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BIOFILM FORMATION AND ANTIBIOTIC RESISTANCE OF *ACINETOBACTER* SPP. ISOLATED FROM SKIN AND WOUND INFECTIONS

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ABSTRACT

Biofilm is a group of cells fixed on a surface and all surrounded by a matrix of organic polymeric materials of microbial origin.

The aim of this study was biofilm formation and antibiotic resistance of *Acinetobacter* spp. isolated from skin and wound infections.

All isolates were characterized with conventional biochemical methods. Antibacterial sensitivity tests were carried out with disc-diffusion technique. MICs of all isolates were determined against 7 important antibiotics. Cell surface hydrophobicity (CSH) was determined. Biofilm formations of some strains with high CSH were determined on different surfaces of polypropylene, microtiter plates (Polycarbonate), glass and venous catheter.

Twenty three *Acinetobacter*spp. were obtained from clinical specimens like wound, burned skin infection. Antibacterial sensitivity tests were carried out with19 antibiotics by disc-diffusion technique. All *Acinetobacter*strains were resistant to cephalosporins and quinolons (95.6%), aminoglycosides (91.3%), sulfanamids (95.6%), tetracyclin (47.8%), carbapenems (100%) and colistin (8.6%). MIC of seven antibiotics except colistinand tetracyclinagainst all isolates was more than 128 µg/ml. Cell surface hydrophobicity (CSH) was determined. Biofilm formations of some strains with high and low CSH were determined on different surfaces of polypropylene, microtiter plates (Polycarbonate), glass and venous catheter.

Biofilm formation of the two selected isolates on glass and polypropylene tubes indicated denser aggregates on polypropylene than glass surfaces. The number of bacteria that adhered to venous catheter surface was reduced after treatment of culture with colistin. One of the isolates with highest biofilm formation was identified by 16S rRNA technique as *Acinetobacter baumannii* Iliya and registered as a new strain which it is a nosocomial agent and its high colonization activity on medical devices was precisely proved in this research.

Keywords: skin and wound infections, antibiotic sensitivity, hydrophobicity, biofilm formation.

INTRODUCTION

Biofilm is a group of cells fixed on a surface and all surrounded by a matrix of organic polymeric materials of microbial origin (extensive exo-polymer of glycocalyx). These exo-polysaccharides, which are 90% of biofilm dry weight, facilitate the connection to the surfaces of micro-colony formation and resistance against antimicrobial materials. Biofilm includes a mass of bacteria stick together on a solid surface and interact. Around them is an external matrix of polysaccharide substance. *Acinetobacter* spp. have evolved asimportant nosocomial pathogens. They are found in diverseenvironments such as soil, water, food products and are often isolated from medical devices[1].A large number of reports describe the outbreaks of *Acinetobacter* spp. associated nosocomial infections such as secondary meningitis, pneumonia, wound, burn and urinarytract infections (UTI)[2,3].There are many different factors increasing hospital infection level that the most important of them include age, surgery, immune system problems, use of immunosuppressive drugs, some chronic diseases like diabetes, renal failure, and cancers and use of wide range of antibiotic[1].*Acinetobacter*is known to show resistance to a majority of commercially available antibiotics (penicillins, aminoglycosides, cephalosporins and quinolones) and therefore raises an importanttherapeuticproblem[4].Biofilm formation on materials and hospital instruments is the other problem which is followed by bacteria adhesion to the different surfaces[5].

The aim of this study was biofilm formationon different surfaces and antibiotic resistant of *Acinetobacter* spp. from skin and wound infection in Kerman hospitals.

PATIENTS AND METHODS

Isolation and identification

Acinetobacter spp. were isolated from the samples of skin and wound infections in Shafa, Afzalipour and Seyedoshohada hospitals. Some biochemical tests including: Gram strain, urea, motility, capsule, catalase, oxidase, indole, methyl red, Simon citrate, triple sugar iron- agar medium were conducted to identify and determine bacteria[6].

Antibacterial susceptibility test

The susceptibility of all isolates to 19 antibiotics (sigma) from different groups was investigated out on Muller-Hinton agar (Hi-Media) using the Kirby-Bauer disc diffusion method.Discswere checked for efficacy against standard strains recommendedby the National Committee for Clinical LaboratoryStandards (1997) as well as others with known antibacterialsusceptibility pattern[7].

