Determination of Antimicrobial Resistance and Resistant Genes in *Acinetobacter baumannii* from Human Clinical Samples

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ABSTRACT

Objective: *The aim of this study is to determine the prevalence, antimicrobial resistance and various resistance genes in* Acinetobacter baumannii (A baumannii) *from human clinical specimens at the payambaran and Baqiyatallah Hospitals in the city of Tehran, Iran, over a period of six months (September 2012 to March 2013).*

Methods: *A total of 500 samples including blood, phlegm, urine, cerebrospinal fluid (CSF) and pus were collected from patients on admission in these hospitals.* A baumannii *was identified by using standard microbiological procedures and conventional polymerase chain reaction (PCR) technique. Antimicrobial susceptibility test was performed according to clinical and laboratory standard institute (CLSI) guidelines using Kirby-Bauer disc diffusion technique, while different resistance genes were detected using PCR method.*

Results: *A total of 121* A baumannii *was detected out of 500 samples, representing 24.20% period prevalence.* A baumannii *was detected from all the sample groups, but a higher prevalence was observed in the blood (43.87%) and phlegm (24.11%). Antibiotic resistance profile showed higher resistance of* A baumannii *to tetracycline (90.90%), trimethoprim (61.98%) and cotrimoxazole (51.23%), followed by aminoglycosides (9.91–31.40%). Relatively low resistance was observed to cephalosporins (16.52–20.66%), quinolones (6.61–9.91%) and macrolides (8.26–14.04%), while the lowest resistance was observed to carbapenems (3.3–5.78%), chloramphenicol and nitrofurantoin. Highest detection for resistant genes was observed for tetA (58.67%), aac (3)-IV (56.19%), sul1 (55.37%) and dfrA1 (48.76%). Relatively low detection was observed for aad A1, bla-genes (SHV, CTX-M, OXA-like, VIM, SIM and IMP) and lowest detection was observed for cat1 and cmlA, while no qnr gene was detected.*

Conclusions: *Multidrug-resistant Acinetobacter infections are posing an increasing threat to the population in these communities. Carbapenems provide an effective option against infections caused by resistant* A baumannii.

Keywords: *Acinetobacter baumannii*, antimicrobial resistance, human clinical samples, resistant genes

Determinación de la Resistencia Antimicrobiana y los Genes Resistentes Frente a la *Acinetobacter baumannii* de Muestras Clínicas Humanas

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RESUMEN

Objetivo: *El objetivo de este estudio es determinar la prevalencia, resistencia antimicrobiana, y varios genes de resistencia frente a la* Acinetobacter baumannii (A baumannii) *de muestras clínicas humanas en el hospital de Payambaran y el de Baqiyatallah en la ciudad de Teherán, Irán, durante un período de seis meses (de septiembre de 2012 a marzo de 2013).*

Métodos: *Se recogieron un total de 500 muestras, incluyendo muestras de sangre, flema, orina, líquido cefalorraquídeo (LCR), y pus de los pacientes al ingresar en estos hospitales. La* A baumannii *fue identificada mediante procedimientos microbiológicos estándar y la técnica de reacción en cadena de la polimerasa (RCP) convencional. La prueba de susceptibilidad antimicrobiana se realizó según las guías*

