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Virulence Factors Profile and Antimicrobial Resistance of *Acinetobacter baumannii* Strains Isolated from Various Infections Recovered from Immunosuppressive Patients

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ABSTRACT

Natural inherent of *Acinetobacter baumanni* to survive in hard conditions in surfaces and its ability to resist against commonly used antibiotics in hospitals caused it to be one of the most prevalent cause of hospital infections. The present study was carried out to research the prevalence, antibiotic resistance pattern and distribution of virulence genes in the *A. baumannii* strains of various infections of immunosuppressive patients. One-hundred and fifty samples were collected and cultured. Their positive results were subjected to disk diffusion and PCR. Of all 150 samples studied, 20 samples (13.33%) were infected with *A. baumannii*. Wound infections had the highest prevalence of *A. baumannii* (16%). *Csga* (70%) and *cnf1* (50%) were the most commonly detected virulence genes. *A. baumannii* strains showed the highest levels of resistance against ampicillin (100%), tetracycline (95%), gentamycin (75%) and cephalexin (60%), while lowest against imipenem (5%) and ceftriaxone (35%). Statistically significant difference was seen between the type of samples and prevalence of *A. baumannii*, prevalence of antibiotic resistance and also distribution of virulence genes ($P < 0.05$). Quick determination of infections caused by *A. baumanni* and its treatment with imipenem can decrease the risk of *A. baumanni*'s infections.

Keywords: *Acinetobacter baumanni*, Virulence genes, Antibiotic resistance pattern, Immunosuppressive patients, Clinical infections.

INTRODUCTION

Healthcare-associated and hospital-acquired infections (HAIs) are common cause of mortality and morbidity al-around the world. Pathogenic bacteria are the most important causes of HAIs. Among all of the, *Acinetobacter baumannii* is one of the most prevalent cause of infections in the hospital environment[1,2].

Acinetobacter species are aerobic gram-negative bacilli that can survive for prolonged periods in the environment and on the hands of healthcare workers[1,3]. Furthermore, *Acinetobacter* infections have become increasingly difficult to treat because of the emergence of strains resistant to various types of antibiotics including cephalosporins, quinolones, sulfonamides, macrolides, aminoglycosides, fluoroquinolones and tetracycline[4,5]. These multidrug-resistant (MDR) strains are responsible for causing various types of infections including endocarditis, wound, skin and soft tissue infections, meningitis, septicemia, pneumonia and respiratory and urinary tract infections (RI and UTIs)[1,3].

Pathogenesis of diseases caused by *A. baumannii* is derived from the presence of latent virulence genes[6,7]. Some of the most significant virulence genes of the *A. baumannii* strains of human clinical infections are colicin V production (*cvaC*), curli fibers (*csg*), siderophores like

aerobactin (*iutA*) and cytotoxic necrotizing factor (*cnf*)[6,7]. Detection of latent virulence genes in the clinical isolates of *A. baumannii* has some great epidemiological outcomes help practitioners to control dissemination of infectious diseases caused by this bacterium.

Up to now, there were no well-conducted previously published data about the prevalence and epidemiology of *A. baumanni* strains in human clinical samples in Iran. Therefore, the present investigation was done in order to study the prevalence, distribution of virulence genes and antibiotic resistance pattern of *A. baumannii* strains isolated from various types of infections recovered from immunosuppressive hospitalized patients.

MATERIALS AND METHODS

Samples and Acinetobacter baumannii isolation

From January 2015 to April 2016, a total of 150 infectious samples including wound (n=50), respiratory (n=40) and urine (n=60) samples were collected from immunosuppressive patients hospitalized in hospitals and health care centers of Iran. Samples were collected from less than 70 years old hospitalized patients. Samples were immediately transferred to the laboratory in cooler with ice packs.

Samples were inoculated on to blood agar (Merck, Germany) and MacConkey agar (Merck, Germany) and incubated aerobically at 37°C for 24 hours. Non-hemolytic, opaque and creamy colonies on blood agar and nonlactose fermenting colonies on MacConkey agar were further sub-cultured on MacConkey agar and incubated for another 24 hours at 37ºC to obtained pure colonies. The isolated organisms were identified based on colonial and microscopic characteristics and various biochemical tests according to standard laboratory methods[8]. Further identification of isolates was done using Gram stain, oxidase test and API 20NE identification strip (Biomérieux, Marcy l'Etoile, France).

