DETERMINATION OF BIOFILM PRODUCTION IN ANIMALS ORIGINATED PSEUDOMONAS AERUGINOSA STRAINS

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Abstract

For the detection of Biofilm formation method, total 56 clinical isolates viz. Pseudomonas aeruginosa were used. Strains were identified using standards microbiological procedure. The susceptibility test of biofilm producing bacteria was performed by using disc diffusion technique. Biofilm detection was tested with Congo Red Agar method (CRA). These method is rapid, sensitive and reproducible, this method is suitable for detection of biofilm formation in the present study. Out of 56 isolates, CRA method detected 17 (30,35%) as high biofilm producer, and 39 (69,65 %) biofilm non- producer. According to the antibiotic susceptibility test, higher antibiotic resistance was observed in biofilm producing bacteria than non-biofilm producers.

Keywords*: Biofilm, Congo Red Agar, Pseudomonas aeruginosa, animal strains*

Introduction

Biofilm are defined as microbial derived sessile communities characterized by the cells that are irreversibly attached to a substratum or to each other. (5) Biofilm are densely packed multicellular communities of microorganisms attached to a surface or interface. Pseudomonas aeruginosa seem to initiate biofilm formation in response to specific environmental cues, such as nutrient and oxygen availability. Biofilm is a source of persistent infections of many Gram negativ bacteria. They are responsible for nosocomial infection and also associated with many medical conditions including indwelling medical device, dental plaque, upper respiratory tract infection and urogenital infection. (4)

All microbes like Gram positive and Gram negative bacteria have capacity to synthesized biofilm. Bacteria commonly involved include *Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus viridans, Escherichia coli, Proteus mirabilis Enterococcus faecalis, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa.* (5)

There are few methods for detect biofilm production. These include the Tissue Culture Plate, Tube method, Congo Red Agar method (CRA),bioluminescent test, piezoelectric sensors, and fluorescent microscopic examination.(Christensen et al. 1995, Freeman J. 1989) MDR organisms have been reported worldwide and are now recognized for dificile healthcare-associated infections to control and to treat. (9)

Materials and methods

A total 56 clinical isolates were subjected to biofilm detection method. Samples were collected from urinary, few pus specimen, sputum samples etc. The entire specimens were received from animals with infection. Isolates were identified by Standard microbiological procedure (Gram staining, cultural characteristics, catalase and oxidase test, biochemical test RapID NF Plus and API 20 NE). (1)

Biofilm formation may be determined in several ways. In this study, biofilm detection was approved by the following technique CRA (Congo Red Agar Method).

CRA medium was established with brain heart infusion broth 37 g/L, sucrose 50 g/L, agar 10 g/L and Congo Red indicator 8 g/L. Congo Red stain was prepared as a concentrated aqueous solution and autoclaved separately from the other medium constituents. Then it was added to the autoclaved brain heart infusion agar with sucrose. Inoculate CRA plates with strains and incubate at 37 °C for 24 h aerobically. Black colonies indicate biofilm production (Fig. 1/ a, b). (6).

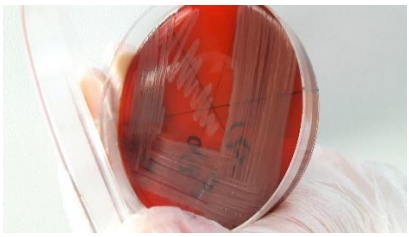
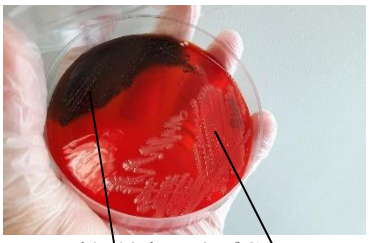


Fig. 1/a Black colonies 24 h at 37 ˚C



b) 48 h at 37 ˚C
Biofilm production
Non biofilm production

Data analysis

The comparisons between different resistance profiles of *P. aeruginosa* strains biofilm producers and non-producing biofilm were carried out by determining the mean and the standard deviation.

