Current Trends in Biotechnology and Pharmacy
Vol. 19(2) 2362-2371, April 2025, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2025.2.22

In Vitro Effects of Homoeopathic *Streptococcus pneumoniae* Nosode on a *Streptococcus pneumoniae* Culture

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Abstract

Globally, antimicrobial resistance is a huge healthcare concern and is projected to cause 10 million deaths worldwide by 2050 if the current trend of irrational utilization of antibiotics continues. The search for new antimicrobials continues to be a pressing need in humanity's battle against bacterial infections. Several in vitro studies have yielded positive results on homoeopathic nosodes and other homoeopathic remedies. However, none of the in vitro or in vivo studies has been conducted on *S. pneumoniae* nosode. To test this paradigm, we assessed the in vitro effects of homoeopathic *S. pneumoniae* nosode on an S. pneumoniae culture. *S. pneumoniae* ATCC49619 was obtained from the National Health Laboratory Service, University of KwaZulu-Natal. The homeopathic remedies tested were *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH. Antimicrobial susceptibility testing was performed using disc diffusion and minimum inhibitory concentration (MIC). The positive control used was ceftriaxone, and the negative control was 20%ethanol. No significant inhibitory effect of any of the tested homoeopathic remedies, *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH including 20%ethanol on *S. pneumoniae* could be found. *S. pneumoniae* demonstrated susceptibility to ceftriaxone. The MIC of ceftriaxone was 2µg/ml. In conclusion, the study revealed that the tested nosode, *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH exhibited no antibacterial potential against *S. pneumoniae*. These findings are in concordance with the hypothesis that homoeopathic remedies are based on host effects: such as activation of the immune system, rather than direct impact on pathogens.

Keywords: Antimicrobial resistance, *Streptococcus pneumoniae*, Homoeopathic nosodes, in vitro Microbiology test

Introduction

Antimicrobial resistance (AMR) is an escalating global challenge, resulting in limited treatment options, and remains a significant concern in healthcare(1, 2). According to the World Health Organization (WHO), 10 million people including 4.1 million people in Africa, are expected to die from AMR organisms by 2050. Bacteria can develop resistance to antibiotics through mutation or acquisition of resistant genes from other bacteria(3, 4). One of the causes of antibacterial resistance is the misuse of antibiotics in agriculture, animal populations, and human medicine, which creates selection

pressure that enables the development of resistant bacterial strains (5, 6).

The situation is aggravated by the increased incidence of infections with multi-drug-resistant bacterial strains, underscoring the need for new treatment options that can eliminate pathogens and prevent drug resistance. Consequently, alternative approaches, such as homoeopathy should be considered (7, 8).

Homoeopathy is an alternative medicinal therapeutic approach based on the Law of Similars. Despite being around for over 200 years, remains one of the most controversial traditional medicine treatments due to a lack of in vitro and in vivo studies(9, 10). Homoeopathic medications are prepared from biological materials, such as live and inactivated organisms, or diseased materials. Nosodes are homoeopathic remedies prepared from inactivated microorganisms such as bacteria, disease products (fluids or tissue), or viruses(11). Using the minimum inhibitory concentration method, *C. albicans K. pneumoniae, E. coli* and S*almonella typhi* polyvalent nosodes exhibited antibacterial effects not only against their respective microorganism but also against other selected organisms (12).

The lack of data and the limited in vitro studies conducted on nosodes revealed a gap that needs to be addressed in the homoeopathy field. Generally, it is accepted that homoeopathic medication works in vivo by stimulating the immune system, thereby enhancing its effectiveness. The study aims to assess the effect of isopathic nosode (*S.pneumoniae*) on the *S. pneumoniae* strain, which has become a serious health problem.

Materials and Methods

Research design

This panel observational study was conducted at the National Health Laboratory Service, Department of Medical Microbiology, Inkosi Albert Luthuli Hospital for 2 weeks in June 2024. Ethical clearance was obtained from the Institutional Research Ethics Committee (IREC). *Streptococcus pneumoniae* ATCC 49619 was utilized.

