# Effect of Xylitol on the Growth and Survival of *Streptococcus pneumoniae*

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## Abstract

**Background:** Xylitol has been applied in dentistry to prevent dental caries for many years. However, it's anti-*Streptococcus pneumoniae* effects are unclear.

**Objectives:** The aim of this study was to determine the anti-*Streptococcus pneumoniae* activity of xylitol.

**Methods:** In this study, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of xylitol were determined by microdilution method and tetrazolium salt-based colorimetric (MTT) assay. The effective concentrations of xylitol in the presence and absence of fructose against growth and survival of *S. pneumoniae* were measured. Data were analyzed using univariate analysis and Kruskal-Wallis test.

**Results:** The results showed that 5% to 7.5% concentrations of xylitol were associated with reduced number of *S. pneumoniae* cells. About 80% to 100% of *S. pneumoniae* cells were killed under laboratory conditions after 3 to 7 hours, respectively. MIC and MBC of xylitol against *S. pneumoniae* were 2.5% and 7.5% after 4 hours, respectively. We found significant changes in the number of viable cells ($P \leq 0.002$).

**Conclusions:** The results showed that the 5% and 7.5% concentrations of xylitol inhibit *S. pneumoniae* growth. However, the emergence of multi-drug resistance in *S. pneumoniae* has become a global concern and the *S. pneumoniae* 23-valent vaccine may not be able to protect against *S. pneumoniae* human pathogenic strains. Finding a non-related antibiotic agent may be useful for controlling *S. pneumoniae* infections.

*Keywords:* Xylitol, *Streptococcus pneumonia*, Growth, MIC, MBC

## 1. Background

Community-acquired pneumococcal infections (CAPI) are health threatening and cause morbidity and mortality in pediatric and elderly populations, but the transformation inhibition and infection control of multi-drug resistant *Streptococcus pneumoniae* strains remain a major health challenge (1, 2). In previous studies, the frequency of pneumococcal meningitis in elderly patients was reported more than 35% (3-5). Furthermore, emergence of *S. pneumoniae* strains with decreasing vancomycin susceptibility represent an important warning sign for health authorities (6). Nonetheless, the introduction of the 23-valent polysaccharide pneumococcal vaccine (PPSV23) has created hope for controlling and preventing pneumococcal infections (7).

Recent findings have corroborated the effectiveness of PPSV23 in preventing hospitalization due to *S. pneumoniae*

community-acquired pneumonia (8). Pneumococcal conjugate vaccines (PCVs) in some countries have reduced the incidence of pneumococcal diseases associated with vaccine serotypes. Rapid increase of non-vaccine serotypes in carriage and pneumococcal infections has become a great concern for physicians (9). In addition to meningitis due to *S. pneumoniae*, pneumococcal infections such as surgical wound infection are reported (10). Therefore, other methods of bacterial growth inhibition are being considered. For example, the results of an investigation showed that Mucin 1 (MUC1) could protect mice against severe pneumococcal diseases, which could be mediated by enhancing macrophage phagocytosis bacterial cells (11).

A previous study showed that oral administration of *Lactobacillus casei* could prevent *S. pneumoniae* lung infection in a mouse model (12). Xylitol, which is a five-carbon sugar alcohol, has been considered as an antibac-

terial agent in some countries and has been used in chewing gums, lozenges, syrups, nasal sprays, toothpastes, and mouthwashes. This compound may be a promising agent in ear, nose, throat (ENT) practices, but further experimental and clinical studies are required (13). The antibacterial activities and synergistic effects of xylitol with other sugars have not been clarified yet. A research reported that xylitol had no significant impact on pneumococcal mucosal colonization (14), while in another study exposure of both epithelial cells and bacteria to 5% xylitol significantly reduced the adherence of pneumococci (15).

