International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 6 (June-2015) pp. 128-134

www.ijcrar.com

Screening of virulence factors in *Acintobacter baumannii* isolated from clinical samples

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KEYWORDS

Acinetobacter baumannii, Virulence factor, Gelatinase, Biofilm, Antibiotics.

A B S T R A C T

Acinetobacter baumannii is a opportunistic nosocomial pathogen, it has a high incidence among immunocompromised individuals, arising largely from its extensive antibiotic resistance spectrum. The present study was focused on evaluate biofilm, Pellicle formation, gelatinase activity, bacteriocin production and antibiogram profile. The present study demonstrate that all *Acinetobacter baumannii* isolates were positive for gelatinase activity, 12 isolates formed biofilm, while 13 isolates formed pellicle, and it were found that three isolates (Ab2, Ab9, Ab12) have the ability to produce bacteriocin. Finally, sensitivity of 15 clinical isolates was tested against 10 antibiotics, results revealed variable resistances against different antibiotics.

Introduction

The genus *Acinetobacter* sp currently consists of more than 40 geno species of which *Acinetobacter baumannii* (Acinetobacter genospecies 2), Acinetobacter genospecies 3 and Acinetobacter genospecies 13TU are clinically most relevant genospecies (Kim et al., 2012). *Acinetobacter* are strictly aerobic gram negative coccobacilli that are widely distributed in soil and water, but also commonly found in the hospital environment Genus, *Acinetobacter baumannii* is considered as an emerging nosocomial pathogen in intensive care units (Karah et al., 2011). The fact that *A. baumannii* is more frequently associated with colonization than infection, even in susceptible patients, emphasizes the relatively low virulence of this bacterium. However, severe *A. baumannii* infections, including pneumonia and bacteremia, do occur in critically ill patients. During the last three decades, clinicians are increasingly faced with infections of *A. baumannii* isolates resistant to almost all clinically applicable antibiotics (Goossens, 2005). The acquisition of antimicrobial resistant in *A. baumannii* strains is also related to their ability to form biofilms, which are assemblages of surface microbial cells that are enclosed in an extracellular polymeric matrix (Cai et al., 2012). Multiple bacterial virulence factors are required for pathogenesis of infections caused by *A.*

baumannii, these include outer membrane proteins family, gelatinase activity, bioflim production, capsular polysaccharides, bacterial phospholipases, penicillin-binding proteins, secreted outer membrane vesicles, siderophores and biofilm formation (Park et al., 2012). The aim of this study to screening of virulence factors in *Acinetobacter baumannii* isolated from clinical samples.

Material and Methods

Bacterial isolates

Fifteen clinical isolates of *Acinetobacter baumannii* were selected from the laboratory of Professor Al thahab at Babylon University, IRAQ. These isolates were identified using conventional techniques, API 20NE system (bioMerieux, France) and vitek 2 system (bioMerieux, France).

Antibiogram test

The antimicrobial susceptibility testing with disc diffusion method was performed on isolates of *Acinetobacter baumannii*. The test was evaluated in *Acinetobacter baumannii* susceptibility to 10 antibiotics Including, Amoxycillin, Amikacin, Ciprofloxacin, Cefalothin, Cefotaxime, Chloramphenicol, Rifampicin, Tetracycline, Trimethoprim/ Sulfamethoxazol, and Imipenem, the standard inhibition diameters as recommended by CLSI (2012).

Biofilm assay

Biofilm formation assay was performed to detect the ability of bacterial isolates to produce biofilm layer**.** It was carried out by inoculating tubes containing 5 ml of nutrient broth with young bacterial isolates. The tubes were incubated at 37°C for 48 hour. After incubation period the bacterial growth was removed and safranin stain was added to each tube for 10 minutes The stain was discarded from tubes and it was left to dry in the air for 15minutes, the staining of tube walls with red color was considered as a positive result (Costerton *et al*., 1999).

Pellicle assay

According to (Marti *et al*., 2011) was performed by inoculating 5 ml of MH broth tubes with a single colony of each isolates of *A. baumannii* in separate tubes, and then incubated for 5 days at 25°C. Pellicle formation appeared as white layer on the surface of MH broth.

Gelatinase assay

According to (Sechi et al., 2004) was achieved using Luria Bertani (LB) agar containing (30 g/L), the growth isolated colony in brain heart infusion broth in sterile tube, incubated at 37°C for 18 hour, one loop of each of the colonies was inoculated onto LB agar containing gelatin, incubated at 37°C.The plates were incubated overnight at 37°C and then cooled for 5 hours at 4°C and the positive result by appearance of a turbid halo for gelatinase production.