Preparation of the homoeopathic nosode

For the in vitro testing, a homoeopathic nosode was utilized in low potencies (6CH and 9CH), medium (30CH), and high potencies (200CH). *S. pneumoniae* nosode 6CH (20% ethanol) was purchased from Comed Health in Pretoria (Waltloo), South Africa. The nosode was prepared according to the German Homoeopathic Pharmacopoeia method 44(13, 14). The *S. pneumoniae* nosode 6CH was diluted and agitated in 20% ethanol to potencies 9CH,30CH, and 200CH(14)

Potencies were stored at the Department of Homoeopathy Laboratory, Durban University of Technology (DUT) at temperatures between 4C and 6C, ensuring they did not freeze. The bio-safety measures were followed during the preparation process. The transfer of 1 drop of the previous potency for preparation of subsequent potency was conducted in the controlled, aseptic environment (in a laminar airflow with a bunsen burner)). *S.pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH were evaluated for antimicrobial efficacy on *S.pneumoniae (15)*.

Preparation of the discs

Filter paper (Whatman® no. 4) was used to prepare discs approximately 5 mm in diameter. These discs were placed in an aluminum foil and autoclaved at 15lbs for 15 minutes to ensure sterilization. Using sterile forceps, the discs were placed in sterile Petri dishes, and each disc was impregnated with an appropriate test substance (*S.pneumoniae* nosodes and 20% ethanol) using a double-impregnation technique. The first impregnation stage con-

sisted of 20 microlitres of the appropriate substance, the second consisted of 10 microlitres. Between each impregnation stage, the discs were dried at 37°C with the lids of each petri dish closed(16).

Disc diffusion assay

The disc diffusion method for antimicrobial susceptibility testing was conducted according to the standard method by Bauer(17) to evaluate the potential presence of antibacterial activities of *Streptococcus pneumoniae* nosode. A 0.5 McFarland standard *Streptococcus pneumoniae* ATCC49619, was used to lawn Mueller Hinton agar supplemented with 5% sheep blood plates. The discs that had been impregnated with tested substances were applied on the center of the Mueller Hinton Agar surface. Each test plate comprised one disc. The tests were done in triplicate to ensure reliability and validity. The medicated disc included *S. pneumoniae* nosode 6CH, *S. pneumoniae* nosode 9CH, *S. pneumoniae* nosode 30CH, *S. pneumoniae* nosode 200CH, and 20% ethanol, and ceftriaxone 30µg. The positive control was Ceftriaxone 30µg discs obtained from JVL Lab Engineering and General Supplies Close Corporation, South Africa. The negative control was 20% ethanol obtained from Shalom laboratory suppliers, in South Africa.

The plates were then incubated at 37°C for 18-48 hours in an inverted position. The plates were observed at 18, 24, and 48 hours of incubation for zone inhibition(18-20). Zone diameters (mm) were determined after 18, 24, and 48 hours of incubation at 35°C and measured the point at which the total growth inhibition zone was noted. The assay was performed twice to ensure reliability. Ceftriaxone zone diameter was interpreted using Clinical Laboratory Standard Institute (CLSI) guidelines(21), where a zone diameter of ≥ 24mm is considered sensitive, while the absence of a zone indicated as (-) is considered resistant(21).

Minimum Inhibitory Concentration Determination

The minimum inhibitory concentration was determined by Epsilometer (E-test) method as per the manufacturer's instructions (Biomeriuex, South Africa). Twenty-four-hour *S. pneumoniae* colonies were suspended in a sterile 0.9% saline solution and adjusted to the McFarland turbidity standard of 0.5. After inoculating the agar plates with the standard inoculum, ceftriaxone E-test scripts were placed on the plates. The plates were incubated at 37°C in a dark incubator for 18-24 hours.

Results and Discussion

The results obtained from this research are presented in figures and tables below. Figures 1 and 2 show the antibacterial assay of *S. pneumoniae* nosode and controls by the Kirby Bauer method in *S. pneumoniae* for both dry and wet medicated discs. The MIC is shown in Figure 3.

The MIC assay results of ceftriaxone for antibacterial activity against *S. pneumoniae* was 2µg/ml. Antimicrobial susceptibility test showed that *S. pneumonia*e was resistant to *S. pneumoniae* nosode 6CH*, S. pneumoniae* nosode 9CH, *S. pneumoniae* nosode 30CH, *S. pneumoniae* nosode 200CH and 20% ethanol as there was an absence of zone of inhibition.

