Evaluation of the effectiveness of *Streptococcus pneumoniae nosode* (6CH, 9CH, 30CH, and 200CH) on the growth of *Streptococcus pneumoniae*

By

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DECLARATION

This certifies that the work is solely my own, except where I have cited both published and unpublished sources. It has not been submitted to the Durban University of Technology or any other institution for evaluation.

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DEDICATION

I dedicate this thesis to my mother and father. While you may not have had much to offer financially, your unwavering support, love, and prayers have been the foundation of my journey and have brought me to where I am today.

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ABSTRACT

Background

The rise in antimicrobial resistance has made bacterial infections increasingly difficult to treat. The issue mostly stems from the misuse of antimicrobials which fosters and accelerates the growth of resistant bacteria. Innovative strategies are required to restrict the use of antibiotics without causing harm to people who truly need this medication. Given the increasing resistance of *Streptococcus pneumoniae* (*S. pneumoniae*) serotypes to new antibiotics and vaccines each year, exploring and incorporating non-antibiotic treatment strategies is crucial. Homoeopathy offers a holistic alternative for treating various common infections. Based on the principle of "*similia similibus curentur*," meaning "like cures like," homoeopathy posits that substances capable of causing symptoms in their raw form, when administered in low doses, can treat diseases with similar symptoms. Homoeopathic nosodes are homoeopathic medications made from biological products, including secretions, diseased tissues, organs, allergens, and microbial products. The use of nosodes for treating and preventing infectious diseases has been extensively researched over the past decade. However, more robust data on their therapeutic efficacy is still needed, which forms the basis for this study. This *in vitro* study aims to determine whether the *Streptococcus pneumoniae* nosode exhibits antimicrobial activity against *Streptococcus pneumoniae*. Various homoeopathic potencies of the *Streptococcus pneumoniae* nosode were evaluated for antimicrobial effects against a *Streptococcus pneumoniae* strain *in vitro* through disc diffusion assay.

Aim of the study

The study aimed to evaluate the antimicrobial effectiveness of *Streptococcus pneumoniae* nosode (at potencies of 6CH, 9CH, 30CH, and 200CH) against *Streptococcus pneumoniae* using the disc diffusion assay method.

Methodology

Measurements were performed using the disc diffusion assay and minimum inhibitory concentration (MIC). Mueller Hinton agar plates supplemented with 5% sheep blood were inoculated with the bacteria. Whatman® filter paper no. 4 discs, each with a diameter of 5 mm, were impregnated with the test substances (*S. pneumoniae* nosode at potencies of 6CH, 9CH, 30CH, and 200CH, as well as 20% ethanol). Antimicrobial

susceptibility discs containing ceftriaxone were obtained from the JVL Lab Engineering and General Supplies Close Corporation, South Africa. Various concentrations of the test substances, including ceftriaxone and 20% ethanol as controls, were utilised to assess the antibacterial activity of the *Streptococcus pneumoniae* nosode potencies through disc diffusion and MIC testing.

Results

The study results showed that the *S. pneumoniae* nosode, derived from *S. pneumoniae*, did not exhibit any inhibitory activity against *S. pneumoniae*. In contrast, the positive control, ceftriaxone, demonstrated a significant inhibitory effect against *S. pneumoniae*. The negative control, 20% ethanol, showed no inhibitory effect.

Conclusion

This study found that *S. pneumoniae* nosode, derived from *S. pneumoniae,* did not inhibit *S. pneumoniae* growth in vitro, as determined by the disc diffusion assay. The results demonstrate that the nosode, across all tested potencies, did not show any measurable antimicrobial activity against *S. pneumoniae* under the conditions of this experiment. This lack of inhibitory effect suggests that the *S. pneumoniae* nosode, as prepared and evaluated, does not possess the antimicrobial properties necessary to impact the growth of this pathogen *in vitro.* Consequently, these results align with the hypothesis that the mechanism of homoeopathic nosodes is attributed to their influence on host factors, such as immune system activation, rather than a direct impact on the pathogens. Further investigation may be warranted to explore different formulations, dosages, or experimental conditions to better understand the potential applications or limitations of homoeopathic nosodes in treating bacterial infections.

Keywords: Nosodes, homoeopathy, *S. pneumoniae, in vitro* Microbiology test, antimicrobial resistance

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LIST OF ACRONYMS

AHPCSA	Allied Health Professions Council South Africa
AMR	Antimicrobial Resistance
ANOVA	Analysis of Variance
AOM	Acute Otitis Media
AYUSH	Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha, and Homoeopathy
CAM	Complementary alternative medicine
CAP	Community-acquired pneumonia
CHC	Community Health Centre
CNS	Central nervous system
DUT	Durban University of Technology
HAP	Health associated pneumonia
KZN	KwaZulu-Natal
LdAPN	Leishmania donovani amastigote nosode
LMICs	Low- and middle-income countries
LytA	Autolysin
MECR-1 gene	Mobilized colistin resistance -1 gene
NESp	Nonencapsulated strains
NGO	Non-Governmental Organisation
NIPD	Non-invasive pneumococcal diseases
PLY	Pneumolysin
PPSV	*Streptococcus pneumoniae* polysaccharide vaccine

PS	Polysaccharide capsule
Psp	Pneumococcal surface protein
Sig A	Immunoglobulin A
SPSS	Statistical Package for the Social Sciences
TEST	Tigecycline Evaluation and Surveillance Trial
UJ	University of Johannesburg
WHO	World Health Organisation

GLOSSARY OF TERMS

Antimicrobial Agent

An antimicrobial agent is a substance that either destroys microorganisms or inhibits their growth, thereby preventing their pathogenic effects (Mustafa 2023: 840).

Antimicrobial resistance

Antimicrobial resistance is the capacity of a microorganism to continue growing despite exposure to an antimicrobial agent that previously was effective in treating infections caused by that microorganism (Mustafa 2023: 840).

Bacteraemia

Bacteraemia is a condition where live bacteria are present in the bloodstream (Wajid, Naaz 2023: 175).

Colonisation

Colonisation is the presence of pathogenic bacteria on a body surface without producing any clinical signs of infection in the host (Sanchez, Rios *et al*. 2023: 1)

Disc Diffusion method

The disc diffusion method is an antimicrobial susceptibility testing technique used to assess the susceptibility of bacteria to various antimicrobial agents (Salam, Amin *et al*. 2023: 4).

Dysbiosis

Dysbiosis is a disruption to the microbiome that leads to an imbalance in the microbiota, resulting in alterations to their functional composition and metabolic activities (Dahiya, Nigam *et al*. 2023: 1).

Homoeopathy

Homoeopathy is a system of medicine grounded in the principle of “like cures like,” which posits that a substance causing symptoms in a healthy individual can be used to treat similar symptoms in an ill individual (Wilhelm, Hermann *et al*. 2024: 2).

Isopathy

Isopathy is the treatment of a disease using the causative agent or a product derived from the same disease (Gujarathi, Korat 2023: 1).

The law of infinitesimals

The law of infinitesimals is a principle in homoeopathy that asserts that the lower the concentration of a homoeopathic remedy, the more potent its therapeutic effect (Wilhelm, Hermann *et al*. 2024: 2).

Meningitis

Meningitis is an acute or chronic inflammation of the protective membrane and fluid that protect the brain and spinal cord, collectively known as meninges (Ibrahim, Saleem *et al*. 2024: 117).

Nosodes

Nosodes are homoeopathic remedies derived from pathological organs or tissues, including diseases-causing agents such as bacteria, fungi, parasites, or viruses (Talele, Shah 2024: 78).

Otitis media

Otitis media is the inflammation of the middle ear caused by an infection (Chan, Stephenson 2023: 376)

Pneumonia

Pneumonia is an infection of the lungs that primarily affects the small air sacs called alveoli (Pochepnia, Grabczak *et al*. 2024).

Virulence factors

Virulence factors are pathogenetic components released by a microorganism that allow them to evade host defences or cause significant damage to the host (Soni, Sinha *et al*. 2024: 2)

Epidemic

Epidemic is a sudden outbreak of an infectious disease that affects a large number of individuals within a specific population or region (Carbone, Lednicky *et al*. 2021: 546).

Ceftriaxone

Ceftriaxone is a third-generation cephalosporin antibiotic utilised for the treatment and prevention of bacterial infections (Heffernan, Curran *et al*. 2021: 207)

Sinusitis

Sinusitis is the inflammation of the mucous membrane lining the paranasal sinuses (Ahern, Cervin 2019: 1)

Virulence

Virulence refers to the extent to which a specific microorganism is capable of causing disease (Strnad, Rudenko *et al*. 2023: 2).

McFarland standard

The McFarland standard is a reference solution utilised to approximate the number of microorganisms in a sample (Chen, Li *et al*. 2024: 2).

Fastidious

Fastidious microorganisms are those that are challenging to cultivate, necessitating specific or enriched media to thrive (Dedysh 2023: 2).

Agar

Agar is a gelati-like substance employed as a medium to support bacterial growth (Nadri, Belattmania *et al*. 2023: 1).

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Streptococcus pneumoniae (*S. pneumoniae*) is a well-characterised Gram-positive opportunistic pathogen associated with a broad spectrum of diseases. These range from non-invasive pneumococcal conditions (mild infections including otitis media, sinusitis, and conjunctivitis) to invasive pneumococcal diseases (life-threatening infections such as meningitis, bacteraemia, and community-acquired pneumonia [CAP]) (Passaris, Mauder *et al*. 2022: 1).

S. pneumoniae, commonly referred to as pneumococcus, is classified as a high-priority pathogenic microorganism by the World Health Organization (WHO) (WHO 2022). It is responsible for approximately 50% of cases of community-acquired bacterial pneumonia (Brazel, Tan *et al*. 2021: 1). Community-acquired bacterial pneumonia is a major cause of morbidity, mortality, and rising healthcare costs worldwide (Brazel, Tan *et al* . 2021: 1).

The majority of *S. pneumoniae* cells are surrounded by a polysaccharide capsule that extends into the extracellular space. This capsule functions as a significant virulence determinant in *S. pneumoniae* pathogenesis. It inhibits both classic and alternative complement pathways, which decrease opsonophagocytic killing. Additionally, the capsule functions by promoting the colonisation of the nasopharynx (Passaris, Mauder *et al.* 2022: 2).

