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Occurrence and molecular characterization of multidrug-resistant *Acinetobacter baumannii* isolated from humans and dogs

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Abstract

This study investigated the occurrence, antibiogram and molecular characterization of *Acinetobacter baumannii* in hospitalized humans and dogs. A total of 120 clinical samples including (64 sputum, 40 urine samples from human and 16 wound swabs from dogs) were cultured on MacConkey agar plates and CHROMagar Acinetobacter medium. Sixty two *A. baumannii* strains were identified from both human and animal sources with a percentage of 51.7%. The occurrence of *A. baumannii* was 56.3% in sputum and 35% in urine and 75% in wound swabs. All 62 *A. baumannii* isolates were tested for the antimicrobial susceptibility by disc diffusion method. Colistin showed a lowest resistance percentage (3.2%), while a complete resistance (100%) of *A.baumannii* strains was for aztreonam. Moreover, the recovered 62 *A. baumannii* strains were screened by PCR for integrase genes (*intI1 & intI2*). Integron-positive isolates (29.03%; 18/62) harbored both *intI1 & intI2* genes. The distribution of integrases was 30.6 % in sputum- recovered strains, 28.5% in urine-originated isolates and 25% in wound-identified strains. This study clarified that multidrug-resistant *A. baumannii* strains of dog and human origin implicated a zoonotic potential; and PCR integrase gene is useful in screening the epidemic strains of *A. baumannii*.

Keywords: *Acinetobacter baumannii,* Multidrug resistance, Humans, Dogs, Integrases.

Introduction

Multidrug-resistant (MDR) *Acinetobacter baumannii* is gram negative coccobacilli causing nosocomial infections in patients[24)]. *A. baumannii* may cause pneumonia in ventilated patients, infections of urinary tract, wound infections[5)]. *A. baumannii* nosocomial spread was demonstrated in pets[13)]. *A. baumannii* isolates were collected from infected surgical wounds in animals hospitalized in a veterinary hospital, confirming *A. baumannii* to be a veterinary nosocomial pathogen[6)]. There is a lack of publications showing the role of animals as a reservoir of *A. baumannii*[4,22)]. Several pets' cases with different *A. baumannii* infections such as urinary, respiratory, wound and reporting an overall attributable mortality of 47%[13)]. The rapid emergence and genetic similarities of MDR bacteria among pet animals and humans implicated a zoonotic transmission[10)]. Integrons are responsible for antibiotic resistance and therefore in the circulating behavior of *A. baumannii*[16)]. The integrons of class 1 enable the genome of *A. baumannii* in capturing and accumulating several resistance genes of antibiotics. An integrase gene (*intI*) encoding the IntI integrase is a

characteristic component of integron[12]. This study aimed to investigate the occurrence, antibiogram and molecular characterization of *A. baumannii* isolated from sputum and urine of patients as well as wounds of dogs.

Materials and Methods

Sample collection:

One hundred and twenty clinical specimens including (64 humans' sputum, 40 humans' urine) from Zagazig Public Health and (16 dogs' wound swabs) from Veterinary Hospital were collected between May and August, 2015.

Isolation and biochemical identification:

Samples were streaked onto MacConkey agar plates (Oxoid) and incubated at 37°C for 24 hrs. Pale colonies were selected and examined microbiologically[25]. The pale colonies were re-cultured onto CHROMagar Acinetobacter medium that was purchased from CHROMagar, France[20]. The microorganisms demonstrating negative results for Gram staining and oxidase test were biochemically confirmed by the API 20E kit (Bio-Mérieux, France).

Antimicrobial susceptibility testing:

The disc diffusion method was carried out as previously documented[3]. The interpretation of the results was done according to Clinical laboratory standard International[8].The plates were incubated at 37°C for 16-18 hrs. Panels of fourteen antibiotic discs were used (Oxoid, England).

Minimum inhibitory concentrations (MICs):

The MICs of the same antimicrobials (Sigma-Aldrich) tested for resistance was detected by the agar dilution method according to Clinical laboratory standard International[7]. The mean and standard deviation were done using SPSS 16 statistical software for Windows.

PCR amplification of integrase genes:

The DNA was extracted from *A. baumannii* isolates as previously described[17]. The program of PCR amplification for integrase genes (types 1&2) were carried out using the following primers (Int1 F 5`CAGTGGACATAAGCCTGTTC 3`; Int1 R 5` CCCGAGGCATAGACTGTA 3`; Int2 F 5` TTG CGAGTATCCATAACCTG3`; Int2 R 5`TTACCTGCA CTGGATTAAGC 3`)[16]. The PCR protocol was simultaneously carried out for 35 cycles as follows: denaturation at 94°C for 30 s; annealing at 55°C for 30 s and finally extension at 72°C for 30 s.

