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Evaluation of different methods to determine the biofilm formation in Pseudomonas aeruginosa isolated from clinical specimens

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Abstract

Background: Antibiotic resistance is one of the global worrying problems that can increase morbidity and mortality. Some antibiotic resistance mechanisms such as biofilm formation can induce resistance to different groups of antibiotics and can cause of presence Multi- Drug Resistant (MDR) strains. The aim of this study was evaluation of different methods to determine the biofilm formation in *P. aeruginosa* isolated from burn- wound colonization.

Materials and Methods: Twenty- five *P. aeruginosa* collected from burn- wound colonization from Motahari hospital, Tehran, Iran. Phenotypic biofilm formation detection was done Congo red agar method and tube test. Congo red agar method: The preparation of Congo Red Agar (CRA) medium was explained previously. CRA plates were inoculated with test organisms and incubated at 375^{o}C for 24 h aerobically. Black colonies with a dry crystalline consistency indicated biofilm production. The experiment was performed in triplicate and repeated three times

Micro-tube method: A loop full of test organisms was inoculated in 1 mL of trypticase soy broth with 1% glucose in test tubes. The micro-tubes were incubated at 37^{o} C for 24 h. Micro-tubes were then stained with crystal violet (0.1%) after washing with phosphate buffer saline. The scoring for tube method was done according to the results of the control strains. Biofilm formation was considered in three types; i) strong, ii) moderate and iii) weak according to the mass of visible film lined the wall of micro-tubes.

Amplification of ppk and modA genes has been done by PCR to molecular detect of biofilm genes. Boiling method used for DNA extraction. The positive results of detection confirmed by Sanger sequencing.

Results: According to the results of the tube test all of isolated showed potential to produce biofilm from score weak to strong. But less than 25% of them showed black colony in CRA assay. All of the strains carried out at least one of the ppk or modA genes according to PCR results.

Conclusion: The results of this study showed that the tube test is more reliable methods for evaluation of biofilm formation in *P. aeruginosa* strains because the some strains with red colonies in CRA method but have one of the responsible genes to produce biofilm. But the results of the tube test confirmed by PCR.

Table 1. Biofilm formation in isolated *P. aeruginosa*

Tube method	Percentage of strains (number)
Weak	50% (25)
Moderate	24% (12)
High / strong	26% (13)

Biography:

Abdolaziz Rastegar Lari is founder of Antimicrobial Resistance Research Center President of Iranian Society for Medical Bacteriology. He is profetional on surveying on antibiotic resistance mechanism, espetialy in gram- negative bacteria.

Speaker Publications:

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