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DOI: 10.7727/wimj.2014.337

de Clinical and Laboratory Standards Institute (CLSI), usando la técnica de difusión en disco de Kirby-Bauer, mientras que los diferentes genes de resistencia fueron detectados usando el método de RCP.
Resultados: *Un total de 121* A. baumannii *fue detectado en las 500 muestras, representando un 24.20% de prevalencia en el período.* A. baumannii *fue detectada en todos los grupos muestrarios, pero la prevalencia más alta se observó en la sangre (43.87%) y la flema (24.11%). El perfil de resistencia a los antibióticos mostró mayor resistencia de* A. baumannii *a la tetraciclina (90.90%), la trimetoprima (61.98%) y el cotrimoxazol (51.23%), seguido por los aminoglucósidos (9,91-31.40%). Una resistencia relativamente baja se observó en las cefalosporinas (16.52-20.66%), las quinolonas (6.61-9.91%) y los macrólidos (8.26 – 14.04%), mientras que la resistencia más baja se observó en las carbapenemas (3.3-5,78%), el cloranfenicol y la nitrofurantoína. La mayor detección de genes resistentes se observó en tetA (58.67%), aac 3-IV (56.19%), sul1 (55.37%) y dfrA1 (48.76%). Una detección relativamente baja se observó en aad A1, genes bla (SHV, CTX-M, tipo OXA, VIM, SIM e IMP), en tanto que la detección más baja se observó en cat1 y cmlA, no detectándose ningún gen qnr.*
Conclusiones: *Las infecciones por Acinetobacter multirresistentes representan una creciente amenaza para la población de estas comunidades. Las carbapenemas proveen una opción efectiva contra las infecciones causadas por* A. baumannii *resistente.*

Palabras claves: *Acinetobacter baumannii,* resistencia a los antimicrobianos, muestras clínicas humanas, genes resistentes

West Indian Med J 2017; 66 (1): 57

INTRODUCTION

Acinetobacter baumanii (*A baumannii*) is a gram-negative, pleomorphic aerobic species commonly isolated from many sources in the environment, including drinking and static water, soil, sewage, food, and the skin of humans and animals. It is usually considered to be an opportunistic pathogen especially in hospitals and healthcare units (1–3). *A baumannii* is a major species, contributing to about 80% of all *Acinetobacter* hospital-acquired infections (4).

It is often difficult to distinguish between infection and colonization with *A baumannii* (5). There is considerable controversy over whether infections caused by this organism leads to unfavourable outcomes; however, it is believed by some clinicians that the recovery of *A baumannii* in the hospitalized patient is an indicator of severe illness, with an associated mortality of approximately 30% (6).

One of the reasons that *A baumannii*-associated disease is predominant among hospitalized patients is its inherent capacity to persist within healthcare facilities, thereby providing reservoirs for transmission and infection. This organism can readily colonize and survive on inanimate surfaces for extended periods of time. It is resistant to desiccation and disinfectants by forming biofilms on abiotic surfaces (7, 8); it develops multi-resistance to antibiotics and can acquire and disseminate antibiotic resistance by virtue of its extraordinary ability to accept or donate resistance plasmids (9–11) and the existence of the virulence factors poses a deleterious effect within the host. As a result, these aforementioned factors have made *A baumannii a* successful pathogen, thus making the management of *A baumanii* infections, a public health problem in many countries (12).

Over the last two decades, *A baumannii* has gained importance as a leading nosocomial pathogen, especially in critical care units. This organism, which was considered a low-virulence opportunistic pathogen, has turned into a multidrug resistant pathogen with increasing cases of morbidity and mortality. These attributes are alarming to healthcare providers worldwide. Despite the large number of studies and publications on the dynamics of this organism, more surveillance is needed, especially in Iran where there is a relative dearth of information on epidemiological distribution of its virulence factors and antimicrobial resistance dynamics. The aim of this study is to determine the prevalence, antimicrobial resistance and various resistance genes in *A baumannii* from human clinical samples in Iran using molecular techniques.

MATERIAL AND METHODS

Sample collection

In this cross-sectional study, 500 clinical specimens including: blood (98 samples), phlegm (141 samples), urine (92 samples), pus (134 samples) and CSF (35 samples) were collected from patients on admission at the Payambaran Hospital and Baqiyatallah Hospital in the city of Tehran, Iran, over a period of six months (from September 2012 to March 2013). These specimens were collected by a laboratory technician, properly labelled and then were transported immediately to the Microbiology laboratory.