Despite the development of several antibiotics and immunisations, *S. pneumoniae* infections persist as a prominent cause of vaccine-preventable death, especially in children below 5 years old. According to the WHO, globally, invasive pneumococcal diseases (IPD) are responsible for more than one million deaths in children under the age of 5 annually, with developing countries bearing the highest burden (WHO 2019; Phongsamart, Srifeungfung *et al.* 2022: 1).

S. pneumoniae conjugate vaccines have demonstrated significant efficacy in reducing pneumococcal diseases worldwide. However, despite this reduction, the emergence

of non-conjugate vaccine (non-PVC) serotypes remains a concern. For instance, after the introduction of PVC7 in the USA in 2000, there was a notable surge in serotype 19A. Similarly, in France, a significant rise in pneumococcal meningitis cases was observed for five years following the implementation of PVC13, with serotype 24F identified as the primary cause. Serotype 24F has also been implicated in serotype replacement across various countries, including Argentina, Germany, Canada, Italy, Lebanon, Norway, Denmark, and Spain (Lo, Mellor *et al.* 2022: e735).

The polysaccharide vaccine for *S. pneumoniae* (PPSV23) provides protection against 23 serotypes and offers moderate long-term immunity. However, it does not protect infants under two years of age, who are most susceptible to severe disease and exhibit the highest rates of carriage (Davies, Cizmrci *et al.* 2022: 1). Protein conjugate vaccines (PCVs), beginning with PCV7 in the early 2000s in the United States, were introduced to address the need for effective protection in infants and to offer prolonged immunity. Current PCVs, such as PCV10 and PCV13, protect against 10 or 13 serotypes (Passaris, Mauder *et al.* 2022: 2). In contrast, higher valency PCVs like PCV15 and PCV20 are available for adults (Passaris, Mauder *et al.* 2022: 2). Notably, PCV15 (V114) has demonstrated acceptable safety and efficacy in preventing pneumococcal disease as part of routine infant vaccination schedules (Lupinacci, Rupp *et al.* 2023: 1142).

In Thailand, a significant rise in the number of serotypes 19A among children below 5 years old between 2009 and 2012 compared to the preceding decade (5.6% to $p = 0.003$). There is a lack of recent data pertaining to the serotype distribution and PCVs coverage that have been published since 2012, emphasising the need for updated information in this area (Phongsamart, Srifeungfung *et al.* 2022: 1). The 24F serotype is the predominant serotype responsible for causing pneumococcal diseases after PVC13 was introduced and its capsule has a significant potential for invasive diseases and a tendency to cause meningitis. The fatality rate for meningitis because of serotype 24F in France was 13% (Lo, Mellor *et al.* 2022: e736). Other countries experienced an increase in the number of serotype 24F, which accompanied a rise in penicillin resistance in invasive pneumococcal diseases, including those caused by non-vaccine serotypes (Lo, Mellor *et al.* 2022: e736).

An increase in drug-resistant *S. pneumoniae* is a worldwide challenge. An economic impact study on drug-resistant infections showed that those caused by multidrug-resistant organisms are linked to higher mortality rates, increased costs, and longer hospital stays (Phongsamart, Srifeungfung *et al.* 2022: 1). The swift dissemination of resistance genes worldwide raises concerns, impacting global public health. To combat microbial drug resistance, increasing research efforts in alternative therapies is a crucial component of the solution. This includes exploring non-antibiotic treatment strategies such as homoeopathy (Phongsmart, Srifeungfung *et al.* 2022: 1)

Homoeopathy is a therapeutic system that employs highly diluted substances to address and potentially cure a wide range of diseases. Despite its widespread use, with approximately 200 million individuals globally utilising homoeopathic remedies daily, experimental research in homoeopathy remains insufficient (Senel 2018: 165). The classical form of homoeopathy, or single-substance homoeopathy, was developed by the German physician Dr Samuel Hahnemann. This approach is grounded in the principle of "*similia similibus curentur*", which translates to "like cures like" (Hahnemann 1991: 3; Hahnemann 1996: 2; Patil, Gandhi 2024: 3).

Extensive discussions have examined the potential preventive role of homoeopathic medicines during epidemics, supported by studies that have shown their preventive efficacy against leptospirosis and influenza (Talele, Vaidhya *et al*. 2021: 50). According to Prasad *et al*. (2023: 73), homoeopathy utilises preparations of substances that, when administered to healthy individuals, produce effects similar to the manifestations of the disease in terms of mental, emotional, physical, and pathological states (Prasad, Aathavan *et al*. 2023: 73). Research involving *in vitro*, animal, and human models has demonstrated the specific anti-infective potential of certain homoeopathic nosodes (Talele, Vaidhya *et al*. 2021: 50). The COVID-19 pandemic provided a chance to assess the potential of homoeopathy using controlled trials. Notably, the Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha, and Homoeopathy (AYUSH) in India had previously advised using *Arsenicum album* 30CH for prevention use among at-risk populations (Talele, Vaidhya *et al*. 2021: 50).

The application of homoeopathy in treating epidemic diseases dates back to Samuel Hahnemann's time, who, for instance, advised using Belladonna for scarlet fever. Remarkably, both homoeopathy and vaccination were introduced in the same year,

1786, with Hahnemann also endorsing vaccination (Jacobs 2018: 159). During the 19th century homoeopathy gained considerable acclaim for its effectiveness in managing epidemic diseases including cholera, yellow fever, and typhus. Homoeopathy treatment strategies include individualisation, combination remedies, isopathy, and genus epidemicus (Jacobs 2018: 159).

Isopathic remedies are prepared from the actual cause of the illness, or its by-products, specifically to treat that same disease. Nosodes, a type of isopathic remedy, are prepared through the homoeopathic method of potentisation, which involves multiple stages of dilutions and succussion. Nosodes can be derived from infected tissues or bacteria; examples include *Tuberculinum*, derived from lung tissue affected by tuberculosis, and *Anthracinum*, made from anthrax toxin. Several studies exploring isopathy in epidemic diseases have shown promising results, For instance, *Oscillococcinum*, demonstrated significant improvements in treating influenza-like illness in a double-blind study (Ferley, Zmirou *et al*. 1989: 329; Jacobs 2018: 159).

In one experimental study, a nosode prepared from mice tissues infected with the tularemia bacterium, primarily affecting animals but occasionally observed in humans, was administered prophylactically to uninfected mice over a period of 30 days. A control group received 70% ethanol. Following exposure to a fatal dose of the bacterium, all nosode-treated groups, excluding those receiving the 1M potency, exhibited a notable reduction in death rates and increased mean times to death. The mortality rate among treated subjects decreased by 22%. This study underscored the need for further research in this area, offering potential solutions for scenarios where pathogens are resistant to conventional antibiotics (Jonas, Dillner 2000: 35; Jacobs 2018: 159).

Therefore, this study aimed to assess the potential antibacterial efficacy of *S. pneumoniae* nosode against *in vitro S. pneumoniae.* The findings could be clinically significant by contributing to the development of non-antibiotic treatment strategies to conventional antibiotics.

1.2 AIM OF THE STUDY

The controlled *in vitro* study assessed the antimicrobial effectiveness of the *S. pneumoniae* nosode at potencies of 6CH, 9CH, 30CH, and 200CH, prepared from

S. pneumoniae, against the growth of *S. pneumoniae* using the disc diffusion and minimum inhibitory concentration (MIC) assays.

1.3 OBJECTIVES OF THE STUDY

The objectives of the study were to:

- Assess the antimicrobial effect of the *Streptococcus pneumoniae* nosode at various potencies (6CH, 9CH, 30CH, and 200CH) on the growth of *Streptococcus pneumoniae,* utilising the homoeopathic principle of “like cures like”.
- Compare the antimicrobial efficacy of the *Streptococcus pneumoniae* nosode with that of the antibiotic ceftriaxone against the growth of *Streptococcus pneumoniae*.
- Compare the antimicrobial effects of the *Streptococcus pneumoniae* nosode with those of 20% ethanol, which served as a negative control, on the growth of *Streptococcus pneumoniae*

1.4 HYPOTHESIS OF THE STUDY

The study hypothesised that *S. pneumoniae* nosode at lower potencies (6CH and 9CH) would demonstrate a significantly greater antibacterial effect on the *in vitro* growth of *S. pneumoniae* compared to nosode at higher potencies (30CH and 200CH). It was anticipated that the higher potencies would exhibit a more limited antibacterial effect relative to the positive control, ceftriaxone. Additionally, the study posited that the various potencies of *S. pneumoniae* nosode would show a significant antibacterial effect in comparison to the negative control, 20% ethanol, regarding their impact on the growth of *S. pneumoniae*.

1.5 SIGNIFICANCE OF THE STUDY

The emergence of antimicrobial resistance (AMR) has made treating bacterial infections increasingly challenging due to the inappropriate use of antibiotics and other antimicrobials which has resulted in the proliferation of drug-resistant bacteria. To address this issue, it is crucial to develop effective strategies that limit the unnecessary use of antibiotics while ensuring that individuals who genuinely require these medications receive them.

The rising resistance of *S. pneumoniae* serotypes to newly developed antibiotics and vaccines highlights the urgent need to explore alternative approaches, such as alternative medicine. Several studies and programmes have focused on policy changes and the development of allopathic antimicrobials; however, these efforts have not yielded the desired outcomes. This underscores the importance of considering non-antibiotic treatment strategies like homoeopathy to complement conventional treatments in the fight against antibiotic resistance. An old discipline like homoeopathy might contribute to tackling the problem, which is the central focus of this research study.

This adds to the discourse taking place regarding homoeopathic *in vitro* studies and antibiotic resistance. The findings expand the existing body of knowledge in the field of homoeopathic *in vitro* studies. Through conducting rigorous research, the findings can contribute to the scientific understanding of how homoeopathic treatments may affect specific biological systems. This can help in addressing the credibility and effectiveness of homoeopathy in a controlled laboratory setting. Understanding the potential effects of homoeopathic treatments on antibiotic resistance is crucial, especially in a time when antibiotic resistance is a global health concern. The study findings shed light on whether homoeopathy has a role to play in addressing this pressing issue or if it can complement existing antibiotic treatments. The study can serve as a valuable resource for homoeopathic students who wish to explore a similar topic.

