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BIOFILM FORMATION BY *Bacteroides fragilis* ISOLATED FROM WOMEN WITH BACTERIAL VAGINOSIS

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ABSTRACT

Many novel, fastidious and uncultivated bacterial species are related with Bacterial vaginosis that is one of the most common genital infections among women in the childbearing age. These are called bacterial vaginosis associated bacteria (BVAB), present in trace amount and have a significant role in the infection. Biofilm forming by these bacteria led to increase chance of bacterial vaginosis incidence due to increase bacterial resistant. A total of 300 high vaginal swabs were obtained from 150 non-pregnant women with bacterial vaginosis depending on Amsel criteria. Two swabs where taken for each patient, these swabs were cultivated anaerobically on selective media, Bacteroides Bile Esculin agar for *Bacteroides fragilis* isolation. Samples were collected from patients those admitted to the out-patient clinics of Gynecology and Obstetrics in Babylon Maternity and Pediatrics teaching hospital and Al-Hillah General Teaching Hospital. Cultivated swabs were gave 31(21%) positive results for Gram negative, anaerobic *Bacteroides fragilis*.

KEYWORDS: Biofilm, *Bacteroides fragilis*, Bacterial vaginosis, Anaerobic conditions.

INTRODUCTION

Biofilm represent one of bacterial life forms during growth and proliferation. Bacteria are organized into sessile aggregates, this form is referred to us the biofilm growth phenotype. Bacteria succeed in forming a biofilm within the human host when the infection often turns out to be untreatable and will develops into a chronic state. The important hallmarks of chronic biofilm-based infections are extreme resistance to antibiotics and many other conventional antimicrobial agents, and an extreme capacity for evading the host defenses.[1]

The microbiological correlate of bacterial vaginosis has been shown to involve a dense, highly structured polymicrobial biofilm, constituted by anaerobic bacteria including *Bacteroides spp*, strongly adhering to the vaginal epithelium.[2]

Then the same isolates were examined for ability to produce biofilm and study the effect of internal douche on the biofilm. The douche used in this study to examined its effect on biofilm was Claradone as recommended by gynecologist, that is antiseptic vaginal douche contains providone-iodine 10% w/v. It have broad spectrum microbicidal effect on vaginal pathogens, which commonly cause specific and non-specific vaginitis, can maintains microbicidal activity even in the presence of blood, pus and vaginal secretions.

MATERIALS AND METHODS

Collection of samples

In this study, 300 samples of high vaginal swabs were collected from 150 women who were in the reproductive age ranging from15 to 45 and have bacterial vaginosis.

The patients had symptoms of abnormal vaginal discharge, odor and itching or burning. These women were attended to out-patient clinics of Gynecology and Obstetrics, in two hospitals that mentioned previously during period from February to October 2016. The sampling was carried out by specialized gynecologist and under sterile conditions.

Clinical diagnosing

Amsel's criteria were used to diagnosis bacterial vaginosis associated bacteria (BVAB), in women which present in trace amount was diagnosed for these patients by standards Amsel's clinical criteria according to.[3,4] And the diagnosis of vaginal flora based on conventional culture-dependent methods and biochemical identification methods depending on.[5] As well as, other characters reported by.[6,9] Tissue culture plate method (TCP) assay (also called semi quantitative microtiter plate test (biofilm assay)) described by.[10] was considered as standard test for detection of biofilm formation and the classification of bacterial adherence and biofilm formation by TCB method by.[11] As mentioned in table(1)

Table 1: Classification of bacterial adherence and biofilm formation by TCB method.

Mean of O.D. value at 630 nm	Adherence	Biofilm formation
< 0.120	Non	Non
0.120 – 0.240	Moderately	Moderate
> 0.240	Strong	High

RESULTS AND DISCUSSION

The identification of *B. fragiles* isolates creates by cultural and biochemical characteristics and also microscopic patterns to colonies of 31 (21%) positive isolates on the selective media Bacteroides Bile Esculin (BBE) agar. The results demonstrates that *B. fragiles* was strict an anaerobic, G –ve. It observed to be rods, capsulate 21 (67.7%) and in some subcultures missed the capsule. It was bile salt resistant that make it able to grows on BBE agar due to contains enzyme required for this reaction, a bile salt hydrolase.[12] and gives small white – greyish colonies and forms brownish –black pigment around the colonies due to hydrolysis of esculin which produces esculetin and dextrose. The esculetin reacts with the iron salt (ferric ammonium citrate) contained in the medium to produce a dark brown to black complex that appears in the medium surrounding colonies of members of the *B. fragiles* as mentioned by.[13]

Biofilm formation on polymetric surfaces was tested by semi quantitative microtiter plate test (biofilm assay). This assay was repeated as triplicate for each isolate to increase the accuracy of assay. According to mean of optical density (OD) value at 630 nm, the results were interpreted as high, moderate and none biofilm former when the mean of OD value were (>0.240,0.120 -0.240 and < 0.120) respectively. The examined isolates gave positive result 31 (100%), 26 (83.9%) were strong biofilm former and 5 (16.1%) were moderate biofilm former as explained in Table (2).

Table 2: Production of biofilm by studied B.fragilis.

Bacterial isolates No.	Biofilm formation					
	Strong	%	Moderate	%	weak	%
B. fragiles (31)	26	83.9%	5	16.1%	0	0%

Crystal violet used in this procedure is a basic dye known to be bind to negatively charged molecules on the surface as well as nucleic acid and polysaccharide, therefor gives an overall measure of the whole biofilm. It has been used as a standard technique for rapidly accessing cell attachment and biofilm formation in a range of Gram – negative bacteria.[14] The production of this system is important for pathogens attachment then initiate infection. Also the biofilm as a system protect the bacteria from the host immune defence as well as the antibiotics and drugs. This identical to the result of.[15] were they founded in patients with BV, a biofilm is usually formed on the vaginal epithelium, so the establishment of a biofilm plays a key role in the pathogenesis of bacterial vaginosis.

Then the same isolates were examined for ability to produce biofilm and study the effect of internal douche of the biofilm, gave variant in activity as mention in the Table (3).

Table 3: Ability of biofilm production by studied *Bacteroid fragilis* with using internal douche.

Bacterial isolates No.	Biofilm formation					
	Strong	%	Moderate	%	Weak	%
	2	6.5%	22	71.0%	7	22.6%

Table (3) shows the ability of biofilm production by *B. fragilis* with douche, it is strong in 83.9% in biofilm without douche while when using douche it is 6.5%. So these agents can give assistant effects along with antibiotics to provide good conditions for recovery and reduce infection returns.

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