The standard deviation is a summary measure of the differences of each observation from the mean. The variance and standard deviation describe how spread out the data is. If the data all lies close to the mean, then the standard deviation will be small, while if the data is spread out over a large range of values, the sample variance will be large. Having outliers will increase the standard deviation. It is used only for distributed uniformly values (symmetrical). https://www.ltcconline.net/greenl/courses

Results and discussions

By Congo red agar method, black colour colonies were observed for the biofilm production. 17*(30,35%)* isolates gave black colour colonies on Congo red agar plate while only 39*(69,65 %)* isolates gave pink colour colonies indicating non biofilm production. (fig. no. 1 b)

All these isolates were identified and characterized by standard microbiological procedure. These isolates mainly isolated from various clinical samples including:perianal abces, otitis, pus eyes, etc. Table no.1 gave an idea regarding biofilm producing microbes mainly resides in particular area.

Table 1

Correlation of biofilm production from various clinical samples

MICROORGANISM	ANIMALS	CLINICAL SAMPLES	BIOFILM PRODUCTION
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus ear	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV

MICROORGANISM	ANIMALS	CLINICAL SAMPLES	BIOFILM PRODUCTION
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Perianal abscess	POSITIV
PSEUDOMONAS AERUGINOSA	cat	Pus eyes	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus eyes	POSITIV
PSEUDOMONAS AERUGINOSA	dog	Pus eyes	POSITIV

Pseudomonas aeruginosa produces strong biofilm, these microbes are highly resistant to various antibiotics. (2) These multidrug resistant biofilm producing microbes given in observation tab. 2.

P. aeruginosa's ability to form antibiotic resistant biofilms is believed to account for the inability of current therapies to eliminate bacterial infections.(3). Biofilm formation, which prevents host defenses and antibiotics from reaching the bacteria. Although the progression of P. aeruginosa colonization makes eradication essentially impossible, early control seems to delay the onset of chronic lung infection (7) Different antimicrobial treatment protocols have been established once the first sign of *P. aeruginosa* colonization is exhibited (7).

The early colonization of these bacteria involves non-mucous colonial morphotypes with low bacterial density. However, once *P. aeruginosa* begins to colonize, the bacteria density increases and switches to a mucous morphotype with a biofilm mode of growth that is less susceptible to antibiotics (8).

Table 2

Antibiotics profile of isolates strains involve in infections Positiv biofilm

	IMP	MEM	TZP	CAZ	FEP	CIP	AK	GN	TOB	ATM	PB	PIP	TAZ
Pus ear	R	R	R	R	R	R	R	R	R	R	S	R	R
Pus ear	S	R	R	R	R	R	S	S	S	R	S	R	R
Pus ear	S	R	R	R	R	R	S	R	S	R	R	R	R
Pus ear	S	S	R	R	R	R	S	S	S	R	R	R	R
Pus ear	S	R	R	R	R	R	S	S	S	S	R	R	R
Pus ear	S	S	S	S	S	S	S	S	S	S	S	R	R
Perianal abscess	R	R	R	R	S	S	S	S	S	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	S	R	S	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R

Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	S	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	S	R	R	R	R
Pus eyes	R	R	R	R	R	R	R	R	R	R	R	R	R
Pus eyes	R	R	R	R	R	R	R	R	R	R	R	R	R
Pus eyes	R	R	R	R	R	R	R	R	R	R	R	R	R