1.6 STRUCTURE OF THE DISSERTATION

The thesis is organised into six chapters, as detailed in Table 1.1.

Table 1.1: Thesis Structure

Chapter	Title	Content description
1	Introduction	Introduces the study and provides its background. Outlines the study's aim, objectives, problem statement, and offers an overview of the research.
2	Literature Review	Definition of homoeopathy, detailing its development both globally and locally. Reviews relevant *in vitro* studies involving homoeopathic nosodes. Additionally, the chapter discusses the disc diffusion method and the MIC.
3	Research Design and Methodology	Methodology, focusing on the disc diffusion method. Research design, study setting, data collection procedures, and data analysis techniques.

4	Presentation of the Findings	Results and interpretation of the findings.
5	Discussion of Findings	Discussion of the findings in relation to the existing literature and aligns them with the objectives of the study.
6	Conclusion, limitations, and recommendations of the study	Conclusion, recommendations, and limitations.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

This chapter presents a literature review focusing on existing literature on the antimicrobial activity of homoeopathic nosodes. The literature review identifies gaps in the field that the study aims to address and elaborates on the study aim and research problem. The chapter begins by outlining the history of AMR and the epidemiology of *S. pneumoniae*, providing a critical background for understanding the study's context. It then proceeds to discuss the principles and background of homoeopathy, including the concept of isopathy. These foundational elements are crucial for comprehensive assessment of the antimicrobial activity of homoeopathic nosodes.

2.2 ANTIBIOTIC RESISTANCE

Antibiotic resistance has become a public health problem globally that has developed into a severe healthcare problem in hospitals (Soltani, Versporten *et al*. 2019: 125). Antibiotic resistance is a natural phenomenon occurring when microorganisms develop the ability to resist antibiotics that were once effective against them. This antibiotic resistance is found in humans, animals, plants, the environment (including soil, water, or air), and food. It can spread between people, animals, or through food of animal origin (WHO 2020: 1).

The global spread of antibiotic resistance genes (ARGS) among clinics, hospitals, and the environment presents a challenge to human and animal health globally. Antibiotic resistance started happening from the beginning, when penicillin was first introduced in the 1940s (Zaman, Khan *et al*. 2021: 1). Most bacterial species had developed the ability to resist antibiotics long before their widespread use in preventing and treating infections. Antibiotic resistance is no longer only confined primarily to hospitals but has become increasingly common in family practice settings, as well as medical and dental communities (Turner, Bishai *et al*. 2020: 1).

A significant factor driving the ancient and continuous evolution of resistance mechanisms is relentless competition for resources between microorganisms, which includes the natural production of secondary metabolites similar to several modern antibiotics. Nearly all available antibiotics have been identified as having bacteria which are resistant toward them (Yusuf, Bax *et al.* 2021: 2). Hence there is an increase in morbidity, mortality, and costs of health care (Menghani, Sanchez *et al*. 2023: 1). Antibiotic resistance is a significant concern due to the growing frequency of treatment failures and higher treatment costs (Ma, Xu *et al*. 2021: 33). Additionally, the use of antibiotics reduces non-pathogenic bacteria and leads to an increase in antibiotic-resistant opportunistic microorganisms in the colon (Huang, Feng *et al*. 2022: 1).

Hou *et al*. (2023: 11) highlight that the quantitative relationship between antibiotic use and resistance is not well understood. Several authors agree that antibiotic resistance is associated with overuse of antibiotics (Weinstein, Zaman *et al.* 2018: 3). This includes uncontrolled use in the clinical setting, pharmacies, and as a result of patient demands (Carvalho, Chenouf *et al*. 2021: 2). A study conducted by Granlund *et al*. (2021: 187) revealed that doctors who are paid per prescription prescribe antibiotics nearly five times more often than other doctors (Hutchinson, Foley 1999: 3; Granlund, Zykova 2021: 187). This finding raises concerns about the potential overuse of antibiotics, which can contribute to the advancement of antibiotic resistance and other related healthcare issues (Hutchinson, Foley 1999: 3; Ma, Xu *et al*. 2021: 33).

The practice of dispensing antibiotics without a prescription continues in certain countries, including Pakistan, India, Zambia, Mozambique, and Spain (Ahmad, Khan *et al*. 2023). For instance, in Zambia, an alarming 97% of antibiotics are provided without a prescription, significantly contributing to the growing problem of antimicrobial resistance (Kalungia, Burger *et al*. 2016). Antibiotic misuse is a leading factor, as highlighted by Ocan *et al*. (2017), who reported that 44.8% of antibiotics used to manage upper respiratory tract infections (URTIs) in Uganda were obtained without a prescription. Most of these countries are lacking standard treatment guidelines, healthcare antibiotic workers and veterinarians frequently overprescribe antibiotics, leading to widespread misuse by the public. Without urgent interventions, we are approaching a post-antibiotic era, where even minor injuries and common infections can result in a rise in mortality rate (Baquero, Martinez *et al*. 2021: 2).

Incorporation of antibiotics into animal feeds contributes to antibiotic resistance and is a major problem (Carvalho, Chenouf *et al*. 2021: 2). This practice is often employed in the livestock industry to promote growth and prevent diseases in animals. However, it contributes to antibiotic-resistant bacteria within the animal population. This is because these antibiotics are not only administered to treat infections but also for disease prevention and growth promotion in animals (Kipper, Mascitti *et al*. 2022: 2). Despite remarkable progress in modern medicine, the optimism surrounding antibiotics is diminishing due to various resistance mechanisms, which present a critical threat to frontline antibiotics (Alsheikh, Sultan *et al*. 2020: 1).

The economic burden of Community-acquired pneumonia (CAP) is steadily increasing, exceeding 17 billion dollars each year in the United States, due to infections caused by resistant bacteria. *S. pneumoniae*, the most commonly isolated bacterium in CAP, is showing increasing resistance to widely prescribed antibiotics for instance penicillin, macrolides, and fluoroquinolones, leading to its classification as drug-resistant *S. pneumoniae* (DRSP) (Stefano, Cook *et al*. 2019: 1).

Many different antibiotics and vaccinations have been developed since AMR. Despite various efforts in recent decades, such as developing new vaccinations and antibiotics annually as a result of micro-organisms developing resistance toward the old treatment, global AMR trends show no signs of slowing down (Larsson, Flach *et al*. 2022: 257). Numerous studies have shown the challenging financial results of AMR including a rise in hospital admissions and drug usage hence causing extremely high healthcare costs. Infection with AMR results in serious diseases, prolonged hospital admissions, and failures in treatment (Shrestha, Cooper *et al*. 2018: 1).

Whenever antibiotic resistance has emerged, the problem has been managed by introducing the latest antibiotics with minimal cross-resistance to existing treatments or by adding different classes (Zaman, Khan *et al.* 2021: 1). The early simplicity and financial incentives of antibiotic discovery led to the widespread, uncritical utilisation of antibiotics without considering the social consequences. However, major scientific challenges in developing new, more effective antibiotics have arisen, particularly due to penetration barriers and efflux mechanisms in gram-positive bacteria, which often need large dosages, leading to toxicity concerns. Given the lengthy research and

advancement timeline, alternative approaches and interventions are desperately required (Zaman, Khan *et al.* 2021: 1).

Resource efficient strategies need to include intensified actions in order to resolve the resistance crisis globally – resistant bacteria do not recognise borders. The development of novel antimicrobial resistant control strategies is crucial in navigating the challenge posed by multi-drug resistant infections globally. The process of horizontal gene transfer and excessive use of antimicrobials in animals or humans helps to expand background populations with resistant bacteria (Liu, Thomsen *et al*. 2022: 556). Therefore, antimicrobial drugs should be controlled to reduce molecular mechanisms leading to bacterial drug resistance and lower antibiotic selection pressure. Nowadays, many antibiotics face resistance, and the threat of bacterial infections can be significantly mitigated if new antibiotics are developed faster than resistance evolves. In addition to developing new antimicrobial drugs, implementing other measures to combat bacterial drug resistance is equally crucial.

2.3 HISTORY OF ANTIMICROBIAL RESISTANCE

Antimicrobials have played a crucial role in treating bacterial infections for instance septicaemia and meningitis that, before their introduction, were untreatable and resulted in death (Mancuso, Midiri *et al*. 2021: 1). However, in recent years, the misuse and overuse of antimicrobials, along with various social and economic factors, have greatly fuelled the proliferation of antibiotic-resistant bacteria, rendering drug treatment less effective. Other major contributors to the rise of AMR include socioeconomic determinants, such as inadequate infection control in hospitals and clinics, poor community hygiene, the build-up of antibiotics in the environment, and their utilisation in the animal and food industries. In 2019, the WHO listed AMR as being one of the top 10 global health threats due to its severe impact on human health (WHO 2019: 1). Sub-Saharan Africa, including South Africa, reported the highest mortality rate due to AMR, with 23.5 deaths per 100 000 people surpassing other regions (Kariuki, Kering *et al*. 2022: 3590). AMR presents a major public health challenge, hindering global economic growth, with developing countries in Africa shouldering the heaviest burden. In sub-Saharan Africa, approximately 1.5 million cases are reported annually, leading to around 19 900 deaths, representing about 17% of worldwide AMR-related deaths (Kariuki, Kering *et al*. 2022: 3590).