Bacteriocin production

Bacteriocin Production was detected according to (Morris and Wells, 1974) the isolates of *A. baumannii* were inoculated in a diametric streak on two Tryptic Soy Agar plates and incubated at 37 °C for 72 h. The macroscopic growth was scraped off the agar surfaces by using a glass slide. The remaining culture on the plates was killed by adding 2 ml of chloroform into the lid of the petri dish containing filter paper and then by inverting the dish in the lid. After approximately 15 min, the plates were aerated by exposing the agar surface for 15 min. The 5 indicator strains (*E. coli, Pseudomonase aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus,*

Proteus mirabilis) were then applied at right angles to the original line of growth. The plates were then incubated at 37 °C for 24 h.

Results and Discussion

Virulence factors

In the present study, antibiotic resistance and virulence factors such as gelatinase activity, biofilm, pellicle formation and bacteriocin production have been detected in fifteen clinical isolates of *Acinetobacter baumannii*. Gelatinase activity, biofilm, and pellicle formation were detected as showed in table 1. Fifteen isolates were positive to gelatinase activity, but the isolated varied in their biofilm and pellicle formation (Table 1).

Once bacteria have adhered to a surface, they can multiply and form microcolonies, followed by the production of exopolysaccharides, resulting in a highly structured microbial community (O'Toole *et al*., 2000). The variation in biofilm formation is possibly related to the variations in *csuA/BABCDE* genes of the tested strains, because these genes have been considered as the most common important factors that can influence biofilm formation among different strains (Howard *et al*., 2012). Results of previous study showed that 16 of 20 Acinetobacter strains were able to form a biofilm (Sechi et al., 2004). While another study, demonstrate that 64 of 86 *A. baumannii* isolates were positive for biofilm formation, with 10 isolates forming strong biofilms, 27 forming medium strength biofilms, and 27 forming weak biofilms (Cevahir et al., 2008). In this study, 12 of 15 *A. baumannii* isolates were positive for biofilm formation. The pellicle formation need different proteins that are associated with pili formation like chaperone-usher system pili and the putative type III pilus (Marti *et al*., 2011). Sara *et al*. (2011) reported that the 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. Gelatinase is a proteolytic enzyme that is capable of hydrolyse gelatin which can cross cell membrane and hydrolyze collagen in subcutaneous tissues during wound infections. Its associated with inflammation, therefore to contribute to virulence in human and animal (Kanemitsu et al., 2001). Cevahir et al. (2008) reported that gelatinase activity occurred in 12 isolates (14%). In this study all isolates were positive for gelatinase activity.

Bacteriocin production

Bacteriocins are proteinaceous and ribosomally synthesized antibacterial compounds produced by bacteria that exhibit bactericidal activity against closely related species (Riley and Wertz, 2002). In recent years, a renewed interest in bacteriocin like activities has led to the discovery, isolation, and purification of bacteriocins from both gram-negative and gram-positive bacteria. They are now being considered for a variety of antimicrobial uses in foods and medicine (Papagianni, 2003; Mahrous et al., 2013). In the present study, the ability of the bacterial isolates to produce bacteriocin was identified and it were found that three isolates (Ab2, Ab9, Ab12) have the ability to produce bacteriocin that had its effect on some isolates of bacteria (*E. coli, Pseudomonase aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Proteus mirabilis*) as showed in table 2.