Table 1: Primer sequence and PCR conditions used for detection of virulence genes in the *A. baumannii* isolates of various types of infections

Gene target	Primer sequence (5'-3')*	PCR product (bp)	PCR Volume (50µL)	PCR programs
cnf1	F: AAGATGGAGTTTCCTATGCAGGAG R: CATTCAGAGTCCTGCCCTCATTATT	498	5µL PCR buffer 10X 1.5 mM $Mgcl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.25 U Taq DNA polymerase (Fermentas) 2.5 µL DNA template	1 cycle:95 ^{0C} — 4 min.30 cycle:95 ^{0C} ——— 50 s ^{0C} ————45 s 72 ^{0C} —— 1 cycle: 8 min
csgA	F: ACTCTGACTTGACTATTACC R:AGATGCAGTCTGGTCAAC	200		
cvaC	F:CACACACAAACGGGAGCTGTT R: CTTCCCGCAGCATAGTTCCAT	680		58 ^{0C} —— 60 s72
iutA	F: GGCTGGACATCATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG	300		

Table 2: Total distribution of *A. baumannii* isolates of various types of infections

Type of samples	No. samples collected	Prevalence of *A. baumannii* (%)
Wound	50	8 (16)
Urine	60	7 (11.66)
Respiratory	40	5 (12.50)
Total	15	20 (13.33)

Antimicrobial susceptibility testing

Pattern of antimicrobial resistance was studied using the simple disk diffusion technique. The Mueller–Hinton agar (Merck, Germany) medium was used for this purpose. Antibiotic resistance of *A. baumannii* strains against commonly used antibiotics was determined using the instruction of Clinical and Laboratory Standards Institute guidelines[9]. Susceptibility of *A. baumannii* strains were tested against levofloxacillin (5 µg/disk), ampicillin (10 u/disk), imipenem (30 u/disk), gentamycin (10 µg/disk), cephalothin (30 µg/disk), cephalexin (10 µg/disk), tetracycline (30 µg/disk), trimethoprim/sulfamethoxazole (25 µg/disk) and ceftriaxone (30 µg/disk) antibiotic agents (Oxoid, UK). All of the inoculated plates were aerobically incubated at 37 °C for 18-24 h in an aerobic atmosphere. Results were interpreted based on the instruction provided by CLSI (2012) (9). In all reactions, the *A. baumannii ATCC* 19605 was used as quality control bacterium.

DNA extraction from the Acinetobacter baumannii isolates

A single colony of *A. baumannii* was inoculated on 5 ml of nutrient broth and incubated over night at 37 ºC. Genomic DNA was extracted from the bacterial colony using the genomic DNA extraction kit (Fermentas, Germany) according to the manufacture instruction. The DNA concentration has been determined by measuring absorbance of the sample at 260 nm using spectrophotometer[10].

PCR-based detection of virulence genes

Table 1 indicates list of primers and PCR program used for detection of virulence factors (11). The DNA was amplified in a programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany). Fifteen microliters of PCR products were resolved on a 1.5% agarose gel containing 0.5 mg/ml of SYBR Green in Tris–borate–EDTA buffer at 90 V for 40 min, also using suitable molecular weight markers.

Table 3. Total distribution of putative virulence genes among the *A. baumannii* isolates of various types of infections

Type of samples (no positive)	Distribution of virulence genes (%)			
	Cnf1	***CsgA***	***CvaC***	***IutA***
Wound (8)	4 (50)	4 (50)	1 (12.50)	1 (12.50)
Urine (7)	3 (42.85)	5 (71.42)	-	1 (14.28)
Respiratory (5)	3 (60)	5 (100)	1 (20)	3 (60)
Total (20)	10 (50)	14 (70)	2 (10)	5 (25)

Table 4: Antibiotic resistance pattern of the *A. baumannii* isolates of various types of infections.