Former studies claimed that if xylitol reduces the growth of *S. pneumoniae* in the nasopharynx, it can also diminish the carriage of this pathogen and thus, have clinical significance in the prevention of pneumococcal diseases (16). Besides, another study suggested that exposure to 5% xylitol changed the ultrastructure of the pneumococcal capsule through reducing the expression of capsule gene (*cps*B gene), which could explain the high clinical efficacy of xylitol in preventing otitis media (17).

Another study pinpointed that xylitol lowered LytA expression level, partly explaining the efficacy of xylitol in preventing acute otitis media (18). Nonetheless, the duration of bactericidal effect of xylitol as well as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of this compound have not been described in detail yet. It is worth mentioning that *S. pneumoniae* strains colonization and their adherence to the nasopharyngeal epithelial cells are critical in triggering infectious diseases and invading other tissues (19). Therefore, any factor that can reduce bacterial populations in the nasopharynx may be able to prevent pneumococcal infections.

## 2. Objectives

The aim of this study was to evaluate the antibacterial effect of xylitol on bacterial growth and survival via tetrazolium salt-based colorimetric (MTT) assay.

## 3. Methods

### 3.1. Ethics Statement

This project was approved by the Ethics Committee of Baqiyatallah University of Medical Sciences (November 2, 2014, code No.: 37).

### 3.2. Bacterial Strains

In this investigation, five *S. pneumoniae* clinical isolates were studied. In a previous study, all isolated strains were identified and their serotypes were determined (6, 7). In addition, the *S. pneumoniae* strain ATCC 6305 (Baharafshan Laboratory, Tehran, Iran) was used as reference.

### 3.3. Bacterial Culture Condition

The lyophilized bacteria in aseptic conditions were dissolved in 1 mL of brain heart infusion (BHI) broth (Merck, Germany). Then, 50 $\mu$L of the suspension was transferred into a blood agar (Merck, Germany) supplemented by 5% sheep blood and incubated at 37°C in a 5% $CO_2$ atmosphere for 24 hours. After overnight incubation, bacterial colonies were harvested and transferred into 3 mL of BHI broth containing 0.2% glucose supplemented with 10% (vol/vol) fetal bovine serum (FBS; Cambrex, Belgium). Then, the solution was incubated at 37°C in a 5% $CO_2$ atmosphere for about 5 hours, and the $OD_{650}$ was reached to 0.5 McFarland. Afterwards, bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland concentration. Then, 300 $\mu$L of this suspension was added to 3 mL of the test media that were prepared by adding sugar alcohol or various sugar concentrations. The test tubes were incubated at 37°C, and the $OD_{570}$ results were calculated after 0, 3, 5, 8, 11, 15, 20, and 24 hours. Each test was carried out in triplicate.

### 3.4. Sugars Stock Solution Preparation and Sterilization

In this research, the 40% stock solution of each sugar was prepared separately. The tested sugars were sterilized by filtration (0.22 mm pore-sized filter; Millipore). Then, certain amount was added to related test tubes. The used media and gradients in this investigation were as follows: BHI broth + 2.5% xylitol, BHI broth + 2.5% xylitol, BHI broth + 7.5% xylitol, BHI broth + 2.5% fructose + 2.5% xylitol, BHI broth + 5% fructose + 5% xylitol, BHI broth + 7.5% fructose + 7.5% xylitol, BHI broth + 5% glucose, and BHI broth alone. Different volumes and concentrations of xylitol, fructose, and glucose were prepared from their stock solutions.

### 3.5. Assay of Xylitol Antibacterial Effects

The effects of different concentrations of xylitol and fructose on growth of *S. pneumoniae* strains were examined both separately and in combination (15). The test media were inoculated and incubated at 37°C in a 5% $CO_2$ atmosphere for 24 hours. During an overnight (at three-hour intervals), 200 $\mu$L of each medium was transferred to a 96-well plate. Afterwards, 20 $\mu$L of MTT solution was added to each well, and the wells were incubated at 37°C in a 5% $CO_2$ atmosphere. After 4 hours, the supernatant was discarded and 50 $\mu$L of dimethyl sulfoxide (DMSO) was added to dissolve blue colored formazan crystals. The plate was again incubated for 30 minutes, and optical density (OD) was measured with ELISA reader at the wavelength of 570 nm.