Isolation and identification of *Acinetobacter baumannii*

Isolation of *A baumannii* was done using MacConkey and blood agar plate culture for all the specimens. The non-haemolytic, opaque, creamy colonies on blood agar and non-lactose fermenting colonies on MacConkey agar were further sub-cultured on MacConkey agar and incubated for another 24 hours at 37 °C to obtained pure colonies (13). The isolated organisms were identified based on colony morphology, microscopic study and various biochemical tests according to standard laboratory methods (13). Stock cultures were main-

tained in both agar slant and 20% sterile buffered glycerin and kept at -70 ˚C. The *Acinetobacter* isolates were tested for antibiotic resistance by Kirby-Bauer disk diffusion method [according to the Clinical and Laboratory Standards Institute guidelines] (14).

Conventional polymerase chain reaction

The reverse and forward primers, size of product as previously published used for the detection of *16S-23S ribosomal DNA,* antimicrobial resistant genes in *A baumannii* in the study are presented in Table 1. Polymerase chain reaction (temperature and volume) for detection of *16S-23S ribosomal DNA,* antimicrobial resistance genes in *A baumannii* are also summarized in Table 2. The PCR amplified products (10 µL) were subjected to electrophoresis in a 1.5% agarose gel in 1X TBE buffer at 80V for 30 minutes, stained with solution of ethidium Bromide and examined under ultraviolet illumination (Uvitec, UK).

Statistical analysis

The prevalence analysis was computed in percentage and presented using descriptive statistics.

Table 1: Primers used for detection of virulence and antimicrobial resistant genes in *Acinetobacter baumannii*

Gene	Primer name	Primer Sequence (5'-3')	Size of product (bp)	Reference
Streptomycin	*aadA1*	(F) TATCCAGCTAAGCGCGAACT (R) ATTTGCCGACTACCTTGGTC	447	15
Gentamycin	*aac(3)-IV*	(F) CTTCAGGATGGCAAGTTGGT (R) TCATCTCGTTCTCCGCTCAT	286	15
Sulfonamide	*sul1*	(F) TTCGGCATTCTGAATCTCAC (R) ATGATCTAACCCTCGGTCTC	822	15
Beta-lactams	*blaSHV*	(F) TCGCCTGTGTATTATCTCCC (R) CGCAGATAAATCACCACAATG	768	15
Beta-lactams	*CTX-M*	(F) TGGCCAGAACTGACAGGCAAA (R) TTTCTCCTGAACGTGGCTGGC	462	15
Chloramphenicol	*cat1*	(F) AGTTGCTCAATGTACCTATAACC (R) TTGTAATTCATTAAGCATTCTGCC	547	15
Chloramphenicol	*cmlA*	(F) CCGCCACGGTGTTGTTGTTATC (R) CACCTTGCCTGCCCATCATTAG	698	15
Tetracycline	*tet(A)*	(F) GGTTCACTCGAACGACGTCA (R) CTGTCCGACAAGTTGCATGA	577	15
Tetracycline	*tet(B)*	(F) CCTCAGCTTCTCAACGCGTG (R) GCACCTTGCTGATGACTCTT	634	15
Trimethoprim	*dfrA1*	(F) GGAGTGCCAAAGGTGAACAGC (R) GAGGCGAAGTCTTGGGTAAAAAC	367	15
Quinolones	*Qnr*	(F) GGGTATGGATATTATTGATAAAG (R) CTAATCCGGCAGCACTATTTA	670	15
Carbiniciline	Imp-F Imp-R	5'-GAATAGAATGGTTAACTCTC-3' 5'-CCAAACCACTAGGTTATC-3'	188	16
Carbiniciline	Vim-F Vim-R	5'-GTTTGGTCGCATATCGCAAC-3' 5'-AATGCGCAGCACCAGGATAG-3'	382	16
Carbiniciline	Sim-F Sim-R	5'-GTACAAGGGATTCGGCATCG-3' 5'-GTACAAGGGATTCGGCATCG-3'	569	16
Oxacillinases	Oxa-51-like-F Oxa-51-like-R	5'-TAATGCTTTGATCGGCCTTG-3 5'-TGGATTGCACTTCATCTTGG-3'	353	16
Oxacillinases	Oxa-23-like-F Oxa-23-like-R	5'-GATCGGATTGGAGAACCAGA-3' 5'-ATTTCTGACCGCATTTCCAT-3'	501	16
Oxacillinases	Oxa-24-like-F Oxa-24-like-R	5'-GGTTAGTTGGCCCCCTTAAA-3' 5'-AGTTGAGCGAAAAGGGGATT-3'	246	16
Oxacillinases	Oxa-58-like-F Oxa-58-like-R	5'-AAGTATTGGGGCTTGTGCTG-3' 5'-CCCCTCTGCGCTCTACATAC-3'	599	16
A baumannii detection	*16S-23S ribosomal DNA*	(F) CATTATCACGGTAATTAGTG (R) AGAGCACTGTGCACTTAAG	208	17