Determination of MIC of antibiotics

Determination of the MIC required inhibit the growth of 23*Acinetobacter*spp. using 7 antibiotics were carried out by agar dilution method. Antibiotics were checkedin the range of 1–1024 µg/mL[8].

Bacterial CSH (Cell Surface Hydrophobicity)

CSH was determined by the affinity to xylene.The hydrophobicity index (HI) was calculatedusing the following equation:

HI A_{600}nm _ B_{600}nm /A_{600}nm x100,

Where A_{600} nm denotes the initial absorbance and B_{600}nmrepresents the absorbance after vortex mixing. The isolateswere considered as strongly hydrophobic when the HI was70% and with hydrophilic character when the HI was30%[9]. Results were statistically analyzedusing the Student's t-test at a 5% significance level.

Biofilm formation on abiotic surfaces

Biofilm formation was analyzed in glass, polypropylene tubes and polycarbonate. The biofilms were formed by adding 0.1 ml of the culture to 10 ml 0.5 × NB (Nutrient Broth) dispensed in test tubes. The cultures were incubated at 37 °Cfor 72 hours under two sets of different conditions:1)shaking at 200 r.p.m and 2) stationary. Biofilms were quantified using the crystal violet method[10-12]. A standard strain of *Pseudomonas* (PAO1) was provided from Medical Sciences University of Kerman.

Bacterial adhesion to venous catheters and effect of colistin and tetracycline antibiotic

The selected isolates used for this study were cultivated for 24 h in 0.5 × NBcontaining 0.25 × minimum inhibitory concentration (MIC) (0.5 µg/ml) and 0.5 × MIC (1 µg/ml) concentrations of colistin (Sigma, USA) and tetracycline (Razak, Iran). After the incubation period, antibiotic was removed from the culture by rinsing twice with sterile saline followed by centrifugation. Cultures without antibiotics were used as the controls. Venous catheters (Becton Dickinson) were cut into 1.5-cm-long segments. The segments were then immersed in tubes containing suspensions of the previously standardized strains 10^8 and kept at room temperature for 30 min. After this contact, each fragment was placed in a tube containing of sterile saline solution, and the tubes were manually inverted 40 times. This procedure was repeated 15 times, transferring the fragment to 15 tubes successively, with the objective of removing the non-adherent bacteria. The catheter fragments were removed from the tube and rolled over the surface of petri dish containing nutrient agar. After an incubation period the bacterial colonies were counted. The number of colonies indirectly showed the number of bacteria that adhered to the catheter surfaces[13].

RESULT

Isolation and identification of *Acinetobacter* spp. from skin and wound infections

By biochemical tests identified 23 *Acinetobacter* spp. Obtained from skin and wound infections.

Antibacterial susceptibility test

The all isolates were testedfor resistance or sensitivity to 19 antibiotics from differentgroups. All*Acineto bacter* strains were resistant to cephalosporins and quinolons (95.6%), aminoglycosides (91.3%), sulfanamids (95.6%), tetracyclin (47.8%), carbapenems (100%) and colistin (8.6%) (Table.1).

Determination of MIC

MICs of seven antibiotics from different groups were tested against 23*Acinetobacter*spp. Isolatesthe majority of isolatestolerated concentrations exceeding 512 µg/mL of antibiotics from β lactams groups. However, 96%of isolates were sensitive to colistin and 60% of isolates were sensitive to tetracycline respectively. It wasobserved that more than 99% of isolateswere highly resistant to β-lactam antibiotics (Fig.1)

Bacterial CSH

CSH indices for all the 23 isolates of *Acinetobacter* obtained from skin and wound infection were determined and theyvaried from 35% to83%.*P. aeruginosa* PA01 wasused as the positive control culture.The difference between the strainsHI values was found to besignificantly different with $P<0.05$.