Type of samples (no positive)	Antibiotic resistance pattern (%)								
	Lev *	**Amp**	**Imp**	**Gen**	**Ceph**	**Cphx**	**Tet**	**Tr-Su**	**Ceft**
Wound (8)	3 (37.50)	8 (100)	1 (12.50)	8 (100)	3 (37.50)	4 (50)	8 (100)	4 (50)	2 (37.50)
Urine (7)	4 (57.14)	7 (100)	-	3 (42.85)	3 (42.85)	5 (71.42)	7 (100)	4 (57.14)	3 (42.85)
Respiratory (5)	3 (60)	5 (100)	-	4 (80)	2 (40)	3 (60)	4 (80)	3 (60)	2 (40)
Total (20)	10 (50)	20 (100)	1 (5)	15 (75)	8 (40)	12 (60)	19 (95)	11 (55)	7 (35)

The products were examined under ultraviolet illumination. *A. baumannii* ATCC 17978 and *A. baumannii* ATCC 19606 and rough strains purchased from the Pasteur Institute (Tehran, Iran) were used as positive controls and distilled water (D.W, Merck, Germany) was used as a negative control.

Statistical analysis

Statistical analysis was performed using SPSS/21.0 software (SPSS Inc., Chicago, IL). The chi-square test and Fisher's exact 2-tailed test analysis were performed in this study. Statistical significance was regarded at a P value < 0.05.

RESULTS

Table 2 represents the distribution of *A. baumannii* isolates of various types of infections. Twenty out of 150 samples (13.33%) were infected with *A. baumannii*. Wound infections had the highest prevalence of *A. baumannii* (16%), while urine had the lowest (11.66%). Statistically significant difference was seen between the type of samples and prevalence of *A. baumannii* (P < 0.05).

Figure 1 represents the results of the gel electrophoresis for detection of the virulence genes of the *A. baumannii* isolates of various types of infections. Table 3 shows the distribution of putative virulence genes among the *A. baumannii* strains of various types of infections. Results showed that bacterial strains of respiratory infections had the highest and also most variable profile of the virulence genes. Totally, *csga* (70%) and *cnf1* (50%) were the most commonly detected virulence genes. Statistically significant difference was seen between the type of samples and prevalence of virulence genes (P < 0.05).

Table 4 indicates the pattern of antibiotic resistance of the *A. baumannii* isolates of various types of infections. *A. baumannii* strains of our study harbored the highest levels of resistance against ampicillin (100%), tetracycline (95%), gentamycin (75%) and cephalexin (60%). Prevalence of resistance against imipenem (5%) and ceftriaxone (35%) were low. Statistically significant difference was seen between the type of samples and prevalence of antibiotic resistance (P < 0.05).

DISCUSSION

Resistant and virulent strains of *A. baumannii* had a high prevalence in various types of human clinical infectious samples of immunosuppressive patients of our study. Totally, 13.33% of samples were infected with *A. baumannii* which was considerable. Some of the most common reasons for the high prevalence of resistant and virulent strains of *A. baumannii* in our study are indiscriminate and unauthorized prescription of antibiotics, daydreaming to the results obtained from the disk diffusion method, prescription of antibiotics

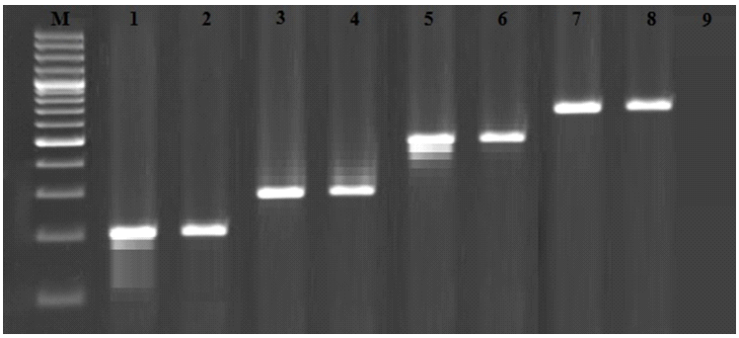

Fig. 1: Results of the gel electrophoresis for detection of the virulence genes of the *A. baumannii* isolates of various types of infections. M: 100 bp ladder (Fermentas, Germany), 1, 2: Positive sample for the *csgA* gene and its positive control, respectively, 3, 4: Positive sample for the *iutA* gene and its positive control, respectively, 5, 6: Positive sample for the *cnf1*gene and its positive control, respectively, 7, 8: Positive sample for the *cvaC* gene and its positive control, respectively, and 9: Negative control.

