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Effected of Xylitol on Biofilm Formation and Growth of Streptococcus Pneumoniae Isolated from Children with Acute Otitis Media in Hilla Province/Iraq

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ABSTRACT

Background: Xylitol is a natural five-carbon- sugar alcohol that inhibits the growth and adherence of *Streptococcus pneumoniae*. In clinical trials, xylitol has been shown to decrease the occurrence of acute otitis media in day-care children but did not decrease nasopharyngeal carriage of the pneumococci. It has also been shown that xylitol affects the ultrastructure of the pneumococcal capsule. Here, it was hypothesized that xylitol might affect the expression of pneumococcal capsular genes. **Objective:** The present study aimsto isolation and identification of *Streptococcus pneumoniae* from children with acute otitismedia. Detection of biofilm formation by *Streptococcus pneumoniae*an Study effect of xylitol on biofilm formation and on growth of *Streptococcus pneumoniae*. **Results:** The results of this study showed that7/8(87.5%) of *S. pneumoniae* isolates were high biofilm former, the moderate biofilm formation were account for 1/8 (12.5%) of*S. pneumoniae*isolates while there is no isolates that express non biofilm formationandxylitol induced pneumococci growth inhibition was observed in 3% concentration of xylitol the growthof *S. pneumonia* reduce to 50 % ,in addition xylitol has a clear inhibitory effect on the formation of the biofilms. **Conclusion:**The results of our study were concluded that *Streptococcus pneumoniae* have ability to produced biofilm and these biofilm can inhibited by xylitol,the decrease in biofilm formation may further explain the efficacy of xylitol in preventing acute otitis media in previous clinical trials. *Streptococcus pneumoniae* growth can inhibition by xylitol.

INTRODUCTION

Xylitol is a natural five-carbon-sugar alcohol (Söderling&Lenkkeri, 2010). It is a non-cariogenic sweetener that can be found in chewing gums, tablets, and oral rinses. It can also be found in fruits and plants; it also exerts anti-ketonic and anti-infection effects (Brown *et al*., 2004).

In addition it is produced from xylose by yeasts, a wide range of yeast species belonging to the genus *Candida* are well-known for their potential industrial applications, these include *Candida boidinii*, *Candida guilliermindii*(Rodrigues *et al*., 2003).

Xylitol can be safely applied as a preventive measure for diseases such as pneumonia, acute otitis media, dental caries, and meningitis (Honkala *et al*., 2006).

However, xylitol decreases the pathogenicity of *Streptococcus pneumoniae* by reducing the growth of these bacteria, xylitol were only a short-term mechanical barrier between bacteria and the host. It has been observed that exposure *S. pneumoniae* to low concentrations of xylitol results in a reduction of cell adherence to nasopharyngeal cells, perhaps due to a disruption of polysaccharide production and mark for significant

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ultrastructure alteration in xylitol-exposed. This effect could persist after short-term xylitol exposure in the oral cavity, explaining the good clinical efficacy of xylitol (Tapiainen, 2001).

The xylitol action mechanism in the otopathogenic bacteria is not justified only by the bacterial growth inhibition. It has also features that make their migration difficult up to the middle ear, an important acute otitis media pathophysiology stage (Söderling *et al*., 2008).

The xylitol influence on the adhesiveness of *Streptococcus pneumoniae* to epithelial cells, after the exposure of epithelial bacteria and cells, associated or not to a concentration of 5% of xylitol, and may also be useful for the prophylaxis of acute otitis media in children, although in tests it did not reduce the nasopharyngeal carriage of pneumococci (Danhauer *et al*., 2010).

Xylitol given in the form of chewing gum or syrup decreased the occurrence of acute otitis media after exposure to xylitol, the cell wall of pneumococci became more diffuse and the polysaccharide capsule became ragged, yet the bacteria remained viable. Xylitol could affect the expression of pneumococcal capsular genes. (Morona *et al.*, 2004).

MATERIALS AND METHODS

Patients:

A total 120 samples, only eight isolates of *Streptococcus pneumoniae* were obtained fromchildren suffering from acute otitis media bystandard bacteriological methods. All sampleswere obtained from patients or individuals whoadmitted to Al-Hilla Surgical Teaching Hospital inBabylon Governorate.

Bacterial Identification:

The samples were processed on blood agar andchocolate plate agar were incubated at $37c_o$ with~5% CO2 (or in a candle-jar). The identificationof Gram-positive bacteria was performed bystandard biochemical methods (catalase test,oxidase test, optochin sensitivity, bile solubility,present of capsule) (Todar, 2003).