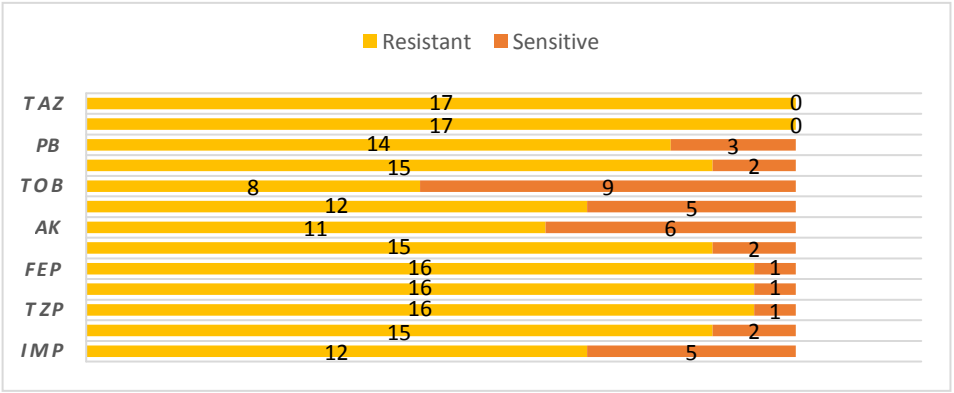


Fig. 1 Resistance profile of antibiotics

Tab. 3

Antibiotics profile of isolates strains involve in infections

	IPM	MEM	TZP	CAZ	FEP	CIP	AK	GN	TOB	ATM	PB	PIP	TAZ
Pus ear	S	S	R	R	R	R	S	S	S	R	S	R	R
Pus ear	R	R	R	R	R	R	S	S	S	S	R	R	R
Pus ear	R	S	R	R	R	R	R	R	S	S	S	R	R
Pus ear	S	R	R	R	R	R	S	R	R	R	S	R	R
Pus tegument	R	R	R	R	R	R	S	R	S	R	R	R	R
Perianal abscess	S	S	R	R	R	S	S	S	S	S	S	R	R
Perianal abscess	R	R	R	R	R	R	R	S	S	S	R	R	R
Pus	S	S	R	R	R	S	S	R	S	R	R	R	R
Pus ear	S	S	R	R	R	R	S	S	S	S	R	R	R
Perianal abscess	S	R	R	R	R	R	S	R	S	S	R	R	R
Perianal abscess	S	S	R	R	R	R	S	R	S	R	R	R	R
Pus eyes	S	S	R	R	R	R	R	R	S	S	R	R	R
Pus	S	S	R	R	R	R	S	R	R	S	R	R	R
Perianal abscess	S	S	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	S	S	R	R	R	S	S	S	S	S	R	R	R
Pus ear	R	R	R	R	R	S	R	S	R	R	R	R	R
Septicemia	R	R	R	R	R	S	R	R	R	R	R	R	R
Septicemia	S	R	R	R	R	R	S	R	S	R	R	R	R
Pus tegument	R	R	R	R	R	S	S	R	S	R	S	R	R
Septicemia	R	R	R	R	R	R	S	R	S	R	R	R	R

Pus ear	R	R	R	R	R	R	S	R	R	S	R	R	R
Pus ear	R	R	R	R	R	R	S	R	R	R	R	R	R
Pus ear	R	R	R	R	R	R	R	R	R	R	R	R	R
Septicemia	R	R	R	R	R	R	R	S	R	R	R	R	R
Pus ear	R	R	R	R	R	R	R	R	S	S	R	R	R
Perianal abscess	R	R	R	R	R	R	S	S	S	R	R	R	R
Perianal abscess	R	R	R	R	R	S	R	R	R	R	R	R	R
Perianal abscess	S	R	R	R	R	S	R	R	R	R	R	R	R
Perianal abscess	S	R	R	R	R	R	S	R	R	R	R	R	R
Perianal abscess	S	R	R	R	R	R	R	R	R	S	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	S	S	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	S	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Perianal abscess	R	R	R	R	R	R	R	R	R	R	R	R	R
Pus eyes	R	R	R	R	R	R	S	R	R	R	R	R	R
Pus ear	R	R	R	R	R	R	R	R	R	R	R	R	R