Table.1 Virulence Factor of *Acinetobacter baumannii*

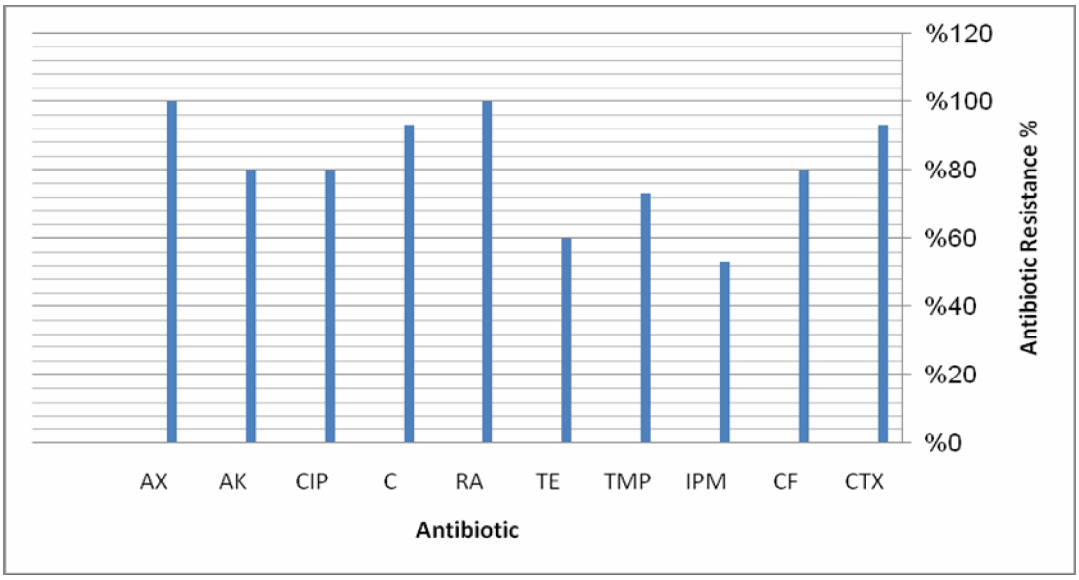
Isolates No.	Gelatinase activity	biofilm formation	pellicle formation
Ab1	+	+	+
Ab2	+	+	+
Ab3	+	+	+
Ab4	+	+	_
Ab5	+	+	+
Ab6	+	+	+
Ab7	+	_	+
Ab8	+	_	+
Ab9	+	+	+
Ab10	+	+	+
Ab11	+	+	+
Ab12	+	+	+
Ab13	+	+	_
Ab14	+	+	+
Ab15	+	_	+

(+):positive, (-):negative

Table.2 Bacteriocin Production of *Acinetobacter baumannii*

	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Ab12	Ab13	Ab14	Ab15
E.coli	_	+	_	_	_	_	_	_	+	_	_	_	_	_
P.aeruginosa	_	_	_	_	_	_	_	_	_	_	+	_	_	_
K.pneumoniae	_	_	_	_	_	_	_	_	+	_	+	_	_	_
S. aureus	_	+	_	_	_	_	_	_	_	_	_	_	_	_
P.mirabilis	_	+	_	_	_	_	_	_	_	_	+	_	_	_

Figure.1 Antibiotic resistance of 15 *Acinetobacter baumannii*



Antibiotic resistances

Susceptibility of *A. baumannii* isolates to 10 antibiotics; amikacin, amoxicillin, chloramphenicol, ciprofloxacin, cefalothin, cefotaxime, rifampin, imipenem, trimethoprime-sulphamethoxazole and tetracycline, were determined by the disk diffusion method in accordance with the clinical and laboratory standards institute guidelines (2012) depending on a diameter of inhibition zone (mm). Figure 1 showed high level resistance of *A. baumannii* clinical isolates to some of the antibiotics. The current study revealed that all *A. baumannii* isolates had 100% resistance to amoxycillin, and rifampin. The results revealed that *A. baumannii* showed a highest resistance to chloramphenicol (93%), cefotaxime (93%), amikacin (80%), ciprofloxacin (80%), cefalothin (80%), trimethoprime-sulphamethoxazole (73%), tetracycline (60%) and imipenem (53%).

A study done by Prashanth and Badrinath, (2004) reported that the isolates of *A. baumannii* were resistant to Ciprofloxacin and Cefotaxime. Hujer *et al*. (2006) showed that *A. baumannii* strains isolated from military and civilian personnel injured in the Iraq/Kuwait war showed 20% resistance to Imipenem. Resistance to quinolones (ciprofloxacin) in *Acinetobacter* species is mostly due to chromosomal mutations in the quinolone resistance determining region (QRDR) of the *gyrA* and *parC* genes with the subsequent production of modified bacterial DNA gyrase and topoisomerase IV enzymes (Bonnin *et al*., 2012). Regarding tetracycline the resistance has been related the bacterial ability to produce the efflux pumps AdeABC (as for β-lactams) and to *tet* genes such as *Tet*(A) and *Tet*(B) (Taitt *et al*., 2013). Sadeghifard *et al*. (2010) reported that *A. baumannii* isolates had 100% resistance to aztreonam, cefotaxime, ceftazidime, ceftriaxon, meropenem and ticarcillin-clavulanate, while the resistance percentages to tobramycin and amikacin were 50% and 56%, respectively.

Conclusion

In conclusion, A. baumannii is an important opportunistic and emerging pathogen that can lead to serious nosocomial infections. Its

pathogenic potential includes the ability to adhere to surfaces, form biofilms, display antimicrobial resistance. Furthermore, new experimental approaches are warranted to develop and evaluate novel therapeutic strategies for dealing with A. baumannii infections.

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