100%) Negative
OX	oxidase test	Cytochrome oxidase	27 (100%) Negative

(n=27)

Table 3: Association between the length of stay for ICU patients and contracting of *Acinetobacter baumannii* complex

Duration (Days)	Number	+ve culture No.	%	OR	CI	χ^2	*P-value*
< 7	198	6	3.03	0.15	(0.05-0.44)	17.2	*p*<0.001
7-14	60	6	10.0	1.63	(0.5-4.8)	0.94	0.33
15-30	18	8	44.4	16.7	(4.9-57.3)	40.3	*p*<0.001
>30	4	0	0	0.0	(0-21)	0.31	*p*=0.57

OR; Odds ratio> 1(at risk), CI; Confidence intervals 95%, χ^2 Chi-square ≥ 3.84, *p*< 0.05 (significant).

Table 4: Association between types of management procedure applied on ICUs patients and contracting of *Acinetobacter baumannii* complex

Procedures	Number	+ve culture No.	%	OR	CI	χ^2	*p-value*
Mechanical ventilation	222	20	9	1.28	(1.20-1.37)	5.6	*p*<0.01
Central venous catheter	116	12	10.3	2.3	(0.83-6.3)	3.1	*p*=0.08
Tracheal tube	118	18	15.2	14.4	(3.12-91.1)	20.2	*p*<0.001
Nasogastric tube	216	18	8.3	2.8	(0.6-12.1)	2.02	*p*=0.26

OR; Odds ratio> 1(at risk), CI Confidence intervals 95 %, χ^2 Chi-square ≥ 3.84 and *p*< 0.05 (significant

Table 5: Association between number of invasive procedures applied on ICUs patients and contracting of *Acinetobacter baumannii* complex

Number of invasive procedures	Number	+ve culture No.	%	OR	CI	χ^2	*p-value*
One invasive	138	6	4.3	0.42	(0.14-1.2)	3.2	*p*=0.07
Two invasive	66	4	6.0	0.0	(0.22-2.7)	0.15	*p*=0.69
Three invasive	76	10	13.1	2.94	(1.1-8.1)	5.7	*p*<0.05

OR; Odds ratio> 1(at risk), CI Confidence intervals 95%, χ^2 ; Chi-square ≥ 3.84 and *p*< 0.05 (significant).

Table 6: Association between underlying diseases among ICUs patients and contracting of *Acinetobacter baumannii* complex

Underlying diseases	Number	+ve culture No.	%	OR	CI	χ^2	*p-value*
Malignancy	16	4	25	5.2	(1.24-20.2)	8.2	*p*<0.001
Pulmonary	48	8	16.7	3.7	(1.27-10.4)	7.9	*p*<0.001
CNS	90	12	13.3	3.5	(1.37-8.9)	7.6	0.001
D.M	26	4	15.4	2.7	(0.7-9.7)	2.9	*p*=0.08
Renal	36	4	11.1	1.8	(0.5-6.2)	0.98	*p*=0.32
Cardiac	76	6	7.9	1.2	(0.4-3.4)	0.09	*p*=0.76
Surgery	58	4	6.9	0.95	(0.3-3.2)	10.0	*p*=0.93
GIT	28	0	0	0	(0-0.9)	5.6	*p*<0.01

OR; Odds ratio> 1(at risk), CI; Confidence intervals 95 %, χ^2; Chi-square ≥ 3.84 and *p*< 0.05 (significant)

Table 7: Association between the ICUs patient's outcomes and contracting of *Acinetobacter baumannii complex*

Outcome	Number	+ve culture No.	%	OR	CI	χ^2	*p-value*
Recovery	224	10	4.5	0.21	(0.1-0.6)	12.1	*p*<0.001
Death	56	10	17.9	4.7	(1.7-12.9)		

OR; Odds ratio> 1(at risk), CI Confidence intervals 95%, χ^2; Chi-square ≥ 3.84 and *p*< 0.05 (significant)

Table 8: Percentage of antibiotics susceptibility pattern of the 20 isolates of *Acinetobacter baumannii complex* from ICUs patients

Antibiotics	S (%)	I (%)	R (%)
Pipracillin-Tazobactam	5	0	95
Levofloxacin	20	20	60
Imepenem	10	0	90
Amikacin	20	0	80
Doxycyclin	20	0	80
Colistin	100	0	0
Polymyxin B	100	0	0
Ticracillin	0	10	90
Cefepime	10	0	90

S; sensitive, I; intermediate and R; resistant

Table 9: The antibiotics susceptibility rates of the 7 isolates of *Acinetobacter baumannii* complex from ICUs environmental samples in the selected hospitals

Antibiotics	S %	I %	R %
Pipracillin-Tazobactam	14.3	14.3	71.4
Levofloxacin	42.9	42.9	14.3
Imepenem	28.6	0	71.4
Amikacin	28.6	0	71.4
Doxycyclin	42.9	0	57.1
Colistin	100	0	0
Polymyxin B	100	0	0
Ticracillin	14.3	0	85.7
Cefepime	0	14.3	85.7