None of the tested substances, *S. pneumoniae* nosode 6CH, 9CH, 30CH, 200CH, and 20% ethanol demonstrated any zones of inhibition at 18, 24, and 48 hours of incubation. The positive control, ceftriaxone 30µg inhibited the growth of *S. pneumoniae.*

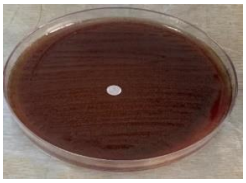
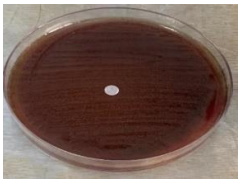
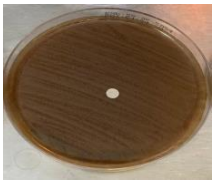
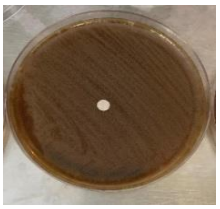
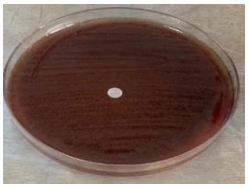
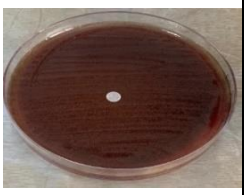
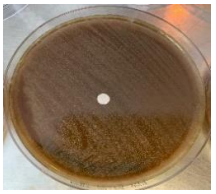
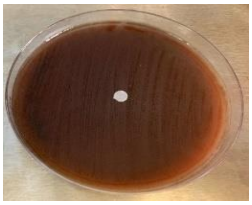
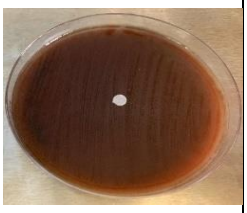
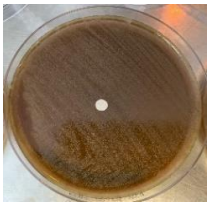
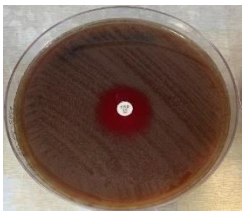
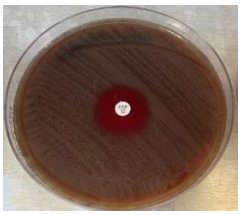
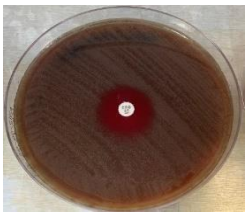
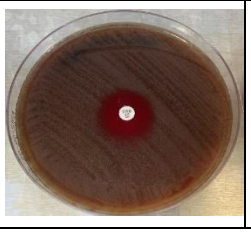
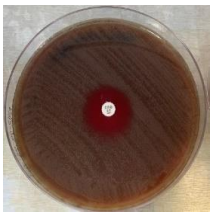
Tested substances	Time		
	18 hours	24 hours	48 hours
S. pneumoniae nosode 6CH Dry disc			
S. pneumoniae nosode 9CH Dry disc			
S. pneumoniae nosode 30CH Dry disc			
S. pneumoniae nosode 200CH Dry disc			
20% Ethanol Dry disc			
Ceftriaxone 30µg			

Figure 1. Antibacterial assay of *S. pneumoniae* nosode and controls by Kirby Bauer method in *S. pneumoniae* (dry medicated discs)