The overuse of antimicrobials remains the main aetiology of AMR (Chauhan 2024: 16). Strategies to combat this issue may include non-antibiotic treatment strategies that can reduce dependence on antibiotics, minimise the spread of resistance, and improve patient health outcomes (Chauhan 2024: 16). One such alternative is homoeopathy, which displays promising results in decreasing the reliance on antibiotics. Studies have explored homoeopathy's potential in addressing AMR. For instance, a study by Weiermayer and Frass *et al.* (2022: 1) on patients with severe sepsis suggested a beneficial effect of homoeopathic treatment on long-term survival (Weiermayer, Frass *et al*. 2022: 1). A randomised trial by Macrì (2019: e37) evaluating the efficacy of homoeopathy in treating acute otitis in children indicated that homoeopathy may reduce the need for antibiotics (Macrì 2019: e37; Tripathi, Bhurupi *et al*. 2024: 6). Similarly, a randomized trial by Taylor and Jacobs (2014:1) evaluated the effectiveness of a homoeopathic ear drop preparation in reducing antibiotic use among children with acute otitis media (AOM) (Perry, Huntley *et al*. 2024: 26). The results indicated that homoeopathic ear drops might help reduce antibiotic usage when AOM is managed with a delayed antibiotic approach (Taylor, Jacobs 2014:1; Perry, Huntley *et al*. 2024: 26) .

In another study, Taylor and Jacobs (2011:109) investigated the effectiveness of homeopathic ear drops in treating otalgia in children with AOM. The findings revealed that the ear drops were moderately effective in alleviating otalgia, particularly during the early stages following an AOM diagnosis (Perry, Huntley *et al*. 2024:25). Furthermore, a randomised double-blind prospective study involving 30 participants compared standard vaccination to *Influenzinum*, a homoeopathic nosode, over 13 weeks (Nayak, Varanasi 2021: 131). The study found both methods equally effective in preventing influenza, though the vaccination group experienced more adverse reactions, suggesting that homoeopathic nosodes may help reduce antibiotic overuse in specific contexts (Nayak, Varanasi 2021: 131).

2.4 STREPTOCOCCUS PNEUMONIAE

*S. pneumonia*e is a gram-positive bacterium causing morbidity and mortality globally. *S. pneumoniae* is one of the major species of *Streptococcus* that are particularly pathogenic. *S. pneumoniae* was first described in 1881 as a major respiratory pathogen that colonises the human nasopharynx causing several diseases

(Phongsamart, Srifeungfung *et al*. 2022: 1). *S. pneumonia*e particularly affects young children, elderly, and immune-compromised patients (Phongsamart, Srifeungfung *et al*. 2022: 1). The age groups that are usually at risk are children who are younger than 5 years old and adults older than 65 years old. *S. pneumoniae* affects immune-compromised individuals for instance patients with chronic diseases, using medical treatment and smoking as they have an elevated risk of infections (Caldera, Mercer *et al*. 2020: 1).

S. pneumoniae has a variety of virulence factors that enable its successful colonisation of the host nasopharynx and immune system. When the host immune response weakens, the bacteria spread to other organs or soft tissues causing diseases ranging from non-invasive pneumococcal diseases to invasive pneumococcal diseases (Gonzalez, Melo *et al*. 2021: 5).

Non-invasive pneumococcal diseases include acute otitis media and, sinusitis while invasive pneumococcal diseases include pneumonia, meningitis, and sepsis which is fatal if left untreated and is a significant cause of death in young children and the elderly, accounting for approximately 12% of deaths annually worldwide (Gonzalez, Melo *et al.* 2021: 5). Despite a consistent decline in pneumonia cases among young children due to vaccination programmes, there are still 3.7 million of severe cases of pneumococcal diseases and over 300 000 deaths among children worldwide (Gonzalez, Melo *et al.* 2021: 5).

S pneumoniae transmission occurs through close contact or aerosols and the ability of *S. pneumonia*e to adhere to the nasopharynx is a significant stage in the process leading to pathogenesis. Despite worldwide pneumococcal vaccine administration, *S. pneumoniae* remains the leading aetiology of community-acquired pneumonia (CAP) (Shoar, Musher 2020: 1; Musher, Anderson *et al*. 2022: 1). Figure 1.1 demonstrates the nasopharyngeal colonisation and transmission of *S. pneumoniae*.

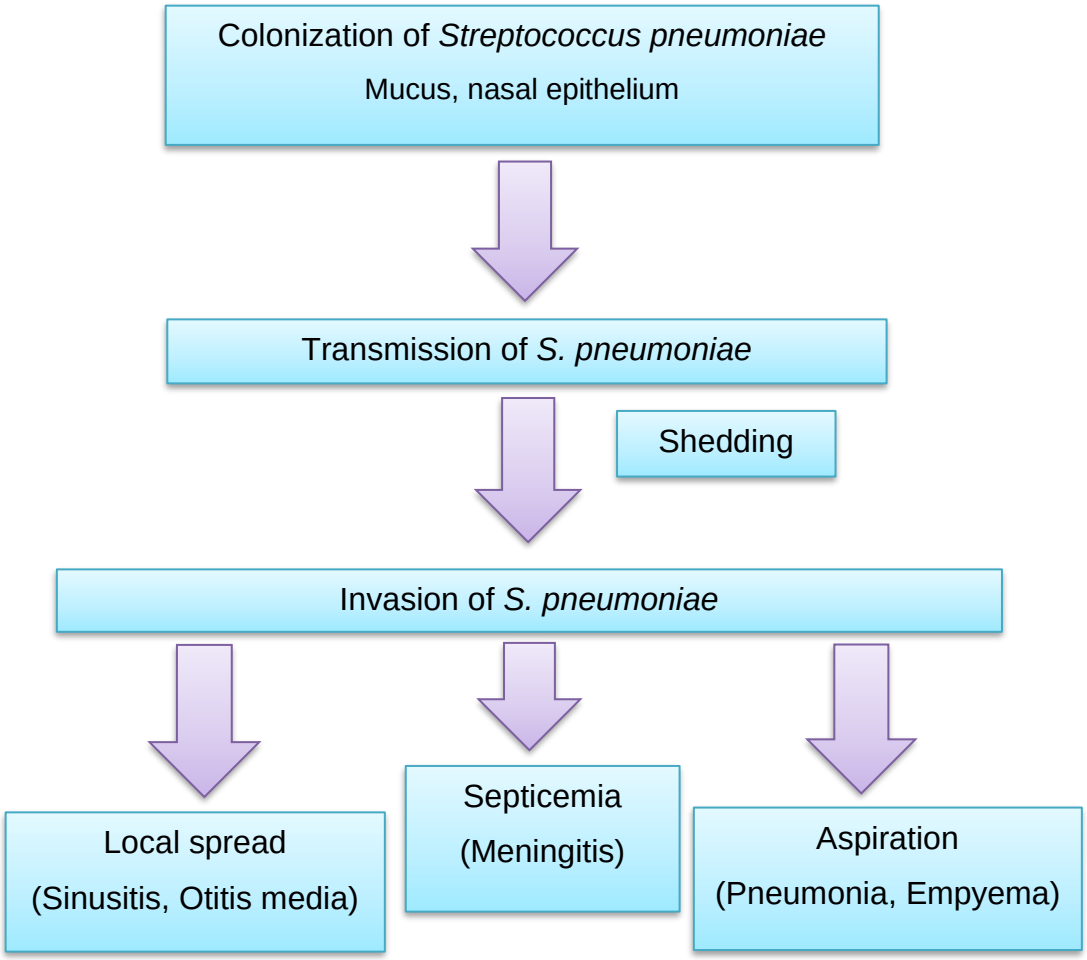


Figure 2.1: Pathogenic route for *S. pneumoniae* infection

In 2017, the WHO classified *S. pneumoniae* among 12 pathogens considered a priority. The continuation of the increasing rate of bacteria resistance to penicillin and other antibiotics such as ceftriaxone has recreated interest in prevention (Weiser, Ferreira *et al*. 2018: 355). The ability to have a combination of high carriage rates makes a bacterium noticeable as it can shift from a commensal to a pathogenic interaction with its host. *S. pneumoniae* is different from most commensals due to its ability to strongly interact with host cells and tissues, hence inducing proinflammatory components of the natural immune system (Kilian, Tetterlin 2019: 1).

2.5 MORPHOLOGY AND IDENTIFICATION

S. pneumoniae is a fastidious non-motile and encapsulated bacterium. The organism usually occurs in pairs as a result are called diplococci and gram-positive (thick peptidoglycan cell wall that takes in purple dye). They can also occur as single cells or in short chains of cocci. The bacteria grow optimally at 35 ℃ to 37 ℃ with

approximately 5% carbon dioxide (or in a candle jar). *S. pneumoniae* grows best on media containing blood and can also grow on chocolate agar plates. When *S. pneumoniae* colonies are cultured on blood agar they appear as tiny, grey in colour, mucoid, or moist colonies. They produce a zone of alpha-hemolysis which appears green. The alpha-hemolytic properties make the organism different from other species, excluding commensal *alpha-hemolytic (viridans) streptococci*. It is difficult to differentiate young pneumococci from *viridans streptococci* as they appear raised which is similar to *viridans streptococci* (Centers for Disease Control and Prevention 2016: 1).

Once *S. pneumonia*e culture ages 24-28 days, they change by becoming flat and have a depressed centre which does not occur with *viridans streptococci*. The difference between *S. pneumonia*e and *viridans* is noted easily when using a microscope (30-50X) or 3X lens. *S. pneumoniae* is also susceptible to optochin, a characteristic that aids in its identification, with the optochin test being one of the key methods used to distinguish *S. pneumoniae* colonies, with bile solubility serving as a confirmatory test (Kellogg, Bankert *et al*. 2001: 1; Castillo, Harcourt *et al.* 2011: 1).

2.6 PROPERTIES OF STREPTOCOCCUS PNEUMONIAE

S. pneumoniae is a well-characterised bacterium with distinct biological properties. *S. pneumoniae* can grow in both the presence and absence of oxygen, However, it thrives in environments where oxygen is present. It does not produce catalase, which differentiates it from other bacterial species, but does produce hydrogen peroxide, a byproduct of its metabolic processes. *S. pneumoniae* does not form spores; this trait is consistent with its classification as a non-sporulating bacterium (Soon, Shyan *et al*. 2020: 168; Mekuria, Tolossa *et al.* 2023: 3512). Figure 2.2 illustrates the list of *S. pneumoniae* properties.

