



ISOLATION AND IDENTIFICATION OF *ACINETOBACTER BAUMANNII* IN HILLA CITY

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ABSTRACT

The nosocomial infections are major medical problems in all areas of the world and they remain one of the main causes of morbidity and mortality in hospitalized patients. A total of 458 samples were collected during 2012, eleven isolates (2.4%) conformed as *A. baumannii*. These isolates were identified by using microscopic examination, biochemical tests, vitek 2 system and a modern diagnostic system, which included genetic test, by using PCR technique which revealed *rpoB* genes. The highest percentage of isolation 6 (6.25%) was from burn specimens and lower percentage was in blood 1 (0.93%). Some virulence factors and antibiotic sensitivity patterns had been studied for all isolates.

KEYWORDS: *A. baumannii*, isolation, *rpoB* genes, antibiotic sensitivity.

INTRODUCTION

Acinetobacter baumannii is Gram-negative, coccobacillus bacterium, non-fermentative has become an increasingly prevalent cause of nosocomial infections^[1-3]. They are the second most common gram negative nosocomial bacilli next *Pseudomonas* encountered in clinical specimens^[4]. These organisms can occur frequently as components of commensal flora of man and animals which are regular contaminants of the hospital environment it is implicated in various ranges of infections includes bloodstream infections, ventilator-associated pneumonia, skin and soft-tissue infections, wound infections, respiratory and urinary-tract infections, endocarditis, and secondary meningitis^[5]. Sequence analysis of four zones of the RNA polymerase β -subunit (*rpoB*) gene and its flanking spacers has been proposed as a useful molecular method for identification of *Acinetobacter* species^[6]. The method, named partial *rpoB* gene sequence analysis. Presence of virulence determinants may play a cardinal role in expressing its resistance pattern. The existence of some of the virulence factors poses a deleterious effect within the host tissue and its ability to adhere to inanimate objects such as medical devices within the hospital environment renders its persistence as a successful pathogen^[7].

MATERIALS & METHODS

Isolation and Identification

A total of 458 samples were collected during 2012, the sample were distributed in urine, blood, sputum, swabs from wound infection and swabs from burn. For this

purpose, different hospitals in Hilla were included in the study. Preliminary identification was performed by gram staining, culturing on MacConkey's agar (Himedia, India) and incubated for overnight at 37°C, non lactose fermenting bacteria or colorless were sub-cultured and incubated for additional overnights. Suspected bacterial isolates which their cells are Gram negative coccobacillary and negative to oxidase, positive catalase further identified by the traditional biochemical test according to^[8,9] then additional confirmed by Vitek 2 system.

Detection of *ropB* Genes by Polymerase Chain Reaction

DNA extraction

Genomic DNA was successfully extracted from *A. baumannii* isolates by using a commercial genomic DNA purification kit (Geneaid/Taiwan). The concentration and purity of extracted DNA was determined. DNA bands were confirmed and analyzed by gel electrophoresis.

PCR Mixture and cycling conditions

The DNA template extracted from *A. baumannii* isolates were subjected to *rpoB* genes by PCR, the protocol was depending on manufacturer's instruction and the right PCR cycling program parameters conditions were installed as in Table (1).

Agarose Gel Electrophoresis

All requirements, technical and preparations of agarose gel electrophoresis for DNA detection and analysis were performed by^[10]. Finally, the gel was photographed using Biometra gel documentation system.

TABLE 1: PCR Protocol for *rpoB* gene

Steps	Temperature	Time	No. of cycles
Initial denaturation	94 °C	3 min	
Denaturation	94 °C	30 sec	30 cycle
Annealing	52 °C	30 sec	
Elongation	72 °C	30 sec	
Final Elongation	72 °C	10 min	

Identification of some virulence factors

Screen for the presence of Capsule, Biofilm, Pellicle formation and various enzymes such as Gelatinase, Lecithinase, Protease and Lipase, Hemolysin production had been done.

Antibiotics sensitivity

Antibiotic susceptibility pattern was analyzed by disc diffusion test as [11] CLSI guidelines 2012.