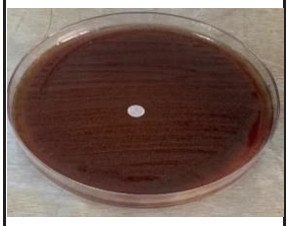
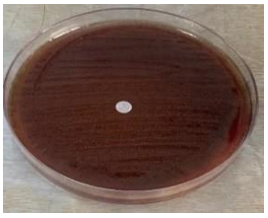
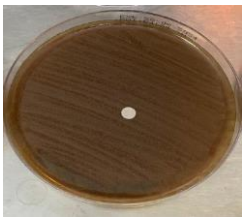
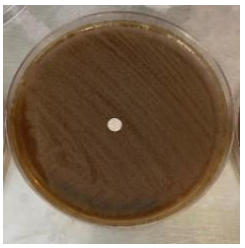
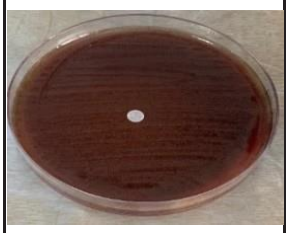
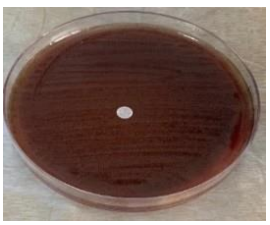
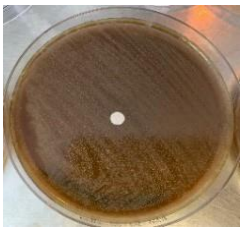
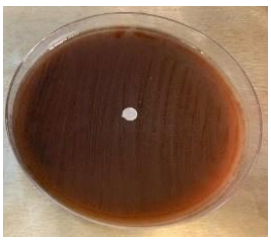
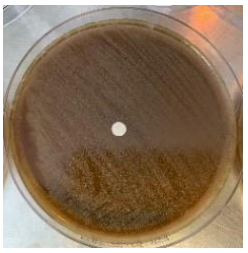
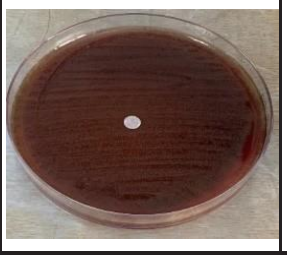
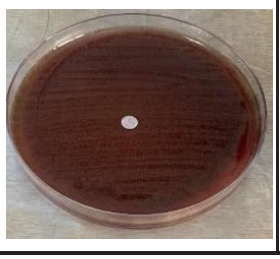
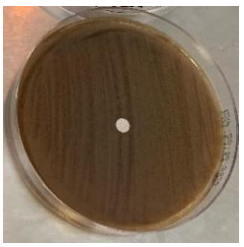
Tested substances	Time		
	18 hours	24 hours	48 hours
S.pneumoniae nosode 6CH Wet disc			
S.pneumoniae nosode 9CH Wet disc			
S.pneumoniae nosode 30CH Wet disc			
S.pneumoniae nosode 200CH Wet disc			
20% Ethanol Wet disc			

Figure 2: Antibacterial assay of *S.pneumoniae* nosode and controls using the Kirby Bauer method in *S. pneumoniae* (wet medicated discs)

Table 1**:** Antimicrobial susceptibility of *S.pneumoniae.*

Tested Substances	Concentration	Time (hours)	Zones Diameter (mm)
S. pneumoniae nosode	6CH	18	-
		24	-
		48	
S. pneumoniae nosode	9CH	18	-
		24	-
		48	-
S. pneumoniae nosode	30CH	18	-
		24	-
		48	-
S. pneumoniae nosode	200CH	18	-
		24	-
		48	-
Ethanol	20%	18	-
		24	-
		48	-
Ceftriaxone	30µg	18	20
		24	20
		48	24

The absence of zone diameter is interpreted as (-) and the presence of zone diameter is interpreted in (mm)

Table 1 shows the zone diameter measured from the *S.pneumoniae* nosode and controls after 18, 24, and 48 hours of incubation. The zone of diameter for ceftriaxone was interpreted using CLSI guidelines, where a zone diameter of ≥ 24mm is considered sensitive, while the absence of a zone indicated as (-) is considered resistant. Antimicrobial susceptibility test showed that *S.pneumoniae* was sensitive to ceftriaxone 30µg, with a zone diameter of 24mm which is within the expected zone diameter. *S.pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH could not inhibit the growth of *S.pneumoniae* and this is shown by the absence of a zone of inhibition after incubation.

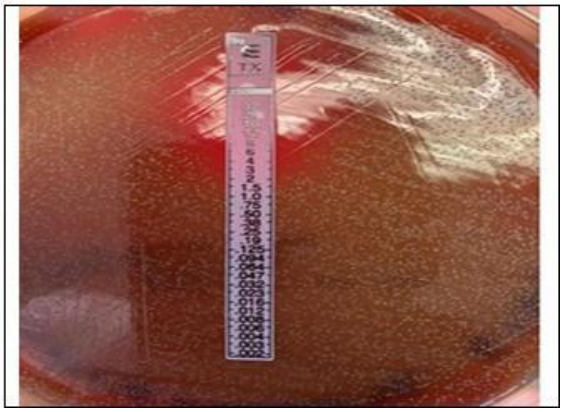


Figure 3: MIC for ceftriaxone using E-test

Discussion

The investigation into the direct microbicidal effects of potentized remedies, especially nosodes, in vitro, is relatively limited compared to the extensive homoeopathic clinical trials in medical research.