Table 2: Polymerase chain reaction conditions for detection of antimicrobial resistance genes in *Acinetobacter baumannii*

Gene	PCR programme	PCR volume (50 µL)
aadA1, aac(3)-IV, sul1, bla$_{SHV}$, cat1, cmlA, Imp	1 cycle: 94 ^{0C} ——— 8 min. 32 cycle: 95 ^{0C} ——— 60 s 55 ^{0C} ——— 70 s 72 ^{0C} ——— 2 min 1 cycle: 72 ^{0C} ——— 8 min	5 µL PCR buffer 10X 2.5 mM $Mgcl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 2 U Taq DNA polymerase (Fermentas) 3 µL DNA template
tetA, tetB, dfrA1, bla$_{CTX-M}$	1 cycle: 94 ^{0C} ——— 8 min. 32 cycle: 95 ^{0C} ——— 60 s 55 ^{0C} ——— 70 s 72 ^{0C} ——— 2 min 1 cycle: 72 ^{0C} ——— 8 min	5 µL PCR buffer 10X 2.5 mM $Mgcl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 2 U Taq DNA polymerase (Fermentas) 3 µL DNA template
qnr, vim, sim	1 cycle: 94 ^{0C} ——— 6 min. 32 cycle: 95 ^{0C} ——— 60 s 55 ^{0C} ——— 70 s 72 ^{0C} ——— 70 s 1 cycle: 72 ^{0C} ——— 5 min	5 µL PCR buffer 10X 2.5 mM $Mgcl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 2 U Taq DNA polymerase (Fermentas) 3 µL DNA template
16S-23S ribosomal DNA	1 cycle: 94 ^{0C} ——— 6 min. 30 cycle: 95 ^{0C} ——— 60 s 58 ^{0C} ——— 60 s 72 ^{0C} ——— 40 s 1 cycle: 72 ^{0C} ——— 5 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 1 µM of each primers F & R 1 U Taq DNA polymerase (Fermentas) 3 µL DNA template

PCR: polymerase chain reaction

RESULTS

During the study period, a total of 121 cultures of *A baumannii* were detected out of 500 samples, representing 24.20% period prevalence. *A baumannii* was detected from all the sample groups, higher prevalence was observed in blood (43.87%), phlegm (24.11%) and urine (23.11%) while relatively low prevalence was detected in CSF (17.14%) and pus (11.94%) [Table 3] (Fig.1).

Table 3: Prevalence of *Acinetobacter baumannii* strains isolated from human clinical samples

Clinical samples	No samples	No *A baumannii*	Prevalence
Blood	98	43	43.87
Phlegm	141	34	24.11
Urine	92	22	23.91
Pus	134	16	11.94
CSF	35	6	17.14
Total	500	121	24.20

No: number; CSF:cerebrospinal fluid

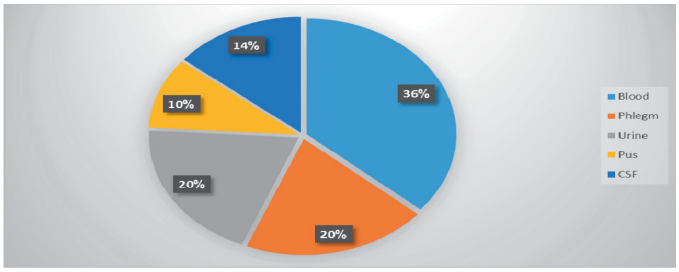

Fig. 1: Prevalence of *Acinetobacter baumannii* strains isolated from human clinical samples

Different antimicrobial resistance genes detected from this study were presented in Table 4a, Table 4b and Fig 2; highest detection for resistant genes was observed for *tetA* (58.67%), *aac(3)-IV* (56.19%), *sul1*(55.37%) and *dfrA1* (48.76%).