### 3.6. Stock Solution of MTT Preparation

A stock solution of MTT at a concentration of 5 mg/mL was prepared in phosphate-buffered saline (PBS) in pH = 7 and was kept at 4°C in the dark until used. In MTT

assay, a colorimetric assay based on the ability of viable cells to reduce a soluble yellow tetrazolium salt (3-[4, 5-dimethylthiazol-2-yl] 2, 5-diphenyl tetrazolium bromide) (MTT) to blue and insoluble formazan crystals by mitochondrial succinate dehydrogenase activity of viable cells was performed with a minor modification ([20]). The amount of color produced is directly related to the number of cells that are metabolically active. Unlike others, in this method washing and harvesting steps, which may result in the loss of a large number of cells, were removed. All the stages of the experiment, from bacterial culture to reading the results with ELISA reader, were performed in a microplate. Therefore, the repeatability, accuracy, and sensitivity of this method are high.

## 4. Results

The measurement of bacterial growth revealed bacterial concentrations of $10^2$ CFU/mL to $10^9$ CFU/mL in various test media containing of 2.5%, 5%, and 7.5% of xylitol with or without fructose. After 24 hours of incubation, OD of the control medium containing 5% glucose was determined by UV-visible spectrophotometer. Table 1 presents means and standard deviations of bacterial growth at $OD_{570}$.

### 4.1. Xylitol Antibacterial Effects

In order for accurate evaluation of the inhibitory effect of xylitol on the growth of *S. pneumoniae* strains, reaction conditions were normalized and same conditions were prepared for all the strains examined. To achieve this, the suspensions of growing bacteria were provided in a logarithmic phase. According to spectrophotometric results, the same volumes of suspensions at an OD of 0.3 - 0.4 (about 0.5 McFarland concentration) were transferred from each strain to various test tubes containing 7.5%, 5%, and 2.5% of xylitol in BHI broth. Results regarding the effect of each of the above-mentioned xylitol concentrations at different times in 24 hours period are illustrated in Figure 1.

Kruskal-Wallis nonparametric test indicated that the 2.5%, 5%, and 7.5% concentrations of xylitol were associated with a reduction in bacterial growth, and the effects of different concentrations in various environments and times were statistically significant ($P < 0.0001$).

Furthermore, Bonferroni's post hoc test showed a significant growth difference in various test media containing 2.5%, 5%, and 7.5% concentrations of xylitol. According to the results of pairwise comparisons, the effects of various media, especially the control medium, compared with the media containing the above-mentioned concentrations of xylitol were significantly different ($P < 0.001$). In addition, counting the number of bacteria by MTT assay indicated that the growth rate of bacteria varied in different environments, but it followed the same trend. How-

ever, the simplicity and efficacy of the MTT assay were suitable for determining the anti-bacterial effect of xylitol. In other words, MTT assay is more precise in viable cell count compared to the serial dilution method and direct counting of bacterial colonies, and it stands in correlative relation to direct OD calculation method at a wavelength of 570 nm.

The results of this study revealed that the least mean of OD, in other words, the lowest number of bacterial cells, was related to the media containing 2.5%, 5%, and 7.5% concentrations of xylitol, respectively. Also, mean OD decreased during time, which indicates the inhibitory effect of xylitol on bacterial growth that was proven in the previous research as well. One of the important findings of this study was that in the presence of fructose, xylitol antibacterial effects declined.

As it was noted in this study, the media containing xylitol and fructose showed a significant difference in the mean OD relative to the medium treated with xylitol alone. However, the highest bacterial growth rate was related to the medium containing 5% glucose. Comparison of bacterial growth between the medium containing 5% glucose and the control medium showed that bacterial growth rate was lower in the medium without 5% glucose.

