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دراسة العلاقة بين تكوين الأغشية الحيوية من قبل *Streptococcus pneumonia* ومقاومة المضادات الحيوية

رسالة مقدمة

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Study of relationship between biofilm formation from *Streptococcus pneumonia* with antibiotics resistant

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Chapter One

Introduction

1.1 Introduction

Streptococcus pneumoniae is a major human pathogen with high morbidity and worldwide mortality, primarily from community-acquired pneumonia, meningitis, bacteremia and otitis medium. The pneumococcus is a transient commensal that asymptomatically resides and proliferates in the human nasopharynx (Kadioglu *et al*, 2008). Colonization of the nasopharynx is importance as it represents server from which bacteria can disseminate throughout the community. In addition, the establishment of a carrier state is accepted to be a prerequisite for disease (Weiser, 2010). Despite the significance colonization of *S. pneumoniae*, little was known about the mechanisms employed via the bacterium to grow and increase in the human nasopharynx. *S. pneumoniae* is a fermentative pneumococcus that depend on a glycolytic metabolism to obtain energy (Paixão *et al.*, 2015). Accordingly, the ability to acquire and the metabolization of sugar is the great importance for the health of this microorganism *in vivo*. In addition, the pneumococcus lacks a complete set of respiratory protein genes and is for this reason unable to generate energy by respiration (Schulz and Hammerschmidt, 2013). The genomic abundance of genes involved in sugar transport further supports the significant role of carbohydrates in the lifestyle of *S. pneumoniae*. Over 30% of the transporters in the *S. pneumoniae* genome were predicted to be involved in the uptake of carbohydrates (David L Hava, LeMieux and Camilli, 2003). And those findings were confirmed by a recent functional genomic approach targeting carbohydrate transport.

The growth *in vivo* needs to rely on alternative nutritional reservoirs. In the human nasopharynx, the glycol proteins lining the epithelial surfaces appear as good candidates to serve as carbon and energy sources for pneumococcal growth. Notable, *S. pneumoniae* is able to grow on mucin as a sole carbon source (Yesilkaya *et al*., 2013).

Mucins are major macromolecular components of the mucus that cover the epithelial surfaces. Each mucin has a unique and characteristic sequence of tandemly repeating amino acids rich in serine and/or threonine, these structures are heavily O-glycosylated glycoproteins (Sakornsakolpat *et al*., 2019). The deglycosylation activity of both exo- and endo glucosidases has been previously demonstrated in *S. pneumoniae* (Hobbs, Pluvinage and Boraston, 2018).

Biofilms are cells that create an extracellular matrix and bind to abiotic or biological surfaces are highly organized groups of cells. Antibacterial resistance is an intrinsic characteristic of biofilms and the defensive matrix of biofilms allows host immune responses to be prevented, persistence to be encouraged and bacteria to spread (Abranches *et al*., 2019*). S. pneumoniae* colonization come first disease and has been found to be harder to eliminate than invasive disease in patients as therapy with antimicrobial mediators do not eliminate the major of bacteria transported in the nasopharynx (Bogaert, de Groot and Hermans, 2004). Moreover, environmental factors which affect the formation of biofilms, like the source of carbon, cell density, the pace of flux and the physical surface properties to which bacteria adhere (Chao *et al*., 2014). The capacity of *S. pneumoniae* to formation biofilms has been tested on abiotic surfaces for a variety of materials including polyvinyl chloride, polystyrene and glass, the last one of which the strongest in terms of the formation of biofilms. The formation of pneumococcal biofilms was observed when using poor culture media, and the difference in pH and temperature, which affects the formation of biofilms (Moscoso *et al*., 2006).

The treatment of bacterial biofilm is complicated by the mechanisms of biofilm growth. Many clinically relevant biofilms are poly-microbial, which caused by different species biofilms have complementary metabolic strategies for obtaining nutrients and degrading host immune molecules (Sojka *et al*,

2018). Therefore, it is important to identify the adaptability to vary growth conditions of biofilm formation to introduce novel or re-emerging effective approaches to combat bacterial biofilms.

1.2 Aims of the study:

1- solation and diagnosis of bacteria *Streptococcus pneumoniae* and diagnose them by phenotypic, biochemical and molecular methods using diagnostic gene*16SrRNA.*

2- To evaluate the biofilm formation in the suitable culture media by *Streptococcus pneumonia*.

3- To identify the correlation with different parameters such as gender, age, and smoking.

Summary

Streptococcus pneumoniae is one of the major general causes of important diseases world-wide such as pneumonia and meningitis with a high morbidity and mortality in adults especially in elderly people. This bacterium plays an important role in pneumonia due to its seriousness and the lack of successful solutions to treat it. Pneumococcal biofilms are essential for colonization and persistence, and it is important to continue to investigate the environmental conditions which affect their formation. Biofilm's importance depends on the reduced sensitivity of this bacterium to antibacterial agents and their capacity to withstand the immune defense mechanisms of the host. This study came to find out the adaptability of biofilm formation in *S. pneumoniae* that causes pneumonia in Diyala city and diagnose them with PCR method by using *16SrRNA* for pneumococcal detection in addition to the roads bacteriology culture test.

In this study, 192 sputum samples were collected from Bacteriology Unit of Baquba teaching hospital, Chest Diseases Consultation Clinic and Al-Khalis General Hospital from October 2019 to January 2020. It appeared that 122 (64%) samples showed no growth while 70 (36%) showed positive growth. *Streptococcus pneumoniae* isolates were obtained from 32 isolates and diagnosed using culture, biochemical tests and microscopic assays as well as using molecular methods including, detection *16SrRNA*.

The antibiotic susceptibility test was applied to all pneumococcal isolates by using 14 antibiotics. The results showed that all the isolates were resistant to Tobramycin 100%. The variation in resistance among isolates to several antibiotics including Gentamycin, Erythromycin, and Azithromycin was a percentage of 87.5%. Tetracycline showed 75.5% resistance, Chloramphenicol 62.5%, Streptomycin 50%. The Clindamycin showed

37.55% resistance. Imipenem reflected 25%, Rifampin 50% and Cefotaxim, Penicillin, Amoxicillin and Vancomycin showed 12.5% resistance.

In the molecular method, the genomic DNA was extracted from 32 samples of broth culture (BHI) and used directly for PCR to detect *Streptococcus pneumoniae.* The results of this study showed of (*16SrRNA* gene) by PCR, which was positive in 32 (100%). In addition, the PCR product of the samples were sent for sequencing to identify the *Streptococcus pneumoniae* strains. The results appeared that the strains of *S. pneumoniae* that cause pneumonia in Diyala city are K10 (12) and D39 (20) strains. Using NCBI program to analyze genetic sequences and the identity was 100% for all isolates

The prevalence of pneumonia infection in this study was more in males than in females as the percentage were (53.1%) and (46.9%), respectively, and the incidence of *S. pneumoniae* in smoking patients was (53.1%), while it was (46.9%) among non-smokers.

Biofilm formation assay was measured biofilm by using microtiter plate biofilm assay in three different media and the biofilm formation was measured at the four bacterial growth phases to show the adaptability of biofilm formation in different culture media. Data of the current study showed that the ability of pneumococcal to form biofilm was better when using a poor medium such as nutrient broth, and the highest value was at the stationary phase.