We were not able to demonstrate any significant effect of homoeopathic drugs in high potencies on *S.pneumoniae* growth in vitro. The hypothesis that *S.pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH have an in vitro efficacy against *S.pneumoniae* bacteria was therefore rejected.

Only a few previous studies evaluated the in vitro effects of homoeopathic drugs on bacteria; the majority of these studies detected significant inhibition of bacterial growth. Passeti (22) examined the effects of Belladona (12CH and 30CH) and *S. pyogenes* (12CH and 30CH) on *S. pyogenes* strains. The results showed that both remedies inhibited bacterial growth in vitro. Additionally, the authors found that treating MRSA cultures with Belladonna or MRSA nosode (6CH and 30CH) reduced bacterial growth in vitro, decreased enzymatic activity, and increased bacterial susceptibility to antibiotic treatment(22).

The absence of a positive anti-microbial response against *S.pneumoniae* contradicts the findings of the study by Simi and Bencitha regarding the effectiveness of nosodes on microorganisms. Their study results showed that *pyroginium* 200CH, 1M, and A*nthracinum* 200CH have a significant antimicrobial effect against *methicillin-resistant Staphylococcus aureus* (MRSA)(23).

In contrast, the lack of growth inhibition in *S.pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH on *S.pneumoniae* is consistent with the findings of Pareek and Jadhav(24). The study assessed whether Sulphur, Senega, Lobelia inflata, and *Klebsiella pneumoniae* nosode(6CH, 12CH, 30CH, 200CH, and 1M) possess antimicrobial effects against *Klebsiella pneumoniae*. According to the results, it was concluded Sulphur, Senega, and Lobelia inflata showed an inhibitory effect. However, no statistically significant results were demonstrated by *Klebsiella pneumoniae* nosode (24).

The study aimed to evaluate the efficacy of Apis mellifica, Graphites, Arsenicum album, and Pulsatilla against different diseases at the potencies of 30C and 200C. The tested homoeopathic remedies did not exhibit antimicrobial effects against *Staphylococcus spp* using the agar well diffusion method(10).

A study by Bruna(25) on the verification of antimicrobial activity of *S. sclerotiorum* nosode and sulphur on the mycelial growth of *S. sclerotiorum* indicated that neither *S. sclerotiorum* nosode nor sulphur reduced the growth of *S. sclerotiorum.*

An in vitro study analyzing the micro-sclera and mycelial growth of the fungi showed that the *M.phaseolina* nosode did not reduce the micro-sclerotia and mycelial growth(26).

Additionally, an in vitro study examined the antimicrobial activity of *Streptococcinum* nosode 6CH, 30CH, 200CH, 0/6, 0/30 and 0/60 LM potencies. It was found that *Streptococcinum* nosode in infinitesimal dilutions does not exhibit antimicrobial activity in either the broth dilution method or the disc diffusion method, however, it does exhibit promicrobial activity in both methods (27).

According to the study findings, the negative control ethanol 20% did not display an antibacterial effect against *S. pneumoniae*. Sauerbrei(28) indicate that the effective bactericidal concentrations of ethanol ranged from 60% to 85%, with required exposure times between ≤0.5 and ≥5 min. Ethanol concentrations of 30%–50% have significantly lower bactericidal activity, and the exposure times tested (5–30 min) are partly insufficient for a significant bactericidal effect(28, 29). Therefore, 20% ethanol is considered too low to produce a significant antibacterial effect. Centers for Disease Control and Prevention (CDC) recommends ethanol concentrations of 60% and 90% for disinfec-

tion(30).

A study on the efficacy of calendula officinalis tincture 60% (v/v) against *Pseudomonas aeruginosa* found no significant difference between the activity of calendula officinalis tincture 60% (v/v) ethanol and 60% ethanol on in vitro *Pseudomonas aeruginosa*. With mean activities of 6.88 and 6.69mm, respectively. Based on these results, it can be concluded that the antibacterial properties of Calendula officinalis tincture 60% (v/v) ethanol are attributed to the 60% ethanol present in the tincture.

Conclusion

Nosodes face criticism and controversy as there is a lack of scientific evidence supporting their efficacy. In the present study, no significant inhibitory effect of the diluted homoeopathic nosode samples was found against *Streptococcus pneumoniae* ATCC49619 Hence, the Findings of the current study could not draw any supportive evidence for the in vitro antibacterial potential of the homoeopathic nosode sample. The outcomes also portray the need for further thorough in vitro investigation of homoeo pathic nosodes in different diluted forms or conducting several in vivo trials in model organisms to claim the potential effectiveness of homoeopathic remedies as a suitable therapeutic agent against pathogens.

