Original research

Detection of carbapenem resistance and virulence genes among *Acinetobacter baumannii* isolated from hospital environments in center of Iran

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Abstract

Carbapenem-resistant *Acinetobacter baumannii* are the top urgent antibiotic resistance threat in the world. The aims of this study were the determination of carbapenem-resistant genes and virulence genes among isolates from hospital environments. In this study, *A. baumannii* isolated from hospital environments and evaluated its antibiotic resistance, virulence factors, and resistance genes. Of 258 samples, 58 showed growth of the target organism. Antibiotic susceptibility test results considered all the *A. baumannii* to be multidrug-resistant isolates with the highest resistance being 36.2% to ciprofloxacin; while the most effective antibiotics with 98.3% susceptibility was piperacillin-tazobactam. Of these 58 hospital environment isolates, 18 isolates were positive for Metallo beta-lactamase. Overall, 65% of the isolates from hospital environments had many virulence factors. PCR assays demonstrated the highest and lowest positive results in *csgA* and *cvaC* gene among hospital environment isolates. Results indicate that the determination of carbapenem-resistant genes and virulence genes among isolates from hospital environments is very important.

Keywords: Carbapenem resistance, Virulence gene, Non-clinical isolates, *Acinetobacter baumannii*

1. Introduction

Multidrug-resistant *Acinetobacter baumannii* is an opportunistic pathogen that causes nosocomial infections [1-3]. Infections caused by this bacterium have a high prevalence in hospitalized and immunocompromised patients who are admitted to intensive care units [4, 5]. These infections are ventilator-associated pneumonia, soft-tissue, urinary tract, and meningitis infections [6-8]. *A. baumannii* has an ability to survive for long periods the surfaces, sometimes even for several years [9]. Due to resistance to a broad range of antimicrobial agents can long-term persistence in the clinical settings, surviving on nutrient sources and transmission by healthcare staff [10, 11].

Carbapenems and colistin are the last choices of antibiotic therapies against multi-drug resistant (MDR) *A. baumannii* strains [12]. The first carbapenem-resistant *A. baumannii* (CRAB) originated in the USA and was reported in 1991 [13]. Recent studies have reported that CRAB is a major causative organism in hospital-acquired infections [14]. Several mechanisms are responsible for resistance to carbapenems in CRAB [15]. The

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production of carbapenemase enzymes is one of the important mechanisms of carbapenem resistance. These enzymes are class A, B, and D according to molecular Ambler classification. The members of class A carbapenemases include SME, IMI, NMC, GES, SFC, and KPC families. Also, class B enzymes are called metallo-beta-lactamases (MBLs), including IMP, VIM, SIM, and NDM. Class D β-lactamases referred to OXA-type enzymes or oxacillinases which are the most prevalent carbapenemases in *A. baumannii* [16, 17]. Prevalence of virulence Factors (VF) is contributed to pathogenesis in *A. baumannii* [18]. Some of the most significant VF of the *A. baumannii* strains are curli fibers (*csg*), colicin V production (*cvaC*), siderophores like aerobactin (*iutA*), and cytotoxic necrotizing factor (*cnf*) [18, 19]. The characterization of latent virulence genes, antimicrobial resistance, and molecular detection of carbapenemases of this bacterium in the hospital environments on abiotic and biotic surfaces are the important epidemiological issue. The aims of this study were the determination of antimicrobial resistance and molecular detection of antibiotic resistance and virulence genes among *A. baumannii* isolated from hospital environments in Qom hospitals.

2. Materials and Methods

2.1 Bacterial strain collection and identification

The study was conducted at Pasteur Institute in the period from October 2019 to December 2019. Totally, 58 isolates were collected from different hospital environments of Qom hospitals (Kamkar and Beheshti hospitals) (Table 1). Samples were washed with PBS and transferred to brain heart infusion (BHI) media for further incubation at 37 °C. Samples were inoculated into blood agar and MacConkey agar for standard aerobic growth and placed at 37 °C overnight. The isolates were identified by the standard biochemical tests. The final confirmation of *A. baumannii* isolates was performed by PCR bla_{OXA-51}-like gene [20].