Strains	HI (%)*
***Acinetobacter* spp1**	**83%**
***Acinetobacter* spp2**	**79%**

***HI = (A600nm–B600nm) _100/A600nm.**
Control:*Pseudomonasaeruginosa*PAO1.

Biofilm formation by *Acinetobacter* isolates

The biofilm formation abilities of somestrains with high CSH weredetermined. Quantitative analysis of biofilms formed by *Acinetobacter* isolates on glass and polypropylene surfaces showedthat shaking conditions were suitable for biofilm formation(Fig. 2).The biofilm formation by strains of *Acinetobacter* with high hydrophobicity was higher and significant differencewas observed compared to strains to low hydrophobicity with less biofilm-forming ability with$P<0.001$

Bacterial adhesion to venous catheters and effect of colistin and tetracycline antibiotic

The number of bacteria that adhered cm^{-2} of catheter surface varied from 4500(3.65 log10) to 5050 (3.70 log10).treatment of cultures with 0.5×MIC (1 µg/mL) and0.25×MIC (0.5 µg/mL) of colistin and tetracycline antibiotic significantly reduced the adhesion ability of all isolates (Table 2, 3). Under asimilar set of conditions, cultures treated with 0.5×MICcolistin and tetracycline concentration could reduce the biofilms more than cells treated with 0.25×MIC. One of the isolates with highest biofilm formation was identified by 16S rRNA technique as *Acinetobacter baumannii* Iliya (*Acinetobacter* spp1) (Fig.3).

Table.1. Percentage of antibiotic resistance of 23*Acinetobacter*strains

Number	Antibiotics	Per of resistance
1	imipenem	100%
2	cefepime	100%
3	nalidixic acid	100%
4	norfloxacin	100%
5	ceftazidime	95.6%
6	gentamicin	100%
7	ceftriaxone	100%
8	ceftizoxime	100%
9	sulfamethaxazole	95.6%
10	cefazolin	100%
11	ciprofeloxacin	95.6%
12	cephalexin	100%
13	amikacin	91.3%
14	nitrofurantoin	100%
15	cefixime	100%
16	tetracycline	47.8%
17	cefalotin	100%
18	cefotaxime	100%
19	Colistin	8.6%

Table .2.Effect of antibiotic colistin on number of isolates (log_{10}) adhered to the catheter surfaces

Strain	Control	Culture (0.25× MIC)	Culture (0.5× MIC)
*Acinetobacter*spp1	3.70	1.80	1.17
*Acinetobacter*spp2	3.65	2.96	2.56

Control represents cell count of bacteria without antibiotic treatment

Table .3.Effect of antibiotic tetracycline on number of isolates (log_{10}) adhered to the catheter surfaces

Strain	Control	Culture (0.25× MIC)	Culture (0.5× MIC)
*Acinetobacter*spp1	3.70	3.62	3.62
*Acinetobacter*spp2	3.65	3.08	2.99

Control represents cell count of bacteria without antibiotic treatment

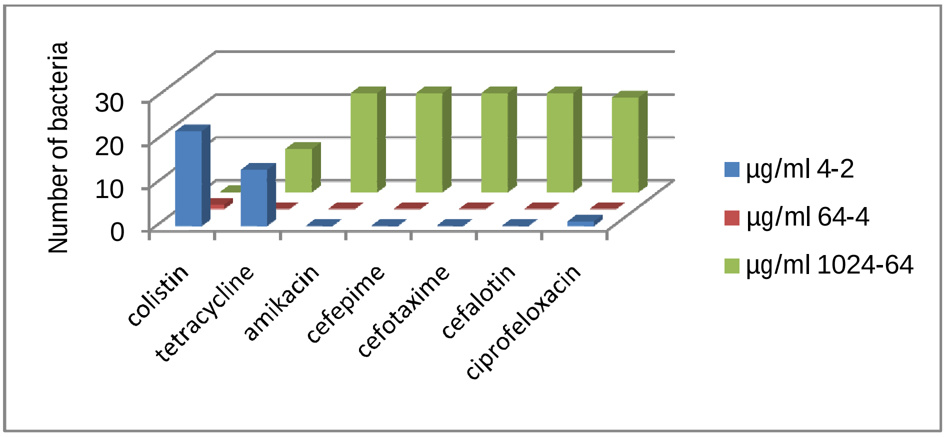

Fig.1. Minimum inhibitory concentration of 23*Acinetobacter*strains with 7 antibiotics Antibiotics were checked in the range of 1-1024 µg/ml