S sensitive,**I** intermediate,**R** resistant

The rate of susceptibility of *Acinetobacter baumannii complex* from ICUs environmental samples were 100% to Colistin and Polymyxin B, 42.9% to Levofloxacin and Docxycyclin. 28.6% to Imepenem and Amikacin. Finally, 14.3% were sensitive to Pipracilin-Tazobactam and Ticracillin. Additionally, the isolates exhibited 42.9% intermediate sensitivity to Levofloxacin, 14.3% to Pipracilin-Tazobactam and Cefepime. Furthermore, 85.7% of isolates were resistant to Ticracillin and Cefepime, 71.4% to Pipracilin-Tazobactam, Imepenem and Amikacin, 57.1% to Doxcycyclin and 14.3% to Levofloxacin (Table 9).

DISCUSSION

Acinetobacter baumannii complex (Abc) is a key nosocomial pathogen that generally causes infections associated respiratory, blood, and surgical health issues (Roca et al., 2012). This organism quickly acquires antimicrobials resistance genes. Abc with multi-drug resistant (MDR) is generally associated with significant morbidity and mortality outcome as a result of the intricacy of its treatment (Chua and Alejandria., 2008). However, there has been no published data on the prevalence of *Acinetobacter* and its antimicrobials susceptibility patterns in Yemen. Therefore, this study is regarded as the first to determine the prevalence and antibiotics susceptibility patterns of Abc in ICUs of selected hospitals in Sana'a city, Yemen. The prevalence rate of Abc among ICUs patients recorded in this study is 7.1%, which is consistent with similar studies performed in Spain (8%) (Corbella et al., 2000) and India (9.6 %) (Joshi et al., 2006), although, this measured rate is relatively higher compared to that of Philippines (5%) (Chua and Alejandria., 2008). On the other hand, higher prevalence rates were reported in Nigeria (14 %) (Ugochukwu et al., 2013) and Iran (17%) (Yadegarinia et al., 2013).

More specifically, the prevalence rates of Abc in respiratory and blood specimens were determined to be 12.9 and 1.4%, respectively. However, preceding studies in Greece (41 studies) reported comparably different prevalence rates of *A. baumannii* ICU-acquired pneumonia and/or bacteremia with significant disparity between different countries (Asian range 4-44%, European countries (0-35%) and United States (6-11%) (Falagas et al., 2008). However, significant correlations with age were only found in the age group >69 years, where there was a significant risk without a statistical significance. The present results were consistent with the results of related studies carried out in Turkey (Alp et al., 2009) and Philippines (Chua and Alejandria., 2008). This result is also similar to that of a study conducted in Taiwan (Kuo et al., 2012). Where it was found that Abc is significantly associated with age group of 65 years and older. As regards sex, this study discovered that Abc was higher in females compared to males with percentages of 8.3 % *versus* 6.3%, although without statistical significance. This result is in agreement with a study on isolated *Acinetobacter* in ICUs patients carried out in the Philippines, which also found no statistical significance in relation to the sex (Chua and Alejandria., 2008).

This study showed the highest prevalence of Abc in patients who were admitted in ICUs for a period of 15-30 days (p<0.001), whereas no statistical significance was observed in patients admitted in ICUs between 7-14 days. Conversely, statistical significant low rate of 3.0% was determined for patients admitted in ICUs for less than 7 days. These results contradict studies that found no significant risk associated with the duration of stay in ICU in South Korea and Turkey (Jung et al., 2010 and Dent et al., 2010). This disparity in opinion may be attributable to the fact that the above mentioned studies focused on the duration of admission in terms of days, while this study divided the duration of admission into four duration groups that made the relation between duration of admission and prevalence of Abc more clearly significant by a long stay. In this study, no isolate was obtained from patient admitted for a duration exceeding 30 day because the sample size of this group was only 4 patients.

Concerning the invasive procedures, a significant risk and a statistical significance was observed in patients with tracheal tubes. Patients with central venous catheter and nasogastric tube also exhibited a significant risk, but without a statistical significance. These findings are consistent with a study in South Korea that reported a significant risk with a statistical significance for patients with tracheal tube and central venous catheter, but without a statistical significance for those with nasogastric tube (Jung et al., 2010). An additional study carried out in Turkey similarly showed, to some extent, a significant correlation with the central venous catheter, while no correlation was found in cases of tracheal tube (Alp et al., 2009). As regards the mechanical ventilation, the prevalence rate of Abc was found to be 9.0% with a statistical significance. These findings are also consistent with a study carried out in USA that similarly reported a significant relationship between prevalence rate of Abc and the mechanical ventilation (Dent et al., 2010).