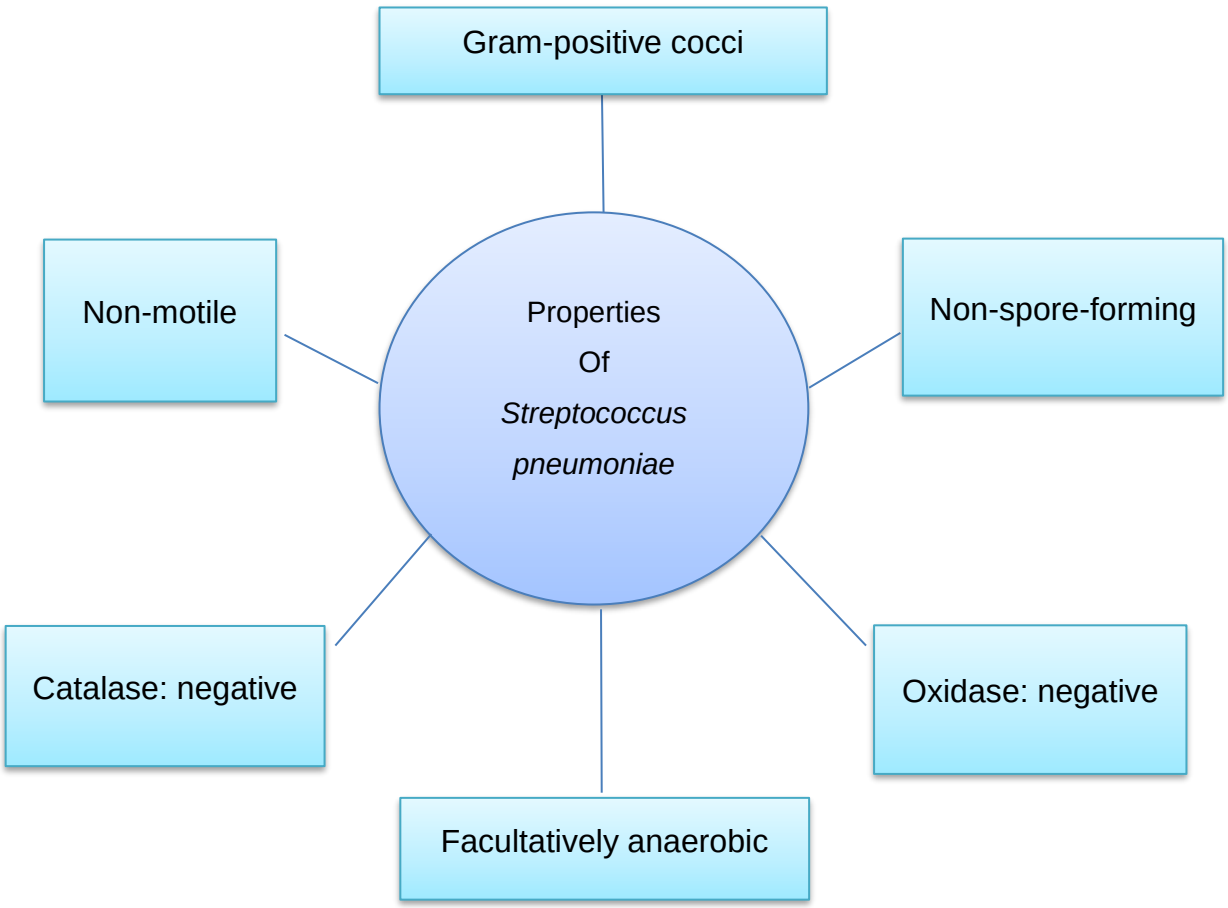


Figure 2.2: Characteristics of *S. pneumoniae*

2.7 EPIDEMIOLOGY

S. pneumoniae is a highly invasive gram-positive organism, also called pneumococcus, an extracellular bacterium that is responsible for causing the majority of community-acquired pneumonia (Sheam, Syed *et al*. 2020: 395). While the exact number of identified *S. pneumoniae* serotypes is changing, there are currently around 97 serotypes known and characterised (Kerorsa 2020: 2). *S. pneumoniae* is a pathogen that colonises the mucosal surfaces of the upper respiratory tract in humans (Weight, Venturini *et al*. 2019: 1). Worldwide, this bacterium is the second most common cause of fatal bacterial infections. Individuals at higher risk are the smallest children, the elderly, and immunocompromised people (Subramanian, Henriques *et al*. 2019: 2)

S. pneumoniae can cause invasive pneumococcal disease (bacteraemia, meningitis, etc) and non-invasive pneumococcal diseases for instance pneumonia, sinusitis, and

otitis media. This does not only occur under the condition of immunocompromised but also microflora imbalance (Zhao, Pan *et al*. 2019: 1; Peng, Guo *et al*. 2023: 2). It is known as a leading aetiology of morbidity and mortality worldwide, with global estimates indicating that in 2015, 81% of all pneumococcal deaths in HIV-negative children were due to pneumonia (Wahl, Brien *et al*. 2018: 744). Pneumonia is classified into two types depending on the location of infection: CAP which develops outside of the hospital setting, and healthcare-associated pneumonia, which occurs during or after being in a healthcare facility (Zhao, Pan *et al.* 2019: 1).

A review study conducted in South Africa on the diversity and epidemiology of human respiratory syncytial virus among patients hospitalised with severe respiratory illness between 2012-2015 showed that among patients under 5 years old, coinfection with *S. pneumonia*e was common. A surge in human respiratory syncytial virus activity was identified in patients admitted to Edendale Hospital located in KZN (Afzal, Ahmed *et al*. 2022). Despite advancements in antibiotics, immunisation, and HIV management programmes, pneumonia remains the leading aetiology of mortality in South Africa. In 2017 approximately 320 000 pneumonia episodes occurred worldwide, including 4 100 South African children aged less than 5 years (Zar, Moore *et al*. 2020: 195)

The PCV programme in South Africa began in 2009 as part of the public immunisation programme. By 2012, invasive pneumococcal disease had decreased by 69% in children below 2 years old, with an 89% reduction in PCV7 serotypes and a significant decline in PVC13 serotypes in 2012 (Marangu, Zar 2019: 4). Pneumonia is estimated to cause more deaths in children below the age of 5 compared to tuberculosis, malaria, and acquired immune diseases combined. More than 90% of *S. pneumoniae*-related deaths take place in developing countries, with the highest incidence in Africa and Asia (Zaman, Khan *et al.* 2021: 1).

2.8 VIRULENCE FACTORS

S. pneumoniae is one of the normal nasopharyngeal microbials but may become a pathogen causing many infections such as septicaemia, meningitis, and mild upper respiratory infections. The virulence genes can be acquired in several ways such as spontaneous mutations and gene exchange through horizontal gene transfer (Afzal, Ahmed *et al.* 2022: 856).

S. pneumoniae has many virulence factors that enable adherence to host cells, promote epithelial cell invasion, and decrease the host's immune response to eliminate the bacterium (Brooks, Mias 2018: 7). If the host cannot clear the bacteria after initial colonisation in the upper respiratory tract, *S. pneumoniae* can multiply and spread to sterile tissues and organs causing infections. The bacteria exploit hosts with immune systems that are compromised or weakened (Brooks, Mias 2018: 6).

The toxins produced by the bacteria are harmful and the bacteria has several surface proteins and physical structures that are crucial to its pathogenesis. *S. pneumoniae* virulence factors operate by inhibiting the host tissues and surface receptors. This interference can affect the development of the immune system in young children and exacerbate deterioration in older or immune-compromised individuals, such as those with chronic diseases or chronic deteriorating diseases (Brooks, Mias 2018: 6; Kerorsa 2020: 8). Table 2.1 outlines the virulence factors of *S .pneumoniae* and their specific functions.

Table 2.1: Selected virulence factors of *S. pneumoniae* and function

Virulence factor	Function	References
Polysaccharide capsule	• Enables the bacteria to escape the nasal mucus • Inhibits phagocytosis by innate immune cells • Allows escape from neutrophil extracellular traps (NETs)	Mathew, Gupta *et al*. 2023: 2; Costa, Cristino *et al*. 2024: 1480; Shi, Lu *et al*. 2024: e00772
Pneumolysin	• Binds to membrane with cholesterol • Forms pores that lead to cell lysis • Facilitates host-to-host transmission	Zou, Wang *et al*. 2023: 1; Hoffet, Tomov *et al*. 2024: 1; Shi, Lu *et al*. 2024: e00772
Autolysin(lytic amidase)	• Cell lysis • Break down peptidoglycan	Wong, Tourlomousis *et al*. 2023: 1; Lee, Lee *et al*. 2024: 2
Pneumococcal surface protein A	• Shields against the host's complement system • Contribute to colonisation by adhering to epithelial cell membranes	Mathew, Gupta *et al*. 2023: 2; Miao, Cui *et al*. 2023: 2
Pneumococcal surface protein C / choline-binding protein A (CbpA)	• Protects against the complement system of the host • Cells adhesion and colonisation of nasopharynx	Li, Zhou *et al*. 2023: 2; Mathew, Gupta *et al*. 2023: 2
Pneumococcal surface adhesion A (PsaA)	• Transport magnesium and zinc into the bacteria cytoplasm • Contribute to invasion of epithelial cells	Bahadori, Shafaghi *et al*. 2024: 2; Miao, Cui *et al*. 2023: 2
Choline-binding proteins: LytB, LytC, CbpC	• Promote bacterial colonisation in the nasopharynx • Modify cell surface proteins, enabling binding to host cell receptors	Puzia, Gawor *et al*. 2023: 2018
Pili	• Inhibit phagocytosis by immune cells • Enhances adherence and colonisation of the epithelial in the nasopharynx	Miao, Cui *et al*. 2023: 2
Neuraminidase	• Degrade mucus • Promotes growth and survival	Mathew, Gupta *et al*. 2023: 3

2.9 ANTIMICROBIAL SENSITIVITY

S. pneumoniae is sensitive to optochin and the optochin test is utilised to distinguish *S. pneumoniae* from *Viridans streptococci*. In the absence of acquired resistance, *S. pneumoniae* is susceptible to vancomycin, ciprofloxacin, and penicillin. *S. pneumoniae* resistance is not the same in every country or region; for instance between 2015 and 2017, Asia was the region with the most severe resistant *S. pneumoniae* globally (Kim, Jung *et al.* 2021: 2).