RESULTS

Among the 458 clinical samples, eleven isolates (2.4%) identification as *A. baumannii*. Vitek 2 assigned eleven isolates as *A. baumannii* with an excellent confidence level (99.99 % probability) however, the distribution of the *Acinetobacterbaumanni* isolates and their sites of isolation are listed in Table (3.2)

TABLE 2: Distribution of *A.baumannii* according to infection

Type of infection(sample)	No. of sample	No. of positive isolate	%
Urinary Tract Infection(Urine)	138	2	1.44
Septicemia(Blood)	107	1	0.93
Respiratory Tract Infection(Sputum)	53	0	0
Wound Infection(Swab)	64	2	3.12
Burn Infection(Swab)	96	6	6.25
Total	458	11	2.40

Detection of some virulence factor

All isolates have the ability to produce biofilm, gelatinase, and pellicle formation (100%). While there're variable production for Lipase 6/11(54%), Protease 8/11 (72%)

and for Lecithinase 4/11(36%). Capsule appears in 6/11 (54%). All isolates have negative result for Hemolysin enzyme production.

TABLE 3: Virulence factor (+): positive, (-): negative

No.	Test	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
1	Capsule	-	-	-	-	+	+	-	+	+	+	+
2	Biofilm	+	+	+	+	+	+	+	+	+	+	+
3	Gelatinase	+	+	+	+	+	+	+	+	+	+	+
4	Pellicle formation	+	+	+	+	+	+	+	+	+	+	+
5	Protease	+	+	-	+	+	+	-	+	-	+	+
6	Lipase	+	+	-	-	-	+	+	+	-	+	-
7	Lecithinase	-	+	+	-	-	+	+	-	-	-	-
8	Hemolysin production	-	-	-	-	-	-	-	-	-	-	-

Molecular Identification of *A.baumannii*

Polymerase chain reaction results showed that 350 bp partial *rpoB* gene sequences were successfully obtained in primers from all eleven isolates of *A. baumannii*.

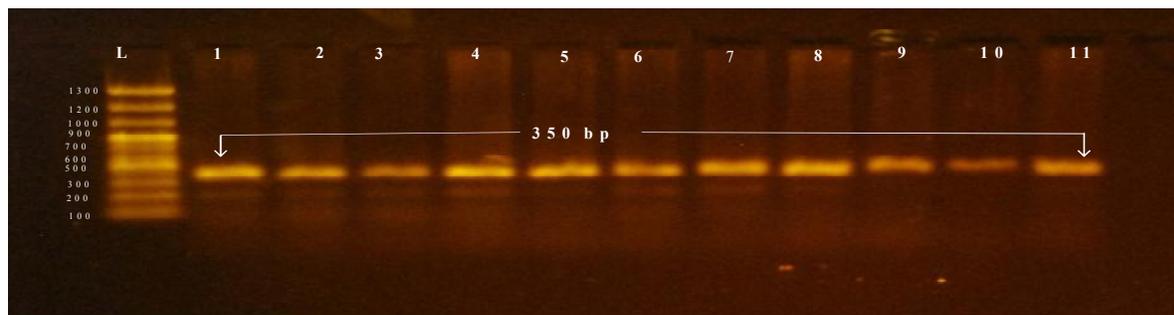


FIGURE1: Ethidium bromide –stained agarose gel of PCR amplified products from extracted DNA *Acinetobacterbaumanni* isolates and amplified with *rpoB* gene primers (forward and reverse). The electrophoresis was performed at 70 volt for 2 hour . Lane (L): DNA molecular size marker (100-bp ladder) , Lanes 1, 2, 3,4,5,6,7,8,9,10,11 isolates of *A. baumannii* show positive results

Effect of Some antibiotics on bacterial isolate

All *A.baumannii* isolates had resistance to Penicillin, Piperacillin, Ampicillin, Amoxicillin, Cefotaxime, Tetracyclin, Ciprofloxacin, Rifampicin, Cefepime, Ceftriaxone and Ceftazidime (100%) whereas the resistance to Tobramycin,

Trimethoprim sulfamethoxazole at (81%). Isolates appear resistance to Amikacin (63%), where (54%) of isolates were resistance to Gentamicin and Imipenem and finally only (9.0%) were resistance to polymyxin B fig (3.2)