Acknowledgment

The author wants to thank the editor, research supervisors, and reviewers of the journal for their constructive comments to improve the paper. Furthermore, the author would like to thank the National Health Laboratory Service for all the necessary facilities and support.

Conflict of Interest

The authors declare no conflict of interest with respect to the authorship or publication of this paper.

Funding

No funding source applicable

References

1. Ugwu MC, Shariff, M., Beri, K., Okezie, U.M., Iroha, I.R., Esimone, C.O. Phenotypic and Molecular Characterization of β-Lactamases among Enterobacterial Uropathogens in Southeastern Nigeria. The Canadian Journal of Infectious Diseases and Medical Microbiology. 2020;2020(1):1-9.
2. Nwobodo DC, Ugwu, M.C., Anie, C.O., Al-Ouqali, M.T.S., Ikem, J.C., Chigozie, U.V., Saki, M. Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. Journal of Clinical Laboratory Analysis. 2022;9(36):1-10.
3. Pereira C, Warsi, O.M., Andersson, D.I. Pervasive selection for clinically relevant resistance and media adaptive mutations at very low antibiotic concentrations. Molecular Biology and Evolution. 2023;8(7):1-13.
4. WHO. World Health Organization [Internet]2019. [15 August 2024]. Available from: https://www.who.int/docs/default-source/immunization/position_paper_documents/pneumococcus/who-pp-pcv-2019-presentation.pdf.
5. Philips BJ, Redman, J., Brennan, A., Wood, D., Holliman, R., Baines, D., Baker, E.H. Glucose in bronchial aspirates increases the risk of respiratory MRSA in intubated patients. Thorax. 2005;60(9):709a-a.
6. Honert MSV, Gouws, P.A., Hoffman, L.C. Importance and implications of antibiotic resistance development livestock and wildlife farming in South Africa: a review. South African Journals of Science. 2018;48(3):401-12.
7. Muller A, Kleynhans, J., Gouveia, L., Meiring, S., Cohen, C., Hathaway, L.J., Gottberg, A.V. *Streptococcus pneumoniae* Serotypes Associated with Death South

Africa 2012-2018. Emerging Infectious Diseases. 2022;28(1):166-79.

8. Kato H, Hagihara, M., Hiramatsu,S.I., Suematsu, H., Nishiyama, N., Asai, N., Mikamo, H., Yamamoto, K., Iwamoto, T. Evaluating the antimicrobial efficacy of ceftriaxone regimens: 1 g twice daily versus 2 g once daily in a murine model of *Streptococcus pneumoniae* pneumonia. JAC- antimicrobial resistance. 2024;6(3):1-6.

9. Ram H, Sharma, R., Dewani, D., Singh, A. Differential Drivers of Antimicrobial Resistance across the World. Account for Chemical Research. 2019;52(4):916-24.

10. Kabir I, Ikram, A., Sikder,B., Tushar, H.H., Munshi, S.K. Determination of the Inhibitory Effects of Commercially Available Homoeopathic Drugs on Pathogenic Bacterial Growth. SVOA Microbiology. 2021:1-6.

11. Preena JJ, Kulsum, S.A.J., Amily, B., Nisha, G., Praveen, R.P., Gopukumar, S.T. A comprehensive Look at Nosode Remedies in Homoeopathic Medicines. International Neurourology Journal. 2023;27(4):326-30.

12. Munshi R, Talele, G., Shah, R. In vitro Evaluation of Antimicrobial Activities of Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Neisseria gonorrhoeae, and Candida ablicans Nosodes. *Homoeopathy*. 2022;111(1):42-8.

13. Benyunes S. German Homoeopathic Pharmacopoeia Supplement: Medpharm; 2005 3 December 2005.

14. Bibliothek D. German Homoeopathic Pharmacopoeia (GHP). publishers Ms, editor: Medpharm scientific publishers.2005. H5.2-H5.4 p.

15. Joshi S, Mukerjee, S., Vaidta, S., Talele, G., Chowdhary, A., Shah, R. Preparation, standardization and in vitro safety testing of Mycobacterium nosodes (Emtact-polyvalent nosode). Homoeopathy. 2016;105(3):225-32.