## 5. Discussion

In this study, the growth inhibitory effect of xylitol on *S. pneumoniae* strains was examined by different methods including the microdilution method ([21]), with minor modified MTT method with tetrazolium violet as a growth indicator ([22]), and gene expression inhibition method (data not shown). The results revealed that adding different concentrations of xylitol, fructose, and glucose to each well containing 200 $\mu$l of enriched BHI broth and $2.5 \times 10^3$ CFU/well caused no significant variation in OD absorption, which was not considered. In the medium containing 5% glucose, after 8 hours of incubation the maximum growth was obtained ($10^9$ CFU/well at an OD of 3 nm) and stagnated for up to 24 hours. However, bacterial growth rate in the media containing 2.5%, 5%, and 7.5% of xylitol was reduced significantly after 3 hours of incubation in comparison with the control medium. The combination of various concentrations of xylitol with 5% fructose inhibited the antibacterial effects of xylitol (Figure 1). These findings support the fact that xylitol alone shows antibacterial activity, but in combination with fructose its antibacterial effect is eliminated. The reason for this finding is unclear. This suggests that using fructose as a preservative or stabilizing agent for xylitol is not appropriate.

Life-threatening infections caused by *S. pneumoniae* still remain a global health problem, especially in children, the population above 50 years of age, and immunocompromised patients. Thus, controlling and preventing

**Table 1.** Means and Standard Deviations of Bacterial Growth Based on Optical Density Measurement at 570 nm Wavelength in Different Media During 24 Hours by Tetrazolium Salt-Based Colorimetric Assay[a]

| Media | 0 Hours | 3 Hours | 5 Hours | 8 Hours | 11 Hours | 15 Hours | 20 Hours | 24 Hours |
|---|---|---|---|---|---|---|---|---|
| **BHI broth (control)** | 0.0238 ± 0.0185 | 0.175 ± 0.0036 | 0.31 ± 0.0017 | 0.288 ± 0.0052 | 0.285 ± 0.0026 | 0.283 ± 0.0036 | 0.28 ± 0.001 | 0.17 ± 0.0036 |
| **BHI broth + 5% glucose** | 0.0142 ± 0.0125 | 0.165 ± 0.0026 | 0.343 ± 0.0036 | 0.315 ± 0.0036 | 0.314 ± 0.0036 | 0.31 ± 0.0036 | 0.3 ± 0.0026 | 0.151 ± 0.001 |
| **BHI broth + 2.5% xylitol** | 0.0175 ± 0.0131 | 0.156 ± 0.0036 | 0.191 ± 0.001 | 0.187 ± 0.0026 | 0.182 ± 0.0055 | 0.18 ± 0.0036 | 0.178 ± 0.001 | 0.157 ± 0.0026 |
| **BHI broth + 5% xylitol** | 0.0077 ± 0.0024 | 0.152 ± 0.0017 | 0.189 ± 0.001 | 0.177 ± 0.0052 | 0.172 ± 0.0026 | 0.171 ± 0.0036 | 0.17 ± 0.0045 | 0.138 ± 0.0043 |
| **BHI broth + 7.5% xylitol** | 0.0066 ± 0.0004 | 0.142 ± 0.001 | 0.186 ± 0.0043 | 0.174 ± 0.0026 | 0.171 ± 0.002 | 0.17 ± 0.0043 | 0.166 ± 0.0036 | 0.135 ± 0.0017 |
| **BHI broth + 2.5% fructose + 2.5% xylitol** | 0.0181 ± 0.0086 | 0.162 ± 0.0036 | 0.275 ± 0.001 | 0.265 ± 0.00608 | 0.261 ± 0.0017 | 0.26 ± 0.0036 | 0.258 ± 0.003 | 0.146 ± 0.0026 |
| **BHI broth + 5% fructose + 5% xylitol** | 0.0059 ± 0.00308 | 0.163 ± 0.0017 | 0.286 ± 0.001 | 0.276 ± 0.0026 | 0.266 ± 0.0026 | 0.264 ± 0.0043 | 0.262 ± 0.0017 | 0.13 ± 0.0075 |
| **BHI broth + 7.5% fructose + 7.5% xylitol** | 0.0429 ± 0.0201 | 0.165 ± 0.001 | 0.299 ± 0.0026 | 0.284 ± 0.0036 | 0.277 ± 0.0017 | 0.275 ± 0.0036 | 0.262 ± 0.0055 | 0.123 ± 0.0026 |