Relatively low detection was observed for *aad A1, bla-genes (blaSHV, blaCTX-M, blaOXA-like, blaVIM, blaSIM and blaIMP)*, lowest detection was found for *cat1* and *cmlA* while no *qnr gene* was detected in this study. Antibiotic resistance profile of *A baumannii* is presented in Table 5a, Table

Table 4a: Distribution of antibiotic resistance genes in *Acinetobacter baumannii* strains isolated from clinical samples in human

Sample	*aadA1*	*aac(3)-IV*	*sul1*	*bla$_{SHV}$*	*bla$_{CITM}$*	*tetA*	*tetB*	*dfrA1*	*qnr*
Blood (43)	12	34	30	8	12	31	11	28	0
Phlegm (34)	8	13	18	8	10	20	12	27	0
Urine (22)	7	14	12	5	2	14	7	12	0
Pus (16)	12	7	5	3	1	6	9	10	–
CSF (6)	2	–	2	–	3	–	5	2	0
Total (121)	**41 (33.88%)**	**68 (56.19%)**	**67 (55.37%)**	**24 (19.83%)**	**28 (23.14%)**	**71 (58.67%)**	**44 (36.36%)**	**79 (48.76%)**	**0 (0.00%)**

CSF:cerebrospinal fluid

Table 4b: Distribution of antibiotic resistance genes in *Acinetobacter baumannii* strains isolated from clinical samples in human

Sample	***Vim***	***Sim***	***Oxa-51-like***	***Oxa-23-like***	***Oxa-24-like***	***Oxa-58-like***	***cat1***	***cmlA***	***Imp***
Blood (43)	4	7	3	–	1	–	–	–	2
Phlegm (34)	2	–	3	4	8	3	–	–	4
Urine (22)	7	5	2	3	2	6	4	1	2
Pus (16)	3	5	–	1	–	2	1	2	2
CSF (6)	–	–	1	–	1	3	–	–	–
Total (121)	**16 (13.22%)**	**17 (14.04%)**	**9 (7.43%)**	**8 (6.61%)**	**13 (10.74%)**	**14 (11.57%)**	**5 (4.13%)**	**3 (2.47%)**	**10 (8.26%)**

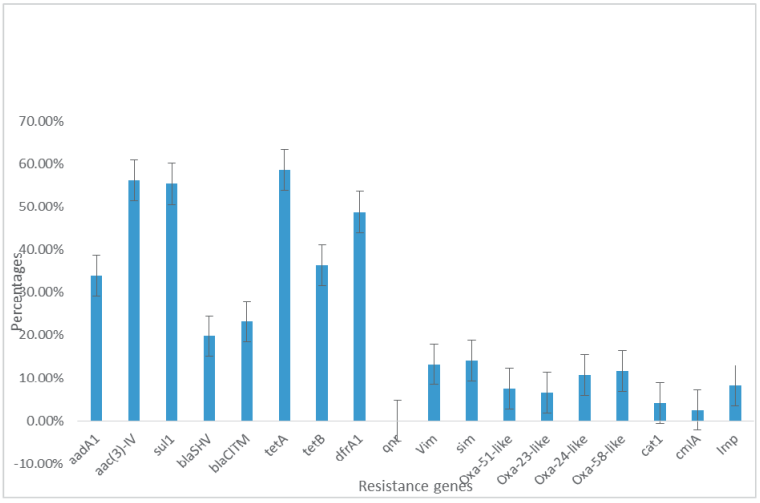

Fig. 2: Antibiotic resistance genes in *Acinetobacter baumannii* strains isolated from clinical samples in humans.