Furthermore, this study found a statistical significant Abc prevalence rate of 13.1% with a significant risk in patients who had undergone three invasive procedures. In cases of patients who went through one and two invasive procedures, their rates were 6% and 4.3%, respectively, without statistical significances. These results are compatible with a study in Brazil that similarly found a significant correlation between prevalence rates and invasive procedures exceeding two (Prata-Rocha et al., 2012). This study also showed statistical significant Abc prevalence rates of 25.0%, 16.7% and 13.3% with significant risks for patients who had malignancy, pulmonary and CNS diseases, respectively. For patients with diabetes mellitus (DM) and renal diseases, prevalence rates with significant risks were also found, although they were statistical non-significant. These results are consistent with a study in Turkey that reported a significant risk in respect to DM, renal and cardiac diseases (Alp et al., 2009). On the other hand, these results are inconsistent with a study in Thailand that studied the same underlying diseases in relation to Abc prevalence; however they reported no statistical significance for all diseases, with the exception of renal diseases. In addition, a study in Saudi Arabia also found no significant correlation between Abc prevalence and pulmonary, cardiac and renal diseases (Babay et al., 2003). This study showed antibiotics resistant rates of 95% for Piperacillin-Tazobactam, 90% for Imipenem, Cefepime and Ticracillin, 80% for Amikacin and Doxcycyclin and 60% for Levofloxacin. Meanwhile, no resistance was observed to Colistin and polymyxin B by all the isolates. Given the fact that all isolated Abc were resistant to over two classes of antibiotics, it was classified a multi-drug resistant (Babay *et al*., 2003). Comparable observation was reported in Philippines, where resistant rates of Abc were determined to be 94% for Amikacin, 85% for Cefepime, 78% for Imipenem, 25% for Doxcycyclin and zero (0.0)% for Polymyxin B. A different study carried out in Iran is also consistent with this study with resistant rates of 97.1% for Pipracillin-Tazobactam, 100% for cefepime, 94.3% for Imipenem Amikacin and zero (0.0)% for Colistin (Yadegarinia et al., 2013). Findings of this study emphasis that isolated multi-drug resistant Abc had wide mechanisms for antibiotics resistance towards many classes of antibiotics. Therefore, experimental treatment with antibiotics will not be valuable solution and may instead increase the mortality and cause the emergence of resistant strains. Thus, several theories can be developed to elucidate the disparities in resistant rates from one country to another. The inconsistency in resistant rates can be attributed to differences in antibiotic prescribing policies and infection control practices in addition to predominant local strain.

Mortality rates of 15 to 46% have been reported for *Acinetobacter* bacteremia, which was relatively low as compared to pneumonia (Rungruanghiranya et al., 2005). This study recorded a mortality of 17.9%, which is statistically significant and somewhat analogous with a study in the USA that reported a mortality rate of 26% (Sunenshine et al., 2007).

Hospital surfaces are frequently in contact with and contaminated during regular patients care, and serve as a source of nosocomial spread for Abc in ICUs. In this study, the prevalence of Abc in ICUs environment was determined to be 8.8%. All of the isolated Abc from patients and ICUs environments were impossible to differentiate using both biotyping and antibiogram susceptibilities. This result is in conformity with a study in the USA (Thom et al., 2011) that reported Abc prevalence rates of 9.8% and 85% of environmental isolates were genetically similar to the clinical isolates.

Knowledge of risk factors would aid the prediction of nosocomial infection and allow implementation of infection control practices, as well as a cautious application of invasive procedures particularly in seriously ill patients affected by complicated diseases. In addition, attempts should be made to get rid of invasive devices such as tracheal tube or central venous catheter at the soonest possibility in order to avert the surfacing of MDR Abc infections. The prevalence of Abc may be attributed to poor infection control practices in the hospital's ICUs, while the high recovery rate from respiratory tracts may be attributable to the process of clearing up the respiratory airways. The Abc isolates were also found to be multi-drug resistant. Among the studied risk factors, the statistically significant factors are the duration of stay, mechanical ventilation, use of tracheal tube, use of more than two invasive procedures and co-morbidity with underlying diseases.

This study concludes that the Abc is prevalent among ICU's patients, particularly in respiratory specimens rather than blood specimens. In addition, both clinical and environmental isolates were impossible to differentiate based on their biotyping and antibiotics susceptibilities. Patients subjected to prolonged admission in ICU, mechanical ventilation and tracheal tube as well as patients who underwent more than two invasive procedures was significantly at a higher risk of contracting Abc. Patients who had malignancy, pulmonary diseases or central nervous system disorders, were also at a significantly higher risk of acquiring Abc. Another important point is that the data suggests that acquisition of Abc appears to be related to increased mortality for ICU patients. Based on the results, healthcare workers should take into the consideration the local trends in nosocomial infections attributable to Abc and its susceptibility patterns in hospital settings and among patients. A stringent observance of the infection control procedures and particular awareness comprising regular training for the ICU workers, constant screening of ICU environment and precise prescription of antibiotics are of vital significance to decrease the prevalence of Abc in the hospital environment. In addition, constant antibiotic resistance observation and rational implementation of invasive procedures are essential in averting Abc nosocomial infections.

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Conflict of interests

The authors declare that they have no any competing of interests in this study.

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