Abbreviation: BHI, brain heart infusion.
[a] Values are expressed as mean ± SD.
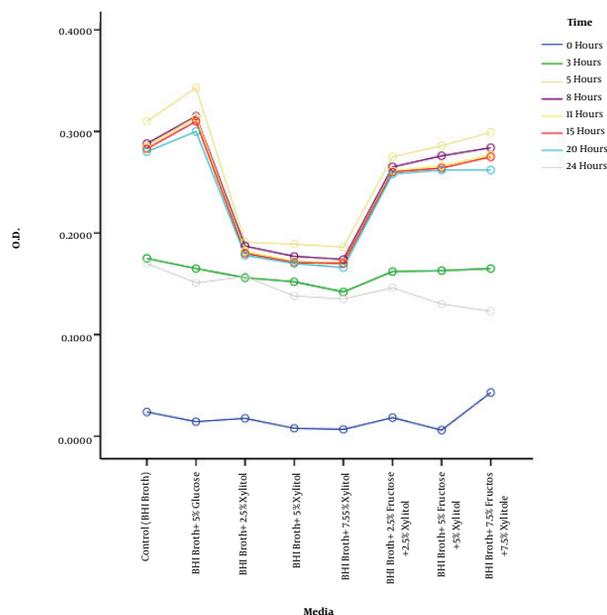


**Figure 1.** Evaluation of the effects of various test media containing 2.5%, 5%, and 7.5% concentrations of xylitol with or without fructose, along with test media containing 5% glucose and control medium during a period of 24 hours by MTT assay

pneumococcal infections in different groups is one the most important health priorities. In this regard, the administration of a dose of 13-valent pneumococcal conjugate vaccine first and then 23-valent polysaccharide pneu- mococcal vaccine (PPV23) at least two months later has been recommended (23). PPV23 protects elderly patients from hospitalization due to SpCAP, but female sex drives the effectiveness showed needed future analysis of vaccine

trials should necessitate (8). Nonetheless, the diversity of *S. pneumoniae* capsular polysaccharides results in more than 91 serotypes, of which at least 23 are virulent in vaccines (24). Despite the existing effective vaccines, they cannot cover all *S. pneumoniae* pathogenic strains. Furthermore, emergence of multi-drug resistant *S. pneumoniae* causing community-acquired pneumonia has added to the problems (25). In this regard, finding a non-related antibiotic therapy may prove useful for controlling *S. pneumoniae* infections worldwide.

### 5.1. Conclusions

Based on our findings, xylitol alone at 5% to 7.5% concentrations showed antibacterial activity against *S. pneumoniae*, and it killed about 80% to 100% of cells under laboratory conditions after 3 to 7 hours, respectively. Thus, the use of xylitol as a food additive may be effective in controlling pneumococcal superficial or gastrointestinal tract infections. However, based on our study, it must be noted that when mixed with fructose, antibacterial effect of xylitol is decreased.

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## Footnotes

## References

1. Andrade DC, Borges IC, Vilas-Boas AL, Fontoura MSH, Araujo-Neto CA, Andrade SC, et al. Infection by Streptococcus pneumoniae in children with or without radiologically confirmed pneumonia. *J Pediatr (Rio J)*. 2018;**94**(1):23–30. doi: 10.1016/j.jped.2017.03.004. [PubMed: 28668258].

2. Habibian S, Mehrabi-Tavana A, Ahmadi Z, Izadi M, Jonaidi N, Darakhshanpoure J, et al. Serotype distribution and antibiotics susceptibility pattern of Streptococcus pneumonia in Iran. *Iran Red Crescent Med J*. 2013;**15**(10). e8053. doi: 10.5812/ircmj.8053. [PubMed: 24693373]. [PubMed Central: PMC3950785].