Negativ biofilm

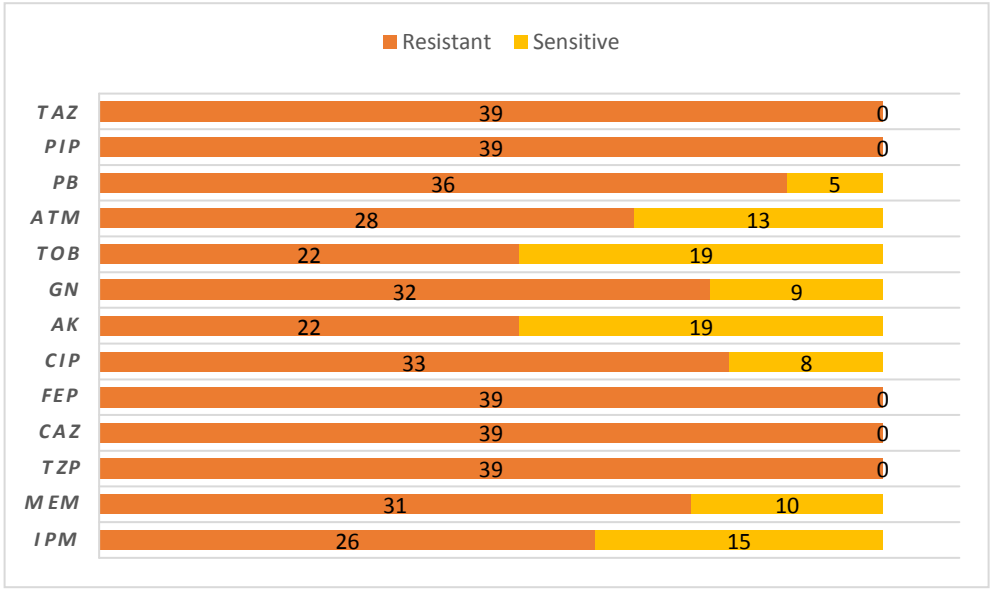


Fig. 2 Resistance profile of antibiotics

Statistical analysis highlighted the following issues:

According to test of normality result; B (-) and B(+) are found normal distribution. So we do independent sample t test. The result is below:

B	N	MEAN	STANDART DEVIATION	P
B(-)	9	9	2,739	0,508
B(+)	6	9,33	3,983	

95% Confidence Interval of the Difference	
Lower	Upper
-4,061	3,394
-4,621	3,954

That has been searched, whether Between B(-) and B(+) is a significiant difference. According to p (0,508) value. There aren't any differences between them significantly.

strain	N	MEAN	STANDART DEVIATION	P
S(-)	9	4,33	3,428	0,086
S(+)	6	2,83	2,401	

95% Confidence Interval of the Difference	
Lower	Upper
-2,000	5,000
-1,755	4,755

That has been searched, whether Between S (-) and S(+) is a significant difference. According to p (0,086) value. There aren't any differences between them significantly.

For our samples, statistical assays have shown that there are no significant differences between the resistance profiles of *Pseudomonas aeruginosa* strains biofilm producers and non-producing biofilm. It seems that the antibiotic resistance to a wider scale of antibiotics is not necessarily influenced by the *Pseudomonas aeruginosa* bacterium property to produce biofilm.

Conclusions

1. *P. aeruginosa* is involved in a variety of animal infections ranging from pus eyes,pus tegument, to perianal abscess and septicemia.
2. Most of the microbes are from common sources like pus samples.
3. *Pseudomonas aeruginosa* growing in a biofilm are highly resistant to antimicrobial agents.
4. Biofilm-producing Pseudomonas strains have been isolated from a chronic infection, as a consequence of inadequate treatment.
5. It is necessary to apply a suitable method for the detection of microbial strains that produce biofilm.
6. Antibioresistance an ever growing panel of antibiotics is not strictly influenced bythe Pseudomonas aeruginosa bacterium property to produce biofilm.

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