Results and Discussion

In this study, 62 *Acinetobacter baumannii* isolates were identified from 120 clinical samples from dogs and humans with an overall prevalence of 51.7%. Also, the occurrence of *A. baumannii* in humans was 56.3% (36 out of 64) in sputum and 35% (14 out of 40) in urine samples (Table 1). Other studies reported different prevalence rates of *A. baumannii* at different geographic areas. The isolation percentage of *A. baumannii* was 13.6% in urine specimens and 54.5% in bronchoaspirates of patients attending Italian hospital[9]. In Saudi Arabia, the isolation rates of *A. baumannii* were 35.4%, 27.1% and 25% from wound swabs, sputum and urine samples from patients attending King Fahd Hospital, respectively[11]. Lower isolation rate of *A. baumannii* (13.6%) was from urine and sputum of patients in Saudi Arabia[1].

Little data is available regarding pets colonization with *A. baumannii* and few literatures have demonstrated the importance of pet animals as a reservoir host for transmission of potentially zoonotic *A. baumannii*[4]. Concerning

the occurrence in dogs (Table 1), *A. baumannii* was isolated from wound swabs with a percentage of 75% (12 out of 16). On the other hand, lower isolation rate of *A. baumannii* was 8.5% from pharyngeal and wound swabs of dogs in Reunion Island[20]. This study contrasted the lower prevalence rate of *A. baumannii* (6. 5%) in rectal, oral and wound swabs from pets at veterinary clinics[4]. Higher occurrence of *A. baumannii* in both clinical samples from humans and dogs in our study may be due to that the sample collection was conducted during the summer season. This finding was in accordance with the study, which showed an increase in *A. baumannii* prevalence rate (17%) monthly per each 10°C elevation in temperature[21]. The climatic conditions may influence the prevalence of *A. baumannii* isolated from dogs because the infectious agents, environmental and animal reservoirs and the replication rate of pathogens are sensitive for climatic changes[23].

The MDR strains of *A. baumannii,* are relatively prevalent in the household pets, have implicated a zoonotic potential[10,19]. In Table 1, the results of MDR *A. baumannii* isolates showed resistance to a total number of resisted antimicrobial types (8/14-14/14). The resistance percentages of *A. baumannii* isolates of human and dog origin were ranged from 3 to 50%. With the lowest *A. baumannii* resistance percentage (3%), only one sputum-recovered strain showed resistance to 8 antimicrobials. Nevertheless with the highest *A. baumannii* resistance percentage (50%), seven urine-originated isolates demonstrated resistance to 13 antibiotics. Related studies have been recorded MDR *A. baumannii*[9]. Of interest, the transmission of antimicrobial resistance of potentially zoonotic *A. baumannii* from infected small animal practices to humans either by direct contact or indirectly via the environmental contaminations of households and pet clinics[10].

Table 1. Occurrence of *Acinetobacter baumannii* in different clinical samples and number of the resisted antibiotic types

Source	Samples No	positive		Multi-resistant isolates No (%)						
		No	%	8*	9*	10*	11*	12*	13*	14*
Sputum	64	36	56.3	1 (3)	2(6)	2(6)	3(8)	3(8)	15(41)	10(28)
Urine	40	14	35	1(7)	1(7)	1(7)	1(7)	1(7)	7(50)	2(15)
Wound swabs	16	12	75	1(8)	1(8)	1(8)	1(8)	1(8)	3(25)	4(35)
Total	120	62	51.7	3(5)	4(6)	4(6)	5(8)	5(8)	25(40)	16(27)

*Number of resisted antimicrobials versus the same isolates

Table 2. In vitro antibiotic resistance profile of *Acinetobacter baumannii* isolated from clinical cases

No	Antibiotic	Sourc e of isolates							
		Sputum (No=36)		Urine (No=14)		Wound swabs (No=12)		Total (No=62)	
		No	%	No	%	No	%	No	%
1	Amikacin 30 μg	24	66.7	9	64.3	9	75.0	42	67.7
2	Gentamicin 10 μg	34	94.4	12	85.7	12	100.0	58	93.5
3	Etrapenem 10 μg	34	94.4	14	100.0	12	100.0	60	96.8
4	Imipenem 10 μg	30	83.3	11	78.6	10	83.3	51	82.3
5	Meropenem30 μg	29	80.6	10	71.4	9	75.0	48	77.4
6	Cephalothin 10 μg	35	97.2	14	100.0	12	100.0	61	98.4
7	Cefuroxime 30 μg	35	97.2	14	100.0	12	100.0	61	98.4
8	Cefoxitin 30 μg	35	97.2	14	100.0	12	100.0	61	98.4
9	Ceftazidime 30 μg	34	94.4	12	85.7	11	91.7	57	91.9
10	Ceftriaxone 30 μg	34	94.4	12	85.7	11	91.7	57	91.9
11	Cefepime 30 μg	22	61.1	5	35.7	8	66.7	35	56.5
12	Aztreonam 30 μg	36	100.0	14	100.0	12	100.0	62	100.0
13	Ampicillin 10 μg	34	94.4	14	100.0	12	100.0	60	96.8
14	Colistin 10 μg	1	2.8	1	7.1	0	0.0	2	3.2

With regard to the in vitro antibiogram sensitivity test, the colistin showed a lowest resistance percentage (3.2%, 2 out of 62) for *A. baumannii* in clinical samples (Table 2). However, higher resistance percentages of *A. baumannii* in this study were found to be 100% for aztreonam, 98.4% for each cephalothin, cefuroxime and cefoxitin and 96.8% for each etrapenem and ampicillin.