16. Prajapati S, Sharma, M., Gupta, P., Kumar, M., Dwivedi, B., Arya, B.S. Evaluation of antifungal activity of different homoeopathic mother tinctures against Candida albicans. Indian Journal of Research in Homoeopathy. 2017;11(4):237-43.

17. Bauer AW, Kirby, W.M.M., Sherris, J.C., Turck, M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. American Journal of Clinical Pathology. 1966;45(4):493-6.

18. Invernizzi JRR. A controlled in vitro study of the effectiveness of Tulbagia Violacea in herbal tincture and Homeopathic dilution (1X and 6X) against gram-positive and gram- negative bacteria. Durban. Durban University of Technology; 2002.

19. Sikdar S, Saham S.K., Bukhsh, A.R.K. Relative Apoptosis-inducing Potential of Homeopathic Condurango 6C and 30C in H460 Lung Cancer Cells In vitro. Journal of Pharmacopunture. 2014;17(1):59-69.

20. Catarina A, Valle, V., Brunel, H.S.S., Malard, P.F., Villarroel, C.L., Andrade, R.V. Homeopathic Viscum Album at Potencies D3 and 200CH Presents Cytokine Modulatory Effect Produced by in vitro Culture of Mesenchymal Stem Cells. Current Research in Complementary and Alternative Medicine. 2022;6(155):1-7.

21. Institute CaLS. M100-Performance Standards for Antimicrobial Susceptibility Testing. 33 ed: Clinical and Laboratory Standard Institute; 2023.

22. Passeti TA, Manzoni, J.A., Ambrozino, L.G.P., Ferreira, M.L., Rodrigues, P.F.P., Bissoli, L.R., Diniz, S.N., Beltrame, R.L. Action of homeopathic medicines Arnica montana, Gelsemium sempervirens, Belladonna, Mercurius solubillis, and nosodium on the in vitro growth of the bacterium *Streptococcus pyogenes* / Action of

homeopathic medicines Arnica montana, Gelsemium sempervirens, Belladonna, Mercurius solubilis, and nosode in vitro growth of *Streptococcus pyogenes*. homeopathy. 2014;77(1/2):1-9.

23. Simi PS, Horrence, B.M., Priyashree, R., Maalolan, S., Rachana, M.R.S. Anti-bacterial activity of homoeopathic nosodes An*thracinum, Pyrogenium* and *Variolinum* in 30CH, 200CH and 1M potencies against Methicillin-Resistant *Staphylococcus Aureus*- An in vitro study. Chettinad Health City Medical Journal. 2024;13(1):1-9.

24. Pareek S, Jadhav, A.B. In Vitro Evaluation of Antibacterial Activity of Homoeopathic Preparations on *Klebsiella pneumoniae.* International Journal of Health Sciences and Research. 2020;10(3):176-80.

25. Bruna BR, Jose, R.S., Sidiane, C.R., Omari, D.F.D.,Edilaine, D.V.G., Eloisa, L. . In vitro activity of homoeopathic medicines against *Sclerotinia sclerotiorum*. Scientia Agraria Pranaensis. 2016;15(3):1-18.

26. Lorenzetti E, Stangarlin, J.R., Treib, E.L., Heling, A.L., Roncato, S.C., Carvalho, J.C., Hoepers, L., Rissato, B.B., Coppo, J.C., Belmonte, C., Kuhn, O.J., Silva, I.F. Antimicrobial action against of Macrophomina phaseolina and action of the grey stem in soybean by homoeopathic remedies Nosode and sulphur. African Journal of Agriculture Research. 2016;11(36):3412-7.

27. Meneses NRA. Study of the effect of infinitesimal doses of *streptococcinum* nosode on the microbial activity of group a *β-hemolytic streptococcus* in an in vitro model. Microbiology. 2016.

28. Sauerbrei A. Bactericidal virucidal activity of ethanol and povidoneiodine. Microbiology Open. 2020;9(9):1-27.

29. Fallica F, Leonardi, C., Toscano, V., Santonocito, D., Toscano, V., Santonocito, Leornadi, P., Puglia. C. Assessment of Alcohol-Based Hand Sanitizers for Long-Term Use, Formulated with Addition of Natural Ingredients in Comparison to WHO Formulation. Pharmaceutics. 2021;13(4):1-16.

30. CDC. Centers for Disease Control and Prevention [Internet]: Centers for Disease Control and Prevention. 2024. Available from: Hand Sanitizer Guidelines and Recommendations | Clean Hands | CDC.