A study conducted in 2021 in Korea on antimicrobial sensitivity was analysed separately for non-invasive and invasive infections caused by *S. pneumoniae*. For invasive infections, central nervous system (CNS) infection and non-CNS infection for invasive infections were analysed independently (Kim, Jung *et al*. 2021: 2). The results demonstrated CNS infection-causing *S. pneumoniae* strains are highly resistant to penicillin, while non-CNS infection-causing *S. pneumoniae* strains have a low resistance rate. In the absence of acquired resistance, *S. pneumoniae* is susceptible to vancomycin, ciprofloxacin, and penicillin. Resistant *S. pneumoniae* isolates are non-susceptible to penicillin, macrolide, cephalosporin (ceftriaxone), fluoroquinolone, clindamycin, chloramphenicol, tetracycline, including cotrimoxazole and they are also referred to as multidrug-resistant (Kim, Jung *et al.* 2021: 2).

2.10 CEFTRIAXONE

Ceftriaxone is an extended-spectrum third-generation cephalosporin for diseases caused by multi-drug-resistant strains (Epstein, Hasselquist *et al*. 1982: 402; Richards *et al*. 1984: 469; Tewabe, Marew *et al*. 2021: 1). Since its introduction in the mid-1980s, ceftriaxone gained popularity due to its enhanced stability against β-lactamases, broad-spectrum efficacy, favorable pharmacokinetics, and tolerability (Lamb, Ormrod *et al*. 2002; 48; Shirin, Islam 2020: 1) Its once-daily administration simplifies complex treatment regimens in hospital settings, further solidifying its role as a preferred parenteral option in outpatient care (Brogden, Ward *et al*. 1988: 605; Lamb, Ormrod *et al*. 2002: 1081; Shirin, Islam 2020: 1). The antibiotic is usually administered for respiratory bacterial infections such as pneumonia, bronchitis, sinusitis, gastritis, and urinary tract infections (Yimer, Hishe *et al*. 2019: 2). Its high potency and effectiveness against a wide range of gram-positive and gram-negative species have made it a staple in healthcare settings (Tewabe, Marew *et al*. 2021: 2).

Figure 2.3 illustrates the chemical structure of ceftriaxone (Shrestha, Cooper *et al*. 2018: 3).

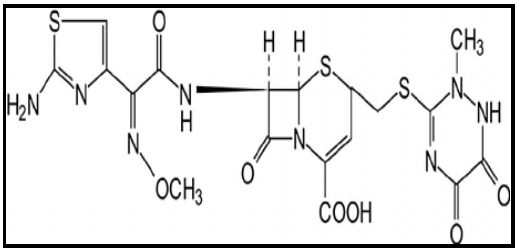


Figure 2.3: Chemical structure of Ceftriaxone
Source: Shrestha, Cooper *et al*. (2018: 3).

2.11 HOMOEOPATHY

2.11.1 MECHANISM OF HOMOEOPATHY

In the context of antibiotic resistance, homoeopathy offers potential as an alternative approach that could reduce reliance on conventional antibiotics, limit the spread of resistance, and improve patient outcomes (Chauhan 2024: 15). Although widely practiced and embraced by millions worldwide, homoeopathy remains subject to scepticism from the scientific and medical communities due to a lack of robust evidence supporting its efficacy and the seemingly implausible mechanism of action it proposes (Lobera, García 2021: 1279). Homoeopathy may serve both as a preventive and therapeutic approach for infectious diseases by addressing underlying vulnerabilities, enhancing the body's innate healing abilities, and restoring balance on physical, mental, and emotional levels (Ram, Sharma *et al.* 2020: 181).

One reason homoeopathy may hold promise in combating antibiotic resistance lies in its unique principles and treatment methods (Chauhan 2024: 16). Unlike conventional antibiotics, which target specific bacteria, homoeopathic remedies are based on the principle of treating the whole person rather than solely focusing on the disease (Swayne 2023: 281). This holistic approach emphasises the importance of supporting the body's natural healing processes, optimising immune function, and restoring balance at all levels of health (Swayne 2023: 281). By considering not only the presenting symptoms but also the individual's overall constitution, vulnerabilities, and general well-being homoeopathy seeks to enhance the body's defences and

strengthen resistance to infections. This is achieved through treating the underlying causes of susceptibility (Ram, Sharma *et al.* 2020: 182).

2.11.2 LAW OF SIMILARS

The homoeopathic system of medicine is based on the principle of "*similia similibus curentur*" which means "like cures likes". According to this principle, a substance that can induce a set of disease symptoms in a healthy person can treat an individual who is ill with a similar set of symptoms. Homoeopathy was developed by Dr Samuel Hahnemann during the late 18th century, and it has been used worldwide for more than 200 years (Drofenik 2022: 1).

Homoeopathy is ranked as the second-most common system of medicine in the world by the WHO (Kaur, Chalia *et al*. 2019: 76). Homoeopathy has been debated because of its routine that involves many dilutions in preparing the medicines. Despite controversies, there is a substantial body of clinical research, such as randomised clinical trials and meta-analyses, indicating that homoeopathy may have effects beyond that of a placebo (Kumari 2022: 353).

The Law of Similars nowadays is often explained through the concept of biochemical equilibrium. This principle is aligned with the Le Chatelier principle, a fundamental thermodynamic concept known in chemistry for nearly a century. It states that, "If a chemical system at equilibrium experiences an alteration in concentration, temperature or total pressure, the equilibrium will shift to minimise that change" (Drofenik 2019: 1).

2.11.3 HOMOEOPATHY'S "LAW OF INFINITESIMALS"

Homoeopathic remedies are medications that are prepared through a process known as potentisation (Bhargaw, Sharma *et al*. 2023: 1). The process includes a series of systemic dilutions with succussion (vigorous shaking) between each dilution (Kumari 2022: 253). These processes eliminate chemical toxicity and increase the substance's therapeutic effect. Homoeopathic potencies are made by a combination of a number and a letter (such as 6X, 6C). The number stands for the number of dilutions that the tincture has undergone in series to prepare the remedy. The letter stands for the proportions or scale used in each dilution of the series (the Roman Numeral X means

10, and the Roman Numeral C means 100 and the number of the succussions that the vial of solution undergoes at each successive stage (Xaba 2018: 52).

The law of infinitesimals in homoeopathy states that the serial dilution process increases the curative power of homoeopathic remedies as the degree of dilution increases (Drofenik 2022: 2). This means that 1-part-per-million solution of a substance is more medicinally powerful than a 1-part-per-thousand solution, which in turn has more curative power than a 1-part-per-hundred solution (Sagar 2007: 156) This is different from many modern drugs as they are ineffective at low doses, with their efficacy increasing with increasing dosage. Many homoeopathic remedies come in 30X or 200C dilutions. If a substance is diluted 30X, it means it contains 1 part of the substance in 10 parts water diluted 30 times. A crucial concept in chemistry is Avogadro's number, which is determined by X-ray diffraction of crystals. It has a value of 6.0221367×10^{23} as calculated by the International Council of Scientific Unions. Taking this constant into account, the limit placed upon dilution, which is the dilution that can be made without losing the original substance altogether, is 12C or 24X (Sagar 2007: 156; Drofenik 2022: 2).

2.12 ISOPATHY

Isopathic remedies are prepared from the actual cause of the illness, or from its by-products, to treat the same condition. Isopathic history was dominated by three homoeopaths, Wilhelm Lux, Denys Collet, and Constantine in the 1800s (Bellavite, Conforti *et al*. 2005: 1; Yaseen 2021: 154). Lux was a German veterinarian physician who further established basic isopathy principles. He proposed the Law of Equals or Sameness ("*aequalia aequalibus curentur*"). In 1833, Lux started treating his animals with the homoeopathic technique and was persuaded that each infectious malady bears inside itself the methods whereby it can be cured (Bellavite, Conforti *et al.* 2005: 1). He realised that the technique of dilution and dynamisation of an infectious substance (bacterium, infection) would enable such a substance to apply a therapeutic effect on the illness coming about because of the disease (Bellavite, Conforti *et al.* 2005: 1).

Isopathic nosodes are somewhat akin to conventional vaccination, except that they are prepared in the homoeopathic manner of potentisation, with repeated dilutions and with succussions between (vigorously shaking) each step (Jacobs 2018: 159). A study

was conducted in Cuba during an epidemic of *Leptospirosis* from 2007 to 2008 to investigate the epidemic control of *Leptospirosis* using isopathy. *Leptospirosis* is caused by a gram-negative bacteria which causes infections such as meningitis and hepatitis. Disease surveillance statistics showed an 84% decreased incidence of the disease in Cuba provinces in 2008 compared with previous years, despite the occurrence of three large hurricanes. The incidence of *leptospirosis* in other untreated provinces of Cuba rose by 21.7% (Jacobs 2018: 159).

There are many other isopathic nosodes that have been developed including *Tuberculinum* from tuberculosis-infected lung tissue and *Anthracinum* from anthrax poison. Isopathic remedies can also be made from toxic chemicals, such as arsenic, allergy-causing agents, mixed-grass pollens, and penicillin, to treat the adverse effects of those substances (Jacobs 2018: 160).

2.13 HOMOEOPATHIC NOSODES

Homoeopathic nosodes are remedies prepared from inactivated diseased products of humans, serum containing the infectious organism, or the isolated organism itself (Herscu, Talele *et al*. 2022: 1). Unlike antibiotics, nosodes as homoeopathic treatments are understood to work by stimulating the immune system in the host rather than directly targeting pathogens (Ram, Sharma *et al.* 2020: 181). Nosodes are used to treat infectious diseases and act as prophylaxis (de Barros, Seugling *et al*. 2019: 157). As a result of potentisation-dilution and succussions, nosodes lose their infectious nature even while their energetic possibilities are raised (Wulfson 2020: 2). The Greek prefix “noso” means disease and is connected to a Latin word “noxa” which is related to noxious or damaging, meaning the use of noxious materials as the basis for a homoeopathic remedy (Galande 2020: 1). The first homoeopathic nosode was discovered by Dr Hahnemann and called *Ambra grisea* (de Barros, Seugling *et al*. 2019: 157).