2.2 Antimicrobial susceptibility testing

According to the CLSI 2019 guidelines, the disk diffusion test was performed on Mueller-Hinton agar using a panel of nine antibiotic disks including ciprofloxacin (CIP), levofloxacin (LVX), gentamicin (GM), imipenem (IMI), piperacillin-tazobactam (PTZ), ampicillin-sulbactam (SAM), ceftriaxone (CRO), and trimethoprim-sulfamethoxazole (SXT) (Mast Diagnostic, UK).

2.3 Phenotypic detection of MBL production

Combined disk diffusion test (CDDT) was performed using an imipenem disk (Mast Diagnostic, UK) and in combination with EDTA (Sigma, UK) to identify MBLs. The inhibition zones of the imipenem, and imipenem + EDTA disks were compared after 20 h of incubation at 37 °C. In the combined disc test, if the increase in inhibition zone with the imipenem + EDTA disk was >7 mm than the imipenem disk alone, it was considered as MBL positive [21].

2.4 Detection of carbapenem resistance and virulence genes

Genomic DNA extractions were performed based on the protocol as described elsewhere [22]. PCR reaction mixtures were prepared in total volumes of 25 µl. The presence of the carbapenemase-encoding genes including bla_{OXA23}-like, bla_{OXA24}-like, bla_{OXA58}-like, bla_{IMP}, bla_{NDM}, and bla_{VIM} genes and virulence genes *cnf1*, *csgA*, *cvaC*, *iutA* were investigated by PCR assays in all isolates [23-28]. All PCR primers are shown in Table 2.

Table 1. Samples source collection

Source	Amount Percent (Number/Total)
Sink	5.1% (3/58)
Floor	6.8% (4/58)
Pillow	12.0% (7/58)
Desk	13.7% (8/58)
Bed	3.4% (2/58)
Under-bed wheels	8.6% (5/58)
Door knob	12.0% (7/58)
Nurses' fingers	5.1% (3/58)
Walls	10.2% (6/58)
Touchpads	22.4% (13/58)

3. Results

Antibiotic susceptibility testing showed out of 58 hospital environment isolated strains, the resistance against to CIP, LVX, GM, IMI, SXT, SAM, CRO, and PTZ were 36.2% (21/58), 31% (18/58), 18.9% (11/58), 15.5% (9/58), 8.6% (5/58), 8.6% (5/58), 5.1% (3/58) and 1.7% (1/45), respectively.

Of these 58 hospital environment isolates, 21/58 (36.2%) showed resistance to imipenem and were therefore further tested for MBL production. Eighteen of these isolates showed positive results for MBL production by CDDT method as shown in Table 3.

PCR analysis in all carbapenem-resistant isolates revealed that prevalence of bla_{VIM}, bla_{OXA-23}-like, bla_{OXA-24}-like, and bla_{IMP} were 10/21 (47.6%), 3/21 (14.3%), 3/21 (14.3%), and 1/21 (4.7 %) of the strains, respectively. None of the hospital environment isolates carried bla_{OXA-58}-like or bla_{NDM} (Table 3).

Also, PCR assays demonstrated positive results in 43.1% (25/58) of strains for *csgA*, 32.7% (19/58) of strains for *cnf1*, 12% (7/58) of strains for *iutA*, and 3.4% (2/58) of strains for *cvaC* genes among hospital environment isolates (Table 3).