3. Ataee RA, Mehrabi Tavana A, Ghorbani GH, Karimi Zarchi AA, Hajia M, Hosseini SMJ, et al. [Determination of bacterial etiology of 100 CSF samples of patients with Meningitis at four military hospitals in Tehran between 2003 and 2005]. *J Mil Med*. 2005;**7**(1):49–56. Persian.

4. Ataee RA, Hossaini SMJ, Karami A, Safiri Z, Allahverdi M. [Simultaneous detection of common bacterial meningitis: Neisseria meningitidis, Haemophilus influenzae and Streptococcus pneumonia by multiplex PCR]. *Trauma Monthly*. 2009;**14**(3):119–26. Persian.

5. Ataee RA, Mehrabi Tavana A, Karami A, Izadi M, Hasani SMJ, Safiri Z, et al. [The assessment of universal primer for rapid detection of bacterial meningitis]. *Hakim Res J*. 2009;**11**(4):27–32. Persian.

6. Ataee RA, Habibian S, Mehrabi-Tavana A, Ahmadi Z, Jonaidi N, Salesi M. Determination of vancomycin minimum inhibitory concentration for ceftazidime resistant Streptococcus pneumoniae in Iran. *Ann Clin Microb Anti*. 2014;**13**(1):53. doi: 10.1186/s1294-014-0053-1.

7. Mehrabi Tavana A, Ataee RA, Esmaili D. Serotyping distribution of invasive pneumococcal disease (IPD) in Iranian patients. *J Pure Appl Microbiol*. 2012;**6**(1):155–60.

8. Wiemken TL, Carrico RM, Klein SL, Jonsson CB, Peyrani P, Kelley RR, et al. The effectiveness of the polysaccharide pneumococcal vaccine for the prevention of hospitalizations due to Streptococcus pneumoniae community-acquired pneumonia in the elderly differs between the sexes: Results from the Community-Acquired Pneumonia Organization (CAPO) international cohort study. *Vaccine*. 2014;**32**(19):2198–203. doi: 10.1016/j.vaccine.2014.02.048. [PubMed: 24613522].

9. Deng X, Church DR, Vanderkooi OG, Low DE, Pillai DR. Streptococcus pneumoniae infection: A Canadian perspective. *Expert Rev Anti Infect Ther*. 2013;**11**(8):781–91. doi: 10.1586/14787210.2013.814831. [PubMed: 23977934].

10. Savini V, Cecinati V, Onofrillo D, Consilvio NP, Polilli E, Crescenzi C, et al. Surgical wound infection by Streptococcus pneumoniae after a cat-scratch disease. *Int J Low Extrem Wounds*. 2012;**11**(4):311–2. doi: 10.1177/1534734612463702. [PubMed: 23222165].

11. Dhar P, Ng GZ, Dunne EM, Sutton P. Mucin 1 protects against severe Streptococcus pneumoniae infection. *Virulence*. 2017;**8**(8):1631–42. doi: 10.1080/21505594.2017.1341021. [PubMed: 28605238]. [PubMed Central: PMC5810494].

12. Racedo S, Villena J, Medina M, Aguero G, Rodriguez V, Alvarez S. Lactobacillus casei administration reduces lung injuries in a Streptococcus pneumoniae infection in mice. *Microbes Infect*. 2006;**8**(9-10):2359–66. doi: 10.1016/j.micinf.2006.04.022. [PubMed: 16904925].

13. Sakallioglu O, Guvenc IA, Cingi C. Xylitol and its usage in ENT practice. *J Laryngol Otol*. 2014;**128**(7):580–5. doi: 10.1017/S0022215114001340. [PubMed: 24999540].