based on the self-experience of medical practitioners, lack of proper disinfection of hospital environment, inherent nature of the bacteria that has the ability to withstand hard conditions and can survive in the surfaces and finally transmission of resistant pathogens from infected patients and workers to hospital environment and also other patients. Momtaz et al. (2015)[11] reported that *A. baumannii* strains were detected in 121 out of 500 human clinical samples (24.2%) which was higher than our results. Jaggi et al. (2012) (12) reported that the prevalence of *A. baumannii* in various types of clinical infections were 9.4% which was lower than our results. Siau et al. (1996)[13] reported that the prevalence of *A. baumannii* in the cases of infections in the Korean hospitals was 11% which was lower than our results. Differences in the type of samples, method of sampling, number of samples collected, method of experiment, sex and age of patients and geographical area which the samples were collected are the main factors for differences in the prevalence of *A. baumannii* in various investigations.

We found that bacterial strains had the high levels of resistance against ampicillin, imipenem, gentamycin, cephalexin, tetracycline and trimethoprim/sulfamethoxazole antibiotics which showed indiscriminate and unauthorized prescription of antibiotics. Management of multidrug-resistant *A. baumannii* infections is a countless challenge for medical practitioners and clinical microbiologists. Moradi et al. (2015)[14] showed that *A. baumannii strains of human clinical infections had a high prevalence of resistance against all types of antibiotics,* with the exception of carbapenems, lipopeptides, and aminoglycosides. Jaggi et al. (2012)[12] reported that the prevalence of antibiotic resistance in the *A. baumannii* strains of clinical samples against amikacin, gentamicin, tobramycin, aztreonem, cefipime, ceftazidime, ciprofloxacin, Levofloxacin and imepenem were 90.3%, 85.8%, 80%, 94.2%, 90.3%, 92.1%, 67.4% and 67.1%, respectively which was similar to our results. Similar findings have been reported from Denmark[15], Iran[16], Colombia[17] and China[18],

Csga, *cnf1*, *cvaC* and *iutA* virulence genes had a considerable prevalence among the *A. baumannii* strains of our clinical infections. Daryanavard and Safaei (2015) (19) reported that the total prevalence of *csga*, *cnf1*, *cvaC* and *iutA* virulence genes among the samples of UTIs were 55%, 40%, 10% and 30%, respectively which was similar to our findings. Momtaz et al. (2015)[11] reported that the prevalence of *csga*, *cnf1*, *cvaC* and *iutA* virulence genes among the *A. baumannii* strains of clinical infections in Iran were 12.39%, 35.53%, 21.48% and 19%, respectively which was lower than our results. Mohajeri et al. (2016)[20] showed that 40 isolates of *A. baumanni* strains of clinical infections had *traT* (80%), 17 isolates had *cvaC* (34%) and 8 isolates had *iutA* (16%) genes. These genes are the most common causes of adhesion and invasion of *A. baumanni* to the epithelial cells of the human organs.

CONCLUSIONS

In conclusion, we identified a large number of resistant and virulent strains of *A. baumannii* in the wound, urinary and respiratory infections of immunosuppressive patients hospitalized in Iranian hospitals and health care centers. Totally, respiratory infections had the highest prevalence of bacteria and also *csga* and *cnf1* were the most commonly detected virulence genes. We found that resistance against ampicillin, tetracycline and gentamycin was maximum. Rapid diagnosis of infections caused by *A. baumanni* and its treatment with imipenem and ceftriaxone can reduce the risk of dissemination of *A. baumanni*'s infections. Judicious prescription of antibiotics according to the results of disk diffusion method can help to decrease prevalence of resistance.

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