Biofilm Production: Tissue culture plate method (TCP):

The TCP assay described by (Christensen *et al*, 1985) is most widely used and was considered as a standard test for detection of biofilm formation. In the present study, all isolates were screened for their ability to form biofilm by TCP method with a modification in duration of incubation which was extended to (24) hours according to (Mathur *et al.*, 2006). Isolates from fresh agar plates were inoculated in tryptic soy broth and incubated for (18 hrs.) at (37^oC) in stationary condition and diluted (1) in (100) with fresh medium. Individual wells of sterile polystyrene (96) well-flat bottom tissue culture plates were filled with (0.2ml) aliquots of the diluted cultures and only broth served as control to check sterility and non-specific binding of media. The tissue culture plates were incubated for (18 hours) and (24 hours) at (37°C). After incubation content of each well was gently removed by tapping the plates. The wells were washed four times with (0.2 mL) of phosphate buffer saline (pH 7.2) to remove free-floating 'planktonic' bacteria. Biofilms formed by adherent 'sessile' organisms in plate were fixed with sodium acetate (2%) and stained with crystal violet (0.1% w/v). Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying. Adherent bacterial cells usually formed biofilm on all side wells and were uniformly stained with crystal violet. Optical density (OD) of stained adherent bacteria was determined with a micro ELISA auto reader at wavelength of 570 nm ($OD_{570\ nm}$). Experiment was performed in triplicate and repeated three times, the data was then averaged, and the results were interpreted according to table (1) (Mathur*et al.*, 2006).

Table 1: Classification of bacterial adherence by TCP method.

Mean OD values	Adherence	Biofilm formation
<0.49	Non	None / Weak
0.49 – 0.6	Moderately	Moderate
>0.6	Strong	High

Effect of Xylitol on biofilm formation:

The same procedure described in (tissue culture plate method for detection biofilm formation) was done with modification. Xylitol (5%) was added to samples in wells of sterile polystyrene 96 well-flat bottom tissue culture plate, after fixed with sodium acetate for half an hour and all steps done as the same steps that described previously (personal communication by Assist. Prof. Dr. LameesA. Abdul-Lateef).

Effect of xylitol on bacterial growth:

1- BHI is prepared and distributed in tubes and xylitol is added to each tube at various volumes to gain the final concentrations (1, 2, 3, 4, 5µg/ml).
2- Positive control is prepared by using BHI free from xylitol.

3- The tubes in item 1 and 2 are inoculated with 0.5 ml of bacterial suspension and then incubation 24hr. at 37°C.

4- After incubation, the absorbance is read at wavelength 650 nm by using spectrophotometer to show the effect of xylitol on the growth of bacteria strain(personal communication by Assist. Prof. Dr. Lamees A. Abdul-Lateef).

Results:

The results revealed that 7/8 (87.5%) of *S. pneumoniae* isolates were high biofilm former, the moderate biofilm formation were account for 1/8 (12.5%) of *S. pneumoniae* isolates while there is no isolates that express non biofilm formation as shown in table(2).

Xylitol induced pneumococci growth inhibition was observed in the presence of xylitol .The mean OD values for the culture growth after 24 hours was (1.283) without xylitol, (1.229) in 1% , (0.903) in 2% ,(0.641) in 3% ,(0.600) in 4%, (0.550) in 5% . The result present in figure (1).

Table 2: Mean formation of biofilm in *S. pneumoniae* by Tissue culture plate.

Bacterial isolates(no.)	Biofilm		
	Strong	Moderate	weak
S. pneumoniae(8)	7(87.5%)	1(12.5%)	0 (0%)

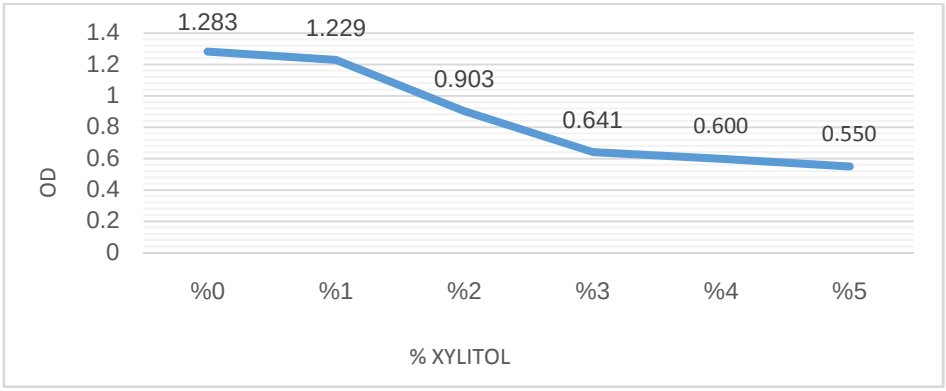

Fig. 1: Effect of Xylitol on the growth of S. pneumonia.