5b and Fig. 3; higher resistance was observed to tetracycline (90.90%), trimethoprim (61.98%), cotrimoxazole (51.23%), followed by aminoglycosides such as tobramycin, streptomycin, gentamycin and amikacin (9.91–31.40%). Relatively low resistance was observed to cephalosporins *vis-a-vis* cephalothin and ceftazidime (16.52–20.66%), quinolones; ciprofloxacin and levofloxacin (6.61–9.91%) and macrolides: erythromycin and azithromycin (8.26–14.04%). While the lowest resistance was observed in: the carbapenems: imipenem and meropenem (3.3–5.78%), chloramphenicol and nitrofurantoin.

DISCUSSION

A baumannii is one of the commonly isolated non-fermenting bacteria in human specimens, especially of nosocomial origin after *Pseudomonas aeruginosa*. Its infections are uncommon but, when they occur, it is usually associated with organ systems with high fluid content *eg* respiratory tract, CSF, peritoneal fluid and urinary tract (18–20). Just as observed in this study, *A baumannii* was detected from all sample groups from organs with fluid content including blood, urine and CSF. Jaggi *et al* (18) and Dash *et al* (21) have also reported the detection of this organism from pus, urine, sputum, blood and body fluids in tertiary hospitals in India. Colonization potentials as well as healthcare associated infection and outbreaks of

Table 5a: Antibiotic resistance profiles in *Acinetobacter baumannii* strains isolated from clinical samples in humans

Sample	streptomycin	gentamycin	Amikacin	tobramycin	co-trimoxazole	cephalotin	ceftazidime	tetracycline	trimetoprim
Blood (43)	11	19	8	7	29	12	8	41	28
Phlegm (34)	7	10	3	2	17	9	2	30	26
Urine (22)	6	7	4	3	11	2	7	20	12
Pus (16)	12	2	–	–	4	–	3	15	9
CSF (6)	2	–	–	–	1	2	–	4	–
Total (121)	**38 (31.40%)**	**38 (31.40%)**	**15 (12.3%)**	**12 (9.91%)**	**62 (51.23%)**	**25 (20.66%)**	**20 (16.52%)**	**110 (90.90%)**	**75 (61.98%)**

Table 5b: Antibiotic resistance profiles in *Acinetobacter baumannii* strains isolated from clinical samples in human

Sample	ciprofloxacin	levofloxacin	imipenem	meropenem	cloramphenicol	nitrofurantoin	azithromycin	rifampin	erythromycin
Blood (43)	5	–	1	1	–	–	4	2	4
Phlegm (34)	4	3	2	–	–	–	2	4	3
Urine (22)	1	2	1	1	1	2	3	4	3
Pus (16)	–	2	2	–	1	–	1	–	7
CSF (6)	2	1	1	–	–	–	–	–	–
Total (121)	**12 (9.91%)**	**8 (6.61%)**	**7 (5.78%)**	**4 (3.30%)**	**2 (1.65%)**	**2 (1.65%)**	**10 (8.26%)**	**10(8.26%)**	**17 (14.04%)**