More than 60 nosodes have been brought into use by homoeopathic practitioners since homoeopathy was discovered (Varanasi, Nayak 2020: 130). This includes homoeopathic nosode such as *Tuberculinum* from the lung tissue infected with tuberculosis and *Anthracinum* prepared from anthrax poison. Cuba continues to be a leading nation in the development and successful implementation of nosodes in its public health system. They have used the nosodes for various diseases such as

hepatitis A, swine flu, cholera including other diseases, especially pandemics (Manchanda 2016: 220; Bala, Srivastava 2020: 216).

A study by Nambison *et al*. (2017: 27) explained that homoeopathic medicines used to treat infections are not antibiotics but may be referred to as “similibiotics” (like bacteria). This implies that these medicines induce symptoms in patients that are similar to those produced by bacteria, thereby stimulating the host's defence system to eliminate the bacteria (Nambison, Nambison *et al*. 2017: 27; Prasad, Aathavan *et al.* 2023: 73). Shah (2015) developed a *HIV* nosode and evaluated its antimicrobial effects on 27 HIV-positive patients. The results showed that nine participants (33.33%) had a surge in their CD4+ count by 20% in the 12th and 24th weeks, and they gained weight (Shah 2015: 25).

Castro *et al*. (1957) investigated the antiviral effectiveness of homoeopathic nosodes during a meningococcal meningitis outbreak in Brazil, demonstrating a 95% efficacy rate over six months when administered as homeoprophylaxis to 18 640 individuals (Castro, Nogueira1957: 2; Nayak, Varanasi 2021: 130). Similarly, Mroninski *et al*. (2001) conducted a study during an outbreak in Blumenau, where *Meningococcinum* nosode was given to 65 826 individuals aged 0-20 years over three days at public health clinics. The results showed 95% protection against severe bacterial infection after six months and 91% after one year (Mroninski, Adriano *et al*. 2001: 1; Nayak, Varanasi 2021: 130). Additionally, de Barros *et al*. (2019: 178) evaluated the effects of homoeopathic medicines and *Cochliomyia hominivorax* nosodes (8CH and 12CH) on the *Cochliomyia hominivorax* parasite, revealing a significant reduction in larval development by up to 67% under controlled laboratory conditions (de Barros, Seugling *et al*. 2019: 178).

2.14 RELATED IN VITRO AND IN VIVO RESEARCH INVOLVING HOMOEOPATHIC NOSODES

A review of the literature shows an important body of knowledge surrounding the *in vitro* and *in vivo* application of homoeopathic nosodes in different disease contexts.

The old nosodes in homoeopathy are undergoing a resurgence, and new nosodes are being introduced by current scientific methods. Nosodes have already shown to have significant therapeutic potential (Shah 2019: 232). *Psorinum* is an old homoeopathic

nosode made from the fluid of blisters from scabies-infested skin. It is known to be one of the most effective remedies for many skin conditions that are resistant to other treatments. Once the substance has been potentised, none of the original fluid remains but the energetic effects of the nosode will treat a range of diseases (Sankar, Jadhav 2017: 2). The homoeopathic nosode was introduced by Dr Samuel Hahnemann around 1835 (Shah 2019: 231).

Recent studies continue to explore the efficacy of nosodes in managing infectious diseases. Talele *et al*. (2022: 049) conducted a study to assess hospitalisation rates and recovery times among participants treated with various homoeopathic preparations, including *Arsenicum album*, *Bryonia alba*, a combination (*Arsenicum album*, *Bryonia alba*, *Gelsemium sempervirens*, and *Influenzinum*), *Coronavirus nosode CVN01*, *Camphora*, and placebo. Participants who were treated with either the CVN01 nosode or *Bryonia* exhibited a lower incidence of laboratory-confirmed COVID-19, a shorter illness duration, and fewer hospitalisations compared to those receiving a placebo ($p < 0.10$). However, no antiviral effects were observed in the other groups treated with different homoeopathic remedies or placebo (Talele, Vaidhya *et al*. 2022: 049).

Similarly, an open-label study was conducted to determine the immune response of the *Coronavirus* nosode BiosimCovex, administered orally on three consecutive days to 10 healthy volunteers. Measures including clinical examinations, immune parameters, and laboratory safety were conducted (Herscu, Talele *et al*. 2022: 1). Interferon-gamma, Interleukin-6, and CD4 were measured. During the study the laboratory tests remained unchanged with non-fatal adverse effects reported (Herscu, Talele *et al*. 2022: 7). A significant difference in IL-6 levels was observed, as determined by repeated measures ANOVA. By day 60, the IL-6 values had returned to normal in nine of the subjects. A surge in CD4 cell count was noted on day 60 compared to day 34 (Herscu, Talele *et al*. 2022: 1).

In a related study, a double-blind, randomised control trial was conducted in India to assess the potential antimicrobial activity of *Emtact* 30CH nosode on 148 patients with recurrent upper respiratory tract (URT) or lower respiratory tract (LRT) infections in a 1:1 allocation ratio (Shaik, Singh *et al*. 2023: 96). Patients were randomly given either *Emtact* 30CH nosode or placebo to be administered three times per day for a period

of 6 months. The results showed a statistically significant reduction in respiratory symptoms including, sneezing, watery nasal discharge, and especially nasal blockage ($p < 0.001$) in the group treated with *Emtact* 30CH nosode. There was also an improvement in sleep ($p = 0.0084$), mood/thinking ability ($p = 0.028$), appetite ($p = 0.0078$), and weight gain. A decrease in weight was observed in the group treated with a placebo. An improvement in haemoglobin and lymphocytes was also observed (Shaik, Singh *et al*. 2023: 96).

Similarly, a study by Ponnam *et al*. (2024:32) demonstrated significant improvements in symptoms and sleep quality in children with adenotonsillar hypertrophy (ATH) over 12 months, with frequently used homoeopathic remedies including *Calcarea carbonicum, Phosphorus, and Tuberculinum* nosode. In addition to symptom relief, improvements were also observed in mood and cognitive function, appetite, and weight gain, whereas a decrease in weight was noted in the placebo group. An increase in haemoglobin levels and lymphocyte count was also recorded.

Beyond respiratory illnesses, nosodes have also been investigated for their potential effect in chronic diseases such as cancer, viral, and parasitic infections. A study evaluating the therapeutic effects of *Hepatitis, HIV,* and *Cancer* nosodes in mice revealed that *HIV* nosode 30C and *Cancer* nosode 30C significantly reduced tumor volume by day 30. In contrast, *Hepatitis C* 30C and *HIV* 100C showed no impact. Additionally, the *HIV* nosode 30C group exhibited improved survival rates from day 25, highlighting its possible role in immune modulation (Shah, Talele *et al*. 2024:1).

Building on the investigations of homoeopathic nosodes against infectious diseases, Joshi *et al*. (2022) performed both an *in vitro* and *in vivo* study to evaluate the anti-leishmanial potential of *Leishmania donovani amastigote promastigote* nosode (*LdAPN* 30CH) on promastigote forms of *Leishmania donovani.* Leishmaniasis is a condition that is caused by intracellular protozoan parasites found in a *Leishmania* family. Although the disease is endemic in several countries, India has the greatest prevalence. The study was conducted in India and, the nosode was prepared from a culture of *Leishmania donovani* (Joshi, Bandral *et al*. 2022: 31). The results showed a significant improvement, with *LdAPN* 30CH exhibiting significant anti-leishmanial activity. An experiment conducted on VL-infected mice demonstrated that *LdAPN* 30CH was successful in treating that infection. The parasiticidal action of the nosode

was evidenced by a shift in the immune response from Th2 to Th1 type. The parasite was eradicated through macrophage activation and the subsequent production of elevated levels of nitric oxide (NO) and pro-inflammatory cytokines (IFN-y and IL-17). Additionally, there was a significant reduction in hepatic parasite load. There were no histological changes observed in the liver or kidney in animals that were treated with the nosode (Joshi, Bandral *et al*. 2022: 40)

Expanding on homoeopathic nosodes with antimicrobial properties, Prasad *et al*. (2023) conducted a study to determine the antimicrobial activity of *Pyrogenium* 30CH and 200CH on *Escherichia coli (E. coli)*. The nosode is made from the product of the decomposition of chopped beef in water. According to the homoeopathic literature, *Pyrogenium* is commonly known for its action in septic conditions (Prasad, Aathavan *et al*. 2023: 73). *E. coli* is a gram-negative anaerobic bacteria from the genus Escherichia, commonly found in the lower intestines (Mueller, Tainter 2023: 1). According to the study findings, *Pyrogenium* 30CH exhibited a moderate zone of inhibition with growth inhibition of 7.14%. On the contrary, *Pyrogenium* 200CH demonstrated an inconsequential zone of inhibition with negligible growth inhibition (Prasad, Aathavan *et al*. 2023: 73).

A similar study was conducted to assess the antiparasitic effect of *Pyrogenium, Cochliomyia hominivorax* (*C. hominivorax)* nosode, and *Sulphur* on *Cochliomyia hominivorax* (de Barros, Seugling *et al*. 2019: 177). *Cochliomyia hominivorax* is a parasite fly known for its larvae (maggots) feeding on the living tissue of warm-blooded animals. *Cochliomyia hominivorax* is the primary species responsible for causing primary myiasis in livestock located in the southern hemisphere, especially in Brazil (de Barros, Seugling *et al*. 2019: 177). The results showed that the mortality rates of larvae treated with the nosodes *C. hominivorax* 8cH and 12cH were 61.3% and 66.6% ($p > 0.05$) respectively. *Pyrogenium* 12CH had the highest mortality rate of larvae (98.6%) followed by *Sulphur* 12CH (94.6%), and trichlorfon (90.8%). The mortality rates in other control groups observed were 2.7% for 30% (v/v) alcohol, 4.3% for distilled water, 3.2% for no substance 2.7% for 30% (v/v) ethanol ($p > 0.05$). The researchers concluded that homoeopathy could be used to treat and prevent humans and animals from being infected with myiasis from *C. hominivorax* (de Barros, Seugling *et al*. 2019: 177).