The effect of xylitol on pneumococcal biofilm formation was studied by growing pneumococcal isolates on polystyrene plates in TSB media supplemented with xylitol at (5% concentration). After an 18-hour incubation time, the xylitol inhibition biofilm formation as shown in table (3)

Table 3: Effected of xylitol in biofilm formation in S. pneumonia.

Bacterial Isolates(no.)	Biofilm after adding 5% xylitol		
	Strong	Moderate	weak
S. pneumoniae(8)	0%	0%	8(100%)

Discussions:

Otitis media (OM) is an inflammation of the middle ear, and is a very common infection in children with a highest rate between 4-7 years of age (Al-Marzoqi*et al*., 2013). The bacterial species most frequently isolated from middle-ear samples are *Streptococcus pneumoniae*, (Guevara *et al*., 2008).

The results of the present study showed that 7/8 (87.5%) of *S. pneumoniae* isolates were high biofilm former, the moderate biofilm formation were account for 1/8 (12.5%) of *S. pneumoniae* isolates while there is no isolates that express non biofilm formation.This result is similar with the result obtained byDavey and OToole, (2000) was detected (100%) of *S. pneumoniae* have ability to formation biofilm in vitro by TCP method, but is disagreement with Fux *et al*., (2005); Donlan and Costerton, (2002) who found that *S. pneumoniae*is able to form biofilm at levels (60%). According to data obtained in this study, the presence of strong or moderate production of biofilm will confer bacteria to adhere strongly to theotitis media. However, weak positive may express the bacteria may be under stress condition or the growth is weak that made the biofilm weak or cannot be produced.

Xylitol induced pneumococci growth inhibition was observed in the presence of xylitol. In 3% concentration of xylitol it seems that the xylitol may accelerate the entrance of bacteria in the stationary phase when it is toxic to this bacteria, so resulted in 50 % inhibition of *S. pneumoniae* growth.

Most sugars cannot be used as antimicrobial agents, as both microbes and host cells are able to utilize them.Xylitol, which differs from most carbon sources due to its five carbon polyol structure, is metabolized*via* the pentose pathway in humans, but is unsuitable as an energy source for many bacteria (Tapiainen and Terhi, 2002). The mechanism of xylitol inhibition pneumococci growth is start by entry into the bacterial cell via the fructose phosphotransferase system, and xylitol is than metabolized to xylitol-5-phosphate, unlike mammal cells, *S. pneumoniae*lacks the ability to metabolise xylitol phosphate further, and which may even be toxic to bacteria (Trahan *et al*., 1985). It must therefore be expelled from the cell. This futile xylitol cycle consumes energy and result in growth inhibition (So¨derling and Pihlanto-Leppa¨la., 1989). Since phosphotransferase systems play a role not only in sugar phosphorylation but also in the regulation of bacterial functions and gene expression in different nutritional and host environments, xylitol exposure may disturb the cellular metabolism in *S. pneumoniae* in addition to inhibiting growth (Vadeboncoeur and Pelletier, 1997).

Xylitol has a clear inhibitory effect on the formation of the biofilms; this result is in agreement with results obtained by Ruiz *et al*., (2011).Xylitol is not only efficient in inhibiting the acid production of cariogenic bacteria, but also in preventing the formation of a multispecies biofilm, in addition xylitol has been found to increase the activity of neutrophils, the white blood cells involved in fighting many bacteria. This effect seems to be quite broad, acting even in cases such as general sepsis(Morona *et al.,* 2004).

The effect xylitol on biofilm formation of pneumococcus may partly explain the efficacy of xylitol to prevent acute otitis media in previous clinical trials. These effects biofilm formation, however, are not likely to be very long-term if alternative carbon sources are available for pneumococci between xylitol doses, as xylitol administered in only three daily doses, i.e. not after each meal, failed to prevent acute otitis media (Hautalahti*et al*.2007).

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