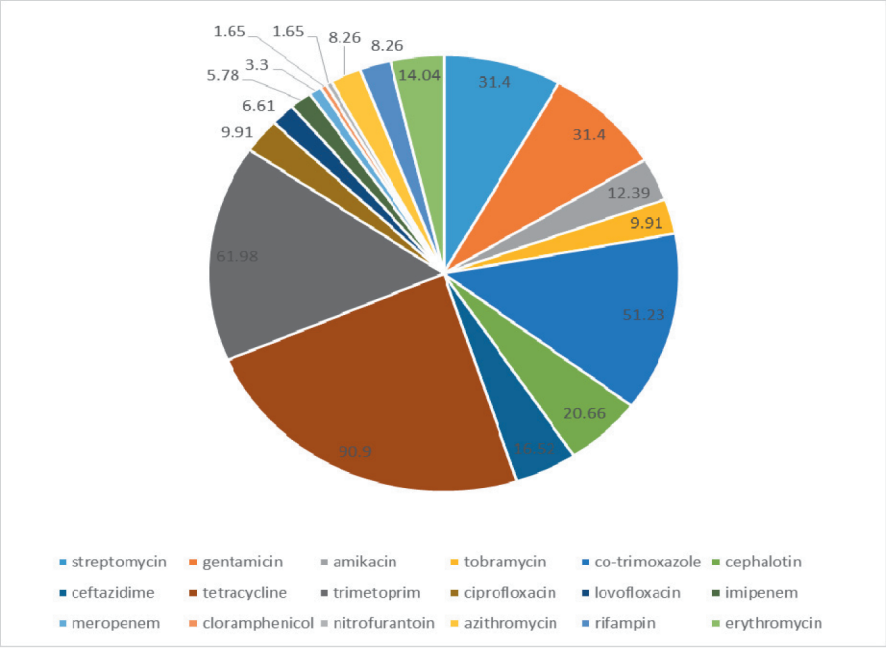

Fig. 3: Antibiotic resistance profiles in *Acinetobacter baumannii* strains isolated from clinical samples in human.

this organism have been reported (12). Detection of *Acinetobacter* from these samples may suggest either an on-going infection or colonization of organ sources with *A baumannii*. However, detection of *A baumannii* in blood samples suggests that *Acinetobacter* cannot exist as a colonizer in blood, it would have a higher pathogenic and septicaemic potential at this site. Since these patients are on hospital admission, there are possibilities that they may have acquired this organism from hospital environments such as surfaces and medical equipments and even beddings. Detection of this organism from the patients from the two hospitals may indicate or suggests that, these hospitals are contaminated with *Acinetobacter* with risk of nosocomial transmission and possibilities of future hospital outbreak.

One of the challenges with *A baumannii* is its capacity to acquire myriads of antimicrobial-resistance genes rapidly, leading to multi-drug resistance, just as observed in this study. *A baumannii* detected in this study was resistant to all antibiotics tested in different proportion and were also found to carry different resistant genes to different classes of ant-

ibiotics, thus, supporting the multi-drug resistant characteristic of *A baumannii*. Widespread use of antimicrobials within healthcare facilities have been considered to be a major factor in the emergence of antimicrobial resistance among Acinetobacter strains, in particular, the wide use of extended-spectrum cephalosporins and quinolones (22).

From this study, *Acinetobacter baumannii* resistant to tetracycline represented the highest antibiotic resistance recorded. Both *tetA* and *tetB* genes represented the most prevalent resistant genes observed in this study. This resistant pattern to tetracycline could be due to the indiscriminate use of this antibiotic either within the hospitals or the communities. The increased tetracycline resistance and tetracycline resistant genes recorded in the present study, support the fact that *tetA* confers resistance to tetracycline only, while *tetB* in addition to tetracycline confers resistance to minocycline (23, 24). This resistance to tetracycline and its derivatives is reported to be due to efflux pumps or ribosomal protective mechanisms (25). In this study, the prevalence of *tetA* was observed to be higher than *tetB*, this is at variant to 66% and 13.6% reported for *tetB* and *tetA,* respectively by Marti *et al* (26). Tetracycline resistant *A baumannii* has been reported from Iran (27, 28), however, it has been reported that, in most clinical practices, use of tetracycline as therapy against *A baumannii* infections is not common but high resistance to tetracycline is common among *A baumannii* isolates (29, 30) and the result of this study confirmed this claim.

The resistance among *A baumannii* strains to β-lactam antibiotics although relatively lower, is of great concern. The β-lactams are broadly accepted for treatment; because of their availability, broad spectrum activities, reduced risk of side-effects, and importantly, their relatively low cost. Low resistance (4–7%) to carbapenems *ie* imipenem and meropenem and close to 20% resistance to cephalosporin (cephalotin and ceftazidime) observed in the present study is lower than over 90%reported to by Jaggi *et al*, in India (18), Japoni-Nejad *et al*, (27) and Safari *et al* (31) in Iran. Reduced resistance to cephalosporins and carbapenems may be due to non-use or judious use of these antibiotics in affected patients. This may also suggests that, carbapenems will be effective drugs in the management of *Acinetobacter* infections or in outbreaks in these hospitals. This finding has also been reported in most studies in Iran (32–34). The detection of β-lactamases such as blaSHV, blaCTXM, blaOXA-like (6.61–23.14%) and Metallo-bêta lactamases (MBL) *ie* VIM, SIM and IMP (8.26–14.04%) indicates and support the fact that *A baumannii* are ESBL and MBL producers.