14. Kontiokari T, Svanberg M, Mattila P, Leinonen M, Uhari M. Quantitative analysis of the effect of xylitol on pneumococcal nasal colonisation in rats. *FEMS Microbiol Lett*. 1999;**178**(2):313–7. doi: 10.1111/j.1574-6968.1999.tb08693.x. [PubMed: 10499281].

15. Kontiokari T, Uhari M, Koskela M. Antiadhesive effects of xylitol on otopathogenic bacteria. *J Antimicrob Chemother*. 1998;**41**(5):563–5. [PubMed: 9630410].

16. Kontiokari T, Uhari M, Koskela M. Effect of xylitol on growth of nasopharyngeal bacteria in vitro. *Antimicrob Agents Chemother*. 1995;**39**(8):1820–3. [PubMed: 7486925]. [PubMed Central: PMC162832].

17. Kurola P, Tapiainen T, Kaijalainen T, Uhari M, Saukkoriipi A. Xylitol and capsular gene expression in Streptococcus pneumoniae. *J Med Microbiol*. 2009;**58**(Pt 11):1470–3. doi: 10.1099/jmm.0.011700-0. [PubMed: 19589898].

18. Kurola P, Tapiainen T, Sevander J, Kaijalainen T, Leinonen M, Uhari M, et al. Effect of xylitol and other carbon sources on Streptococcus pneumoniae biofilm formation and gene expression in vitro. *APMIS*. 2011;**119**(2):135–42. doi: 10.1111/j.1600-0463.2010.02703.x. [PubMed: 21208281].

19. Manning J, Dunne EM, Wescombe PA, Hale JD, Mulholland EK, Tagg JR, et al. Investigation of Streptococcus salivarius-mediated inhibition of pneumococcal adherence to pharyngeal epithelial cells. *BMC Microbiol*. 2016;**16**(1):225. doi: 10.1186/s12866-016-0843-z. [PubMed: 27681377]. [PubMed Central: PMC5041332].

20. Prabst K, Engelhardt H, Ringgeler S, Hubner H. Basic colorimetric proliferation assays: MTT, WST, and resazurin. *Methods Mol Biol.* 2017;**1601**:1–17. doi: 10.1007/978-1-4939-6960-9_1. [PubMed: 28470513].

21. Ataee RA, Mehrabi Tavana A, Hosseini Seyed M, Moridi K, Ghorbananli Zadegan M. A method for antibiotic susceptibility testing: Applicable and accurate. *Jundishapur J Microbiol.* 2012;**2012**(1, Winter):341–5. doi: 10.5812/kowsar.20083645.2374.

22. Gholami M, Mohammadi R, Arzanlou M, Akbari Dourbash F, Kouhsari E, Majidi G, et al. In vitro antibacterial activity of poly (amidoamine)-G7 dendrimer. *BMC Infect Dis.* 2017;**17**(1):395. doi: 10.1186/s12879-017-2513-7. [PubMed: 28583153]. [PubMed Central: PMC5460590].

23. Ludwig E, Meszner Z; Hungarian Society of Infectious Disease; Clinical Microbiology Vaccinology Section. [Prevention of Streptococcus pneumoniae (pneumococcal) infections in adults]. *Orv Hetil.* 2014;**155**(50):1996–2004. Hungarian. doi: 10.1556/OH.2014.30070. [PubMed: 25481502].

24. Gonzalez-Miro M, Rodriguez-Noda L, Farinas-Medina M, Garcia-Rivera D, Verez-Bencomo V, Rehm BHA. Self-assembled particulate PsaA as vaccine against Streptococcus pneumoniae infection. *Heliyon.* 2017;**3**(4). e00291. doi: 10.1016/j.heliyon.2017.e00291. [PubMed: 28435909]. [PubMed Central: PMC5390691].

25. Tiwari K, Raj VS, Upadhyay DJ, Gupta RK. In vitro activity of bioactive extracts from rare actinomycetes against multi-drug resistant Streptococcus pneumoniae. *J Appl Microbiol.* 2015;**118**(6):1306–14. doi: 10.1111/jam.12810. [PubMed: 25810118].