4. Discussion

A. baumannii is an opportunistic pathogen and also has the ability to cause nosocomial diseases due to antibiotic resistance and can survive on surfaces, the body of the treatment staff, and patients [29, 30]. This bacterium has the ability to survive on different surfaces and objects, so it has a high potential for spread and colonization in hospitalized patients [31]. Through the mechanisms of acquisition of determinants of resistance and upregulation of intrinsic resistance mechanisms, this bacterium is able to resistance against a wide range of available antibiotics [30]. *A. baumannii* with multidrug resistance causes severe infections and high mortality, especially in patients with impaired immune systems or immunocompromised [2, 32]. Although the virulence factors and pathogenicity mechanism of *A. baumannii* are not fully understood and require further study and research, but this bacterium has the ability to cause a wide range of infections and deaths in hospitals. The virulence factors of this bacterium play an important role in resisting the host's defense mechanism [33, 34]. Also these factors are important

Table 2. Primers used for amplification

Target gene	Primers sequence (5' to 3')	Size (bp)	Reference
bla_{NDM}	F: CGGAATGGCTCATCACGATC R: CGGAATGGCTCATCACGATC	621	[23]
bla_{IMP}	F: GTTTATGTTCATACWTCG R: GGTTTAAYAAAACAACCAC	432	[24]
bla_{VIM}	F: TTTGGTCGCATATCGCAACG R: CCATTCAGCCAGATCGGCAT	500	[25]
bla_{OXA-23}-like	F: TCTGGTTGTACGGTTCAGC R: AGTCTTTCCAAAAATTTTG	606	[26]
bla_{OXA-24}-like	F: ATGAAAAAATTTATACTTCC R: TTAAATGATTCCAAGATTTTC	246	[26]
bla_{OXA-58}-like	F: ATGAAATTATTAAAAATATTGAGTTTAG R: TTATAAATAATGAAAAACACCCAAC	843	[26]
bla_{OXA-51}-like	F: TAATGCTTTGATCGGCCTTG R: TGGATTGCACTTCATCTTGG	353	[27]
cnf1	F: AAGATGGAGTTTCCTATGCAGGAG R: CATTCAGAGTCCTGCCCTCATTATT	498	[28]
csgA	F: ACTCTGACTTGACTATTACC R: AGATGCAGTCTGGTCAAC	200	[28]
cvaC	F: CACACACAAACGGGAGCTGTT R: CTTCCCGCAGCATAGTTCCAT	680	[28]
iutA	F: GGCTGGACATCATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG	300	[28]

F: Forward; R: Reverse

Table 3. Positive and negative genes among hospital environment isolates

Sample No.	MBL	*bla*VIM	*bla*OXA-23-like	*bla*OXA-24-like	*bla*IMP	*bla*OXA-58-like	*bla*NDM	csgA	cnf1	iutA	cvaC
1	-	-	-	-	-	-	-	-	+	-	-
2	-	-	-	-	-	-	-	+	-	-	-
3	-	-	-	-	-	-	-	+	+	-	-
4	+	+	-	-	-	-	-	-	+	-	-
5	-	-	-	-	-	-	-	+	-	-	-
6	+	-	+	-	-	-	-	+	-	-	-
7	+	-	-	-	-	-	-	+	+	-	-
8	+	+	-	-	-	-	-	+	-	-	-
9	-	-	-	-	-	-	-	+	+	-	+
10	+	+	-	-	-	-	-	+	-	-	-
11	-	-	-	-	-	-	-	-	+	-	-
12	+	-	-	+	-	-	-	+	-	-	-
13	-	-	-	-	-	-	-	+	-	-	-
14	+	+	+	-	+	-	-	+	+	-	-
15	-	-	-	-	-	-	-	+	-	-	-
16	-	-	-	-	-	-	-	+	-	-	-
17	+	-	-	-	-	-	-	-	+	+	-
18	-	-	-	-	-	-	-	-	-	+	-
19	+	+	-	+	-	-	-	+	+	-	-
20	-	-	-	-	-	-	-	-	-	+	-
21	-	-	-	-	-	-	-	-	-	+	-
22	+	-	-	-	-	-	-	+	-	-	-
23	-	-	-	-	-	-	-	+	+	-	-
24	+	-	-	-	-	-	-	+	-	-	-
25	+	+	-	-	-	-	-	-	+	+	-
26	+	-	-	-	-	-	-	+	+	-	-
27	-	-	-	-	-	-	-	+	-	-	-
28	-	-	-	-	-	-	-	+	+	-	-
29	-	-	-	-	-	-	-	-	+	-	-
30	+	+	+	-	-	-	-	+	+	-	-
31	+	+	-	-	-	-	-	+	-	-	-
32	+	+	-	-	-	-	-	+	-	-	-
33	-	-	-	-	-	-	-	-	+	-	-
34	+	-	-	-	-	-	-	+	+	-	-
35	-	-	-	-	-	-	-	-	-	+	-
36	+	+	-	+	-	-	-	+	+	-	-
37	-	-	-	-	-	-	-	-	-	+	-
38	-	-	-	-	-	-	-	-	+	-	-
39 – 58	-	-	-	-	-	-	-	-	-	-	-