Acinetobacter are known to produce a myriad of β-lactamases. The main mechanisms of resistance to extended-spectrum cephalosporins in *A baumannii* have been reported to be due to the over-expression of chromosomal cephalosporinases and plasmid-encoded Ambler class A, B, and D β-lactamases (35). The *blaOXA* gene has been considered as a natural component of the species chromosome and has been used in identification of *A baumannii* (36). *Previous report indicates blaOXA-51* genes are present in the vast majority of isolates of *A baumannii* and may also be associated with resistance to carbapenems (35), however, in this study only 7.43%–11.57% of *A baumannii* carried *blaOXA* genes. There has been some debate as to whether they are present in all isolates of this species (37).

Although, in this study, 4–7% of *A baumannii* are resistant to carbapenems, almost double of this organism (8.26–14.04%) were observed to be a carriers of VIM, SIM and IMP; this may suggest the role of *blaOXA* genes in carbapenem resistance. Also, this may suggest the potentials of this organism to increase resistance to carbapenems in the near future in these hospitals. This increase in the number of MBLs in *A baumannii* is a concerned development in the global emergence of MBL resistance to β-lactams.

In this study, tobramycin and amikacin showed better activities and higher sensitivities to *A baumannii* compared to streptomycin and gentamycin, *A baumannii* resistance to aminoglycosides is relatively low. Akers *et al* (38) have reported higher sensitivities of tobramycin to *A baumannii*. While Dauner *et al* (39) and Mostofi *et al* (40) reported reduced susceptibility to gentamycin against *Acinetobacter*. Amikacin showed higher sensitivity in this study compare to that reported by other studies (41). Susceptibility to aminoglycosides has changed a lot in recent years, gentamycin used to be an active agent against *Acinetobacter* infections, but now this organism has acquired resistance against this antibiotic. This resistance to aminoglycosides in *A baumannii* has been reported to be mediated principally by aminoglycoside-modifying enzymes [AMEs] (42). And the detection of *aac(3)-IV* and *aadA1* AMEs in this study corroborates this report. These AMEs have also been reported from different parts of the world including, Iran (34, 42, 43).

Quinolone resistance in *A baumannii* observed in this study is relatively low; this shows that quinolones have higher sensitivity to *A baumannii.* Non-detection of *qnr gene* in this study corroborates other reports worldwide (44–46). This plasmid mediated-quinolone resistance gene (PMQR) has not yet been reported in *A baumannii. Acinetobacter baumannii* resistance to quinolones has been reported to be due to modifications in the structure of DNA gyrase secondary to mutations in the quinolone resistance-determining regions of the *gyrA* and *parC* genes (47, 48). Higher resistance of *A baumannii* to trimethoprim and co-trimoxazole (> 50%) observed in this study, have also been reported elsewhere (49, 50). Indiscriminate use of these antibiotics and higher detection of *sul1* and *dfrA1* resistant genes in this organism against sulfonamides and trimethoprim may account for this. While chloramphenicol, nitrofurantoin, azithromycin, rifampin and erythromycin all showed higher sensitivities against *A baumannii,* their therapeutic use in *Acinetobacter* infections is not widely reported and in most cases, their use maybe as adjunct therapy.

In conclusion, detection of *Acinetobacter* from different samples suggest either an on-going infection or colonization

of organ sources with *A baumannii.* Resistance was observed in all antibiotics tested in different proportion and there were also different resistant genes to different class of antibiotics, thus, supporting the multi-drug resistant characteristic of *A baumannii.* Multi-drug resistant *Acinetobacter* infections are posing an increasing threat to the population in these communities. Carbapenems provide an effective option against infections caused by resistant *A baumannii.*

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