Lamees A. Abdul-Lateef et al /J. Pharm. Sci. & Res. Vol. 10(12), 2018, 3216-3218

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Biofilm formation by *Streptococcus agalactiae* is affected by pH changes *in vitro*

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Abstract

Background: The capacity of *Streptococcus agalactiae* to cause infections is related to their ability to produce biofilms which allow bacteria to adhere to tissues causing pathogenesis and promote their resistance to antibiotics resulting in prolonged infections. Therefore, the aim of current study was to study the ability of these bacteria to form biofilms and the effects of pH changes on this ability.

Materials and Methods: A total of 25 confirmed isolates of *Streptococcus agalactiae* were collected in this study. The isolates were obtained by vaginal swaps from pregnant women who were complaining from abnormal vaginal discharge. Biofilm was detected by semi- quantitative microtiter plate test (biofilm assay) using TSB supplemented with 1% glucose.

Results: In this study, all strains of GBS were biofilm former and 52% of them were strong biofilm formers while 48% were moderate biofilms formers. Also, results showed that the ability of the studies isolates to form biofilms was weak at low pH and enhanced at high pH. Therefore, high pH plays a critical role in the pathogenesis of chronic vaginal bacterial infections.

Key Words: Streptococcus agalactiae, pH, biofilm, vaginal infection.

1. INTRODUCTION

Group B streptococcus (GBS) or *Streptococcus agalactiae* is a Gram-positive encapsulated bacterium exhibiting β-hemolysis on blood agar. The organism lives commensally in the gastrointestinal and genitourinary tracts of up to 30% of healthy adults. In addition, GBS is facultative anaerobic, catalase-negative and gram-positive cocci. Their metabolism is mainly fermentative and lactic acid is the predominant end product [1].

Colonization and persistence of bacteria in different host niches is dependent on the adherence capacity of GBS to host cells and tissues. This adhesion facilitates bacterial cells aggregation and formation of sessile communities known as biofilms. Bacterial biofilms represent well-known virulence factors with a vital role in persistence and chronic infections [2]. In the host environment, bacteria are often protected from the immune system by building sessile colonies embedded in an extracellular matrix of polysaccharides representing the biofilm. For GBS the bacterial capsule and type IIa pili have been demonstrated to play an important role in biofilm formation [2]. Biofilm formation is a complex aggregation of microorganisms growing on a solid surface. Biofilms are usually found on solid substance submerged in or exposed to some aqueous solution [3].

Biofilms allow long-term bacterial persistence and protect bacteria from recognition by immune system and controlling the expression of bacterial surface-associated structures, such as pili and the capsule, which are both involved in promoting bacterial biofilm formation [4].

A decrease in the number of normal flora lowers the concentrations of H_2O_2 and lactic acid and results in proliferation, and subsequent biofilm formation, of pathogenic bacteria. In addition, biofilms formation is poor in acidic environment while an increase in pH enhances bacterial bacterial ability to form biofilms [5].

Taken together, the aim of current study was to investigate the *in vitro* effects of pH changes on biofilms formation in *Streptococcus agalactiae* bacterial strains.

2. MATERIAL AND METHOD

Bacterial samples

All samples were obtained by vaginal swaps from pregnant women performed by a gynecologist who admitted them to Al-Hilla Surgical Teaching Hospital and Babylon Maternity and Paediatrics Hospital during the period from February to November 2017. however, out of 300 samples collected , only twenty five isolates of *Strep. agalactiae* were obtained.

Bacterial identification

The samples were processed on blood and Edward modified medium agars, incubated at 37 ºC. The identification of gram positive bacteria was performed by standard biochemical methods using Catalase, CAMP and Oxidase tests.

Detection of biofilm formation

Tissue culture plate method (TCP) assay (also called semi quantitative microtiter plate test (biofilm assay) described by [6] was considered as a standard test for detection of biofilm formation as follow:

1- Isolates from fresh agar plates were inoculated in TSB containing 1% glucose and incubated aerobically for 72 hrs at 37°C and then diluted 1:100 with TSB.
2- Individual wells of sterile, polystyrene, 96 well-flat bottom tissue culture plates wells were filled with 150 µl of the diluted cultures and only broth served as control to check non-specific binding of media. Each isolate was inoculated in triplicate.
3- The tissue culture plates were incubated for 24 hrs at 37°C. After incubation, content of each well was gently removed by tapping the plates. The wells were washed four times with phosphate buffer saline (pH 7.2) to remove free-floating bacteria.
4- Biofilms formed by adherent sessile organisms in plate were fixed by placing in oven at 37°C for 30 minutes.
5- All wells stained with crystal violet (0.1 % v/v). Excess stain was rinsed-off by thorough washing with deionized water and plates were kept for drying.
6- 150 µl of acetone/ethanol (20:80, v/v) mixture was added to dissolve bounded crystal violet. The optical density (O.D.) at 360 nm was recorded and the results were interpreted according to [7] (Table 1).

Table 1 Classification of bacterial adherence and biofilm formationby 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

Effect of pH changes on biofilm formation

TSB was prepared and pH was adjusted to 4,5,6 using HCl or NaOH. These pH values were used to reproduce the vaginal pH in normal conditions (pH 4) and in case of infection (pH 5 ,6) [8].