role in binding and invasion bacteria to host cells [28]. According to the contents, it is necessary to study the prevalence of antibiotic resistance and prepare a useful antibiotic treatment model to control and treat diseases caused by *A. baumannii*. The most important factor in resistance to carbapenem antibiotics is the presence of the *bla*OXA genes, which causes the production of carbapenem hydrolyzing enzymes. In the present study, out of 58 isolates, the rate of antibiotic resistance in *A. baumannii* based on antibiotic susceptibility tests was the highest and lowest resistance for CIP and PTZ antibiotics, respectively. According to Nourbakhsh et al. (2018) studies, the highest resistance is related to CIP (97.2%) [35], which is similar to our study in terms of the highest antibiotic resistance. In the study of Shakibaie et al., resistance to CIP and PTZ was reported to be 66% and 93.3%, respectively [36]. Although the

bacterial resistance to CIP was as high as in our study, the resistance to PTZ differed greatly from our study. According to a study by Shirmohammadlou et al., *A. baumannii* is completely resistant to CIP [37], and this result is consistent with our study. These results are consistent with the study of Kabbaj et al., which showed that MBL production is among the 74% of bacteria resistant to imipenem [38].

Among carbapenem-resistant isolates, the frequency of *bla*VIM and *bla*IMP genes had the highest and lowest, respectively. Also none of the hospital environment isolates carried *bla*OXA-58-like or *bla*NDM. In the study of Amudhan et al. [39], the most resistance is related to *bla*VIM in 45%, and in the study of Shirmohammadlou et al. [37], resistance to *bla*IMP was less common, which met with our study.

Regard to prevalence of virulence genes, the results of our study were similar to those of Al-Kadmy et al. (Highest frequency was *csgA* 66.7% and lowest frequency was *cvaC* 9.5%) [40], Momtaz et al. (Highest frequency was *csgA* 55% and lowest frequency was *cvaC* 10%) [41], although our results contradicted the results of Darvishi study (highest frequency was *cnf1*, 35.53% and lowest frequency was *csgA* 12.39%) [28].

The present study had some limitations. Mainly, this was a two-center study; therefore, the generalization of the results to other regions requires further investigations.

In conclusion, increasing antibiotic resistance due to the acquisition of resistance genes and mutations due to selective pressures is a global problem. The increase in antibiotic resistance in *A. baumannii* is spreading, especially against effective antibiotics. Therefore, Studying and determining the pattern of antibiotic resistance for the preparation of a treatment protocol and the use of appropriate antibiotics is effective and reduces the increase in antibiotic resistance. In the current study, we isolated *A. baumannii* from hospital environments and determinated its antibiotic resistance, virulence factor, and resistance gene. Antibiotic susceptibility testing showed the most resistance and sensitivity was against CIP and PTZ respectively. PCR analysis in all carbapenem-resistant isolates revealed a high prevalence of *bla*VIM and *bla*IMP. Also, results demonstrated the highest and lowest positive results for *csgA* and *cvaC* gene among hospital environment isolates.

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