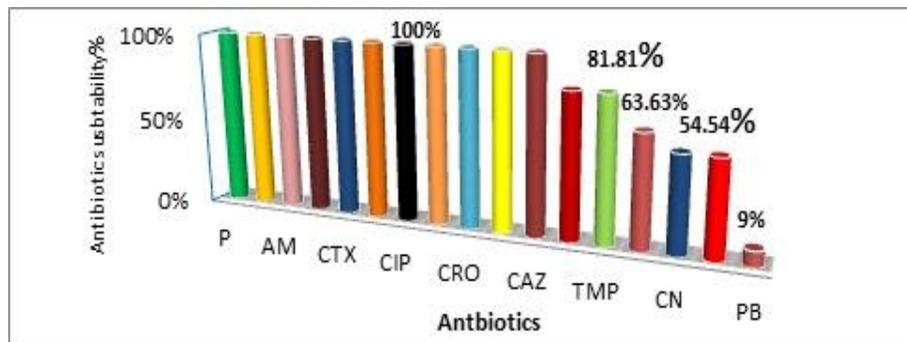


FIGURE 2: Profile of antibiotics susceptibility of *A. baumannii* isolates

DISCUSSION

Evident from many studies that *A. baumannii* holds a repertoire of robust virulence characters which might contribute to its pathogenic potential. Versatility in its ability to grow on a variety of medical devices enables it to establish itself in hospital environment [12]. [6] proposed that sequence analysis of the *rpoB* gene has been particular found to represent a reliable and rapid identification method for *Acinetobacter* species Fig. (1).

The biofilm confers several advantages to bacteria (a) the biofilm is an efficient scavenging system for trapping and concentrating essential minerals and nutrients from the environment [13]. (b) sessile bacteria are more resistant to the effects of antibiotics and to effector cells and molecules of the host defense system [14] the proximity of bacteria within the biofilm provides an ideal environment for the horizontal transfer of genes, including antibiotic resistance genes [15]. The result in table-3, showed that all isolates have the ability to form biofilm and this result has been demonstrated in a number of studies showing that a clinical isolates of the bacterium can attach to and form biofilm on glass surface [16]. Gelatinase is a proteolytic enzyme that hydrolyse gelatin, which can cross cell membrane and hydrolyze collagen in subcutaneous tissues during wound infections, its ability to hydrolyze collagen and certain bioactive peptides suggests its participation in the initiation and propagation of inflammatory process [17], all isolates have the ability to produce gelatinase comparable with [12] showed gelatinase producers in their isolates [18] members of the *A. baumannii* group have a higher ability to form pellicle than other species this feature could be connected to the higher colonization rate of patients by pathogenic *A. baumannii*, and probably contributing to the increased risk of clinical infection. We have observed lipase activity with our isolates which were in accordance with the report of [19]. [20] who indicates that *A. baumannii* is producing protease enzyme, protease enzyme secreted outside of the cell through a process of growth as they accumulate significantly in the phase stability of the bacteria, and it is one of virulence factors important for *A. baumannii* bacteria [21]. Previous study by [22] showed very lesser percentage of lecithinase in *Acinetobacter* species. [23] Established that capsular polysaccharide from the *A. baumannii* surface exposed and is a virulence factor which is conceded to our study [24]. In experiments using the sheep blood agar plate assay in *A. haemolyticus* exhibited strong haemolytic activity (referred to as β -haemolysis) whereas all other strains of *Acinetobacter* exhibited weak (α -haemolysis) or no (γ -

haemolysis) strains that parallel with our results. Results of antibiotic sensitivity Fig. (2) were coincided with [25] who founds that clinical isolates of *A. baumannii* showed resistance 100% to ampicillin, piperacillin/tazobactam, ceftriaxone, ceftazidime, cefepime and ciprofloxacin. Previous studies showed a significant increase in tetracycline resistance in Iran and the results of this study confirmed previous reports [26,27]. Also resistant to Rifampicine, converged with [28], whereas the resistance to Trimethoprim sulfamethoxazole coincided with [29] that referred to all isolates of *A. baumannii* show resistance to Trimethoprim [30] isolated *Acinetobacter* from different body fluids which has good sensitivities for gentamycin. [31] showed gradually increasing resistance of *Acinetobacter*. The antibiotic susceptibility patterns clearly showed the increasing resistance of *A. baumannii* to various antibiotics. Polymixin B is one agent which is active against *A. baumannii*. In study of clinical isolates from the Western Pacific region showed 3.3% resistance of *A. baumannii* to colistin [32, 33]; in a study in Korea there was high resistance to colistin and polymixin.

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