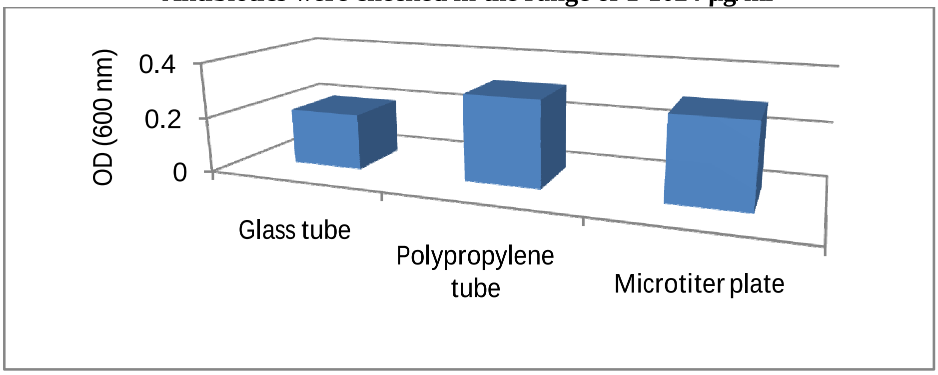

Fig.2: Biofilm formation of *Acinetobacter* spp. on polycarbonate (Micro titer plate), polypropylene and glass surfaces

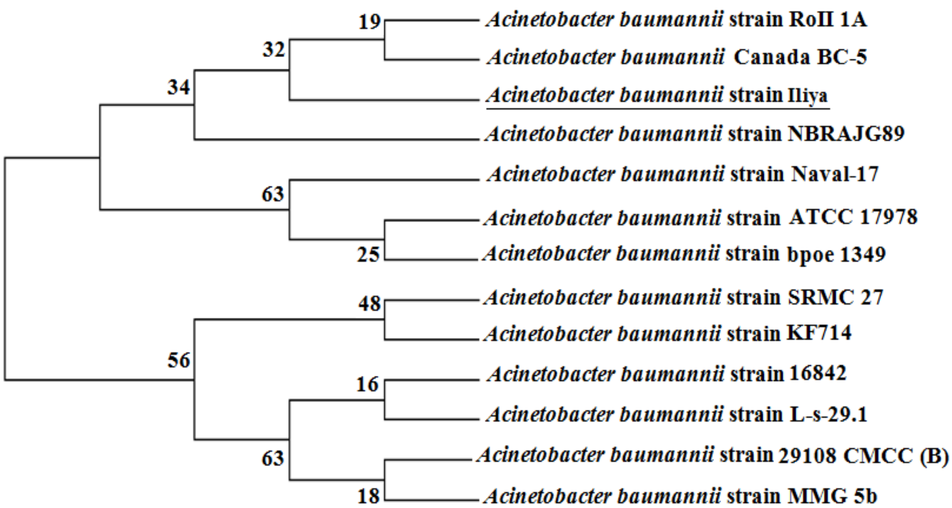

Fig.3.Phylogenic tree of *Acinetobacter baumannii* Iliya

DISCUSSION

In recent decades, increasing involvement of *Acinetobacter* spp.in hospital and their antibiotic resistance nature has been an important observation[14]. We isolated twenty three *Acinetobacter* spp. from clinical specimens like wound, burned skin all *Acinetobacter* strains showed resistant to Cephalosporins, Quinolons, Aminoglycosides, Sulfanamids, Carbapenems more than (90%) except of Tetracycline (47.8%), and Colistin (8.6%).

MICs of seven antibiotics indicated colistin and Tetracycline more effective than other antibiotics. Bacterial CSH is known to be associated with pathogenicity, bacterial adhesion and biofilm formation[15]. Accordingly, we have evaluated the hydrophobicity of the isolates by determining the affinity of cells to xylene[9]. Two isolates showed the highest CHS values as compared with the other strains. *A. baumannii* strains attach to and from biofilm on different surfaces such as glass, polycarbonate, polypropylene and urinary catheters. It is important to note that some of these substances are used widely in the fabrication of medical environments[12]. There is a positive relationship between the degree of bacterial hydrophobicity and adhesion to the abiotic surfaces[13]. In this study biofilm formation of the two selected isolates on glass and polypropylene tubes indicated denser aggregates on polypropylene than glass surfaces. We observed reduction in bacterial adherence to catheter surfaces with sub-MIC concentration of colistin and tetracycline. Strains attach to and production biofilm on different surfaces such as glass, polycarbonate, polypropylene and venous catheters. It is important to note that some of these substances are used widely in the fabrication of medical environments. According to biofilm formation of some bacterial strains in catheters, the above mentioned data can be used in developing programs to prevent and control such a nosocomial infections distributing via medical devices.

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