Table 3. Mean of MIC (μg/ml) of common antibiotics versus *Acinetobacter baumannii* isolated from clinical specimens

No	Antibiotic	Sputum (No=36)	Urine (No=14)	Wound swabs (No=12)	Total (No=62)
1	Amikacin	25.6±9.5	22.9±9.9	26.7±9.8	25.2±9.6
2	Gentamicin	7.9±1.8	7.3±1.9	8.0±0.0	7.8±1.6
3	Etrapenem	3.9±0.3	3.8±0.9	4.0±0.0	3.9±0.5
4	Imipenem	7.5±1.6	7.0±2.5	7.4±2.0	7.4±1.9
5	Meropenem	7.2±1.9	6.7±2.6	6.8±2.4	7.0±2.2
6	Cephalothin	16.0±0.0	15.1±3.2	16.0±0.0	15.8±1.5
7	Cefuroxime	16.0±0.0	15.1±3.2	16.0±0.0	15.8±1.5
8	Cefoxitin	16.0±0.0	15.1±3.2	16.0±0.0	15.8±1.5
9	Ceftazidime	15.8±1.3	15.4±2.1	15.3±2.3	15.6±1.7
10	Ceftriaxone	30.9±4.7	28.6±9.0	30.0±6.9	30.2±6.3
11	Cefepime	15.4±2.7	14.1±4.7	15.0±3.5	15.0±3.4
12	Aztreonam	16.0±0.0	16.0±0.0	16.0±0.0	16.0±0.0
13	Ampicillin	15.8±1.3	16.0±0.0	16.0±0.0	15.9±1.0
14	Colistin	1.1±0.5	1.2±0.8	1.0±0.0	1.1±0.5

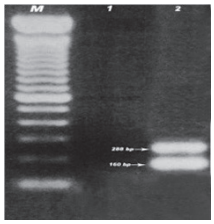

Fig. 1. Representative PCR amplification of *Acinetobacter baumannii* integrase (intI1& intI2) genes from a dog wound. M: DNA marker (100 bp); 1: negative control and 2: *intI1*gene (160 bp) & intI2 gene (288 bp).

In a concordant study, all *A. baumannii* isolates were resistant to cefepime, cefotaxime, ceftazidime, ceftriaxone, gentamicin, and aztreonam but most isolates were sensitive to colistin[2]. Also, the percentages of *A. baumannii* resistance were 8% for ciprofloxacin, 55% for ampicillin- sulbactam and 74% for amikacin[2)]. Also from Table 3, the total mean of MIC of common antibiotics versus *A. baumannii* was ranged from 1.1±0.5 μg/ml (colistin) to 30.2±6.3 μg/ml (Ceftriaxone). In India, 6.4% and 8.3 of *A. baumannii* isolates showed resistant and intermediate results for Meropenem by MIC, respectively[14)].

Six integron classes encoded different integrase genes have been recorded in A. baumannii, and class 1 integrons are more prevalent[15].With regard to PCR amplification of integrase genes in *A. baumannii* isolates (Fig. 1); the overall incidence of integrase genes (*intI1& intI2*)was 29.03% (18 out of 62 isolates).

In this study, all isolate showed positivity for integrons harbored both *intI1* gene (with an amplified fragment of 160 bp) and *intI2* gene (with an amplicon of 288 bp). Also, prevalence of intgerase genes was 30.6 % (11 out of 36) in isolates from human sputum, 28.5% (4 out of 14) in those recovered from urine samples and 25% (3 out of 12) in those isolated from wound swabs of dogs. A nearly similar distribution of the *int1* gene in *A. baumannii* isolates was found to be 19% (12 out of 64) from sputum and wound swabs of patients in Egypt and Saudi Arabia[18)].

However, the integrase gene PCR showed a higher frequency (50%, 24 out of 48) of integron positive *A. baumannii* isolates in Netherland[16)]. This study clarified that the PCR of integrase gene is a rapid tool for identification of *A. baumannii* strains with the epidemic potentials because *A. baumannii* epidemic strains, harboring more integrons than the nonepidemic one, were correlated with the resistance to many antibiotics[16)]. Therefore, this is very important to introduce the specific control measures to minimize the spread of *A. baumannii* nosocomial infections.

In conclusion, this study confirmed that circulating MDR *A. baumannii* strains of dog and human origin implicated a zoonotic potential. Besides, the PCR of integrase gene is a suitable tool for screening of *A. baumannii* strains with the epidemic potentials. Therefore, this MDR of *A. baumannii* promotes a novel development of phage therapy.

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