Using the MIC method, *Candida albicans (C. albicans), Klebsiella pneumoniae (K. pneumoniae), E.coli and Salmonella typhi (S. typhi)* polyvalent nosodes were evaluated for potential antimicrobial activity on their corresponding infection and cross infections (Munshi, Talele *et al*. 2022: 42). The tested nosodes exhibited antibacterial effects not only against their respective microorganism but also against other selected organisms (Munshi, Talele *et al*. 2022: 42). Passeti *et al*. (2014) examined the effects of *Belladona* and *Streptococcus pyogenes* (*S. pyogenes*) nosode on *S. pyogenes* strains. The results showed that both remedies inhibited bacterial growth *in vitro*. Additionally, the authors found that treating methicillin-resistant *Staphylococcus aureus* (MRSA) cultures with *Belladonna* or *MRSA* nosode inhibited growth *in vitro*, reduced enzymatic activity, and raised bacterial susceptibility to antibiotic treatment (Passeti, Manzoni *et al*. 2014).

Simi *et al*. (2024: 1) showed that *Pyrogenum* 200CH, 1M and *Anthracinum* 200CH have a significant antimicrobial effect against MRSA. Sinha *et al*. (2020: 173) showed that *Lachesis* (6CH, 12CH, 30CH, 200CH, and 1M) and *Staphylococcinum* nosode (30CH, 200CH, and 1M) had an antimicrobial effect on *Staphylococcus aureus* (*S. aureus*) (Sinha, Jadhav 2020: 173).

Suri *et al.* (2022: 128) conducted a study in India to examine the anti-plasmodial effect of a novel homoeopathic nosode derived from *Plasmodium falciparum* in both *in vitro* and *in vivo* settings. The treatment substances were a cell-free parasite nosode, an infected RBC nosode, and a mixture nosode (Suri, Walter *et al.* 2021: 125). The study findings showed a significant improvement, under *in vitro* conditions, with 71.42% (3D7) and 68.57% (RKL-9) inhibition produced by the mixture nosode. The cell-free parasite nosode produced 62.85% 3D7 and 60% RKL-9 inhibition and the infected RBCs nosode showed 60.61% 3D7 and 57.14% RKL-9 inhibition (Suri, Walter *et al.* 2022: 128). In addition, the nosodes were found to be harmless to a raw macrophage cell line with > 70% cell viability. Under *in vivo* conditions, on day 35, suppressive efficacy was noticed in mixture nosode-treated mice, with $0.005 \pm 0.001\%$ parasitaemia (Suri, Walter *et al.* 2021: 126). The kidney and liver function biomarkers were observed to be within the normal range, with increased levels of IL-4 and IL-10, while a decrease in IL-17 and IFN-γ was observed in cytokine analysis (Suri, Walter *et al.* 2022: 128).

An *in vitro* study was conducted to evaluate the possible cytotoxic activity of the nosode *Carcinosinum* on tumour cell cultures using cell lines of breast cancer MDA-MB-231, lymphoblastic leukaemia jurjkat, and prostate cancer PC-3 (Botkina, Plotnikov 2023: 2). The *in* vitro results showed the absence of a direct cytotoxic effect of the *Carcinosinum* nosode (Botkina, Plotnikov 2023: 2).

Mondal *et al.* (2016) performed a study to evaluate the anticancer activity of *Hepatitis C* (*Hep C*) nosode against cancer cells. The findings demonstrated that the *Hep C* nosode induced apoptosis, which was marked by typical changes in cell morphology, an increase in reactive oxygen species, and greater DNA fragmentation. Additionally, the *Hep C* nosode elevated levels of pro-apoptotic signal proteins like Bcl-2 and caspase-3. It also altered mitochondrial membrane potential and caused the externalisation of phosphatidylserine. Moreover, the nosode reduced the expression of two cancer biomarkers, topoisomerase II (Top II) and telomerase, aligning with its anticancer effect (Mondal, Das *et al*. 2016: 209).

Additionally, using a minimum inhibition assay, a study was conducted to examine the potential antibacterial activity of *Staphylococcinum* nosode (30CH, 200CH, and 1M), *Lachesis* (30CH, 200CH, and 1M), and *Echinacea* (6CH, 12CH, 30CH, 200CH, and 1M) on *S. aureus*. The findings revealed that *Staphylococcinum* nosode and *Lachesis* in all the tested potencies could inhibit the growth of *S. aureus* when compared to 90% ethanol ($p < 0.05$) (Sinha, Jadhav 2020: 172). Table 2.2 presents a list of both historical and new nosodes that have been evaluated for efficacy *in vitro* and *in vivo* testing.

Table 2.2: List of selected old and new nosodes assessed for antimicrobial susceptibility

Old homoeopathic nosodes			
Homoeopathic Nosode	**Source**	**Testing Method**	**References**
Psorinum	Fluid of blisters from scabies-infested skin	*In vivo, In vitro*	Mondal, Samadder *et al*. 2016: 143; Ahamed, Vaiyapuri *et al*. 2023: 77
Pyrogenium	Decomposition of chopped beef	*In vivo, In vitro*	De Barros, Seugling *et al.* 2019: 177; Prasad, Aathavan *et al*. 2023: 72
Influenzinum	Derived annually from an influenza strain utilised in the flu vaccine for the year	*In vivo*	Nayak, Varanasi 2021: 131
Staphylococcinum nosode	*Staphylococcus aureus*	*In vitro*	Sinha, Jadhav *et al.* 2020: 173
Medorrhinum	Gonorrhoea bacterium	*In vitro*	Nambison, Nambison *et al*. 2017:2
Carcinosinum	Cancer tissue	*In vitro, In vivo*	Botkina, Plotnikov 2023: 2
Hepatitis C nosode	Hepatitis C virus	*In vitro*	Mondal, Das *et al*. 2016:209
New homoeopathic nosodes			
Homoeopathic Nosode	**Source**	**Testing Method**	**References**
Leishmania donovani amastigote promastigote nosode (*LdAPN 30C*)	*Leishmania donovani* culture	*In vivo*	Joshi, Bandral *et al*. 2022: 031
C. albicans nosode	*C. albicans*	*In vitro*	Munshi, Shah *et al*. 2022: 1
K. pneumoniae nosode	*K. pneumoniae*	*In vitro*	Pareek, Jadhav 2020: 1; Munshi, Talele *et al*. 2022: 1
E. coli nosode	*E. coli*	*In vitro*	Munshi, Talele *et al*. 2022: 1
Salmonella typhi nosode	*Salmonella typhi*	*In vitro*	Munshi, Talele *et al*. 2022: 1
S.pyogenes nosode	*S. pyogene*	*In vitro*	Passeti, Manzoni *et al*. 2024: 1

2.15 DISC DIFFUSION METHOD

The primary goal of *in vitro* antimicrobial susceptibility testing is to make guidance available for clinical therapy. With a rise in AMR rates, the use of standardised, flexible, easy, as well as low-cost methods such as the disc diffusion method, has become more crucial (Ahman, Matuschek *et al*. 2020: 1). The disc diffusion method is one of the oldest and most utilised methods for susceptibility testing of an organism. It is applicable to a broad range of bacteria as well as antimicrobial agents (Jonasson, Matuschek *et al*. 2020: 1).

This method has undergone various changes, with the Kirby-Bauer test being one of the most common. Alexander Fleming employed several of these techniques while

researching penicillin in the 1950s. At that time, various procedures were in use, and microbiologists such as Bauer, Kirby, Sherris, and Tuck evaluated all the procedural variables, including media, temperature, and agar depth. In 1966, they published a seminal paper detailing the disc diffusion test that is still in use today. The National Committee for Clinical Laboratory Standards (NCCLS) used the basic procedural steps as the reference method for disc diffusion. Adhering to these steps precisely is crucial for obtaining accurate results (Valgas, Machado *et al.* 2007: 1; Benkova, Soukup *et al*. 2020: 807).

There are many advantages to using the method because it is technically easy to conduct and very reproducible. The method is affordable compared to other antimicrobial-susceptible methods. It does not require any special equipment and is considered to be more versatile than other susceptibility testing methods (Jonasson, Matuschek *et al.* 2020: 1). It provides susceptible category results that are easily understood and interpreted by laboratory clinicians. It is flexible regarding antimicrobial agent selection for testing (Patel, Tenover *et al*. 2011: 2; Gajic, Kabic *et al.* 2022: 2).

In addition, it is used for the screening of plant extracts, and essential oils and other drugs (Balouiri, Sadiki *et al*. 2016: 1). The main limitation of the method is the spectrum of organisms for which it has been standardised. This means that not all fastidious bacteria can be accurately assessed through the assay. Standardisation has been established for certain fastidious bacterial pathogens, such as *Streptococci, Haemophilus influenzae,* and *Neisseria meningitidis*, using specific culture media, different incubation conditions, and interpretive criteria for inhibition zones (Balouiri, Sadiki *et al*. 2016: 1).

The agar disc diffusion method is also unsuitable for determining the MIC because it cannot quantify the amount of antimicrobial agent diffused into the agar medium. The two common diffusion methods are disc diffusion and well diffusion assay (Valgas, Machado *et al.* 2007: 1).

In the disc diffusion method, agar plates are inoculated with an institutionalised inoculum of the test microorganism. Then a filter paper disc (approximately 5 mm in breadth) containing the test substance at the desired concentration is placed on the agar surface (Balouiri, Sadiki *et al.* 2016: 2). The petri dish is stored under appropriate conditions, and antimicrobial agents or treatment diffuse into the agar and inhibit

germination and development of the test microorganism. The diameter of zones of inhibition is then measured (Balouiri, Sadiki *et al.* 2016: 2).

2.16 MINIMUM INHIBITORY CONCENTRATION

MIC is the lowest concentration of an antibiotic expressed in mg/L (µg/mL) at which bacterial growth is completely inhibited under controlled *in vitro* conditions (Kowalska, Dudek 2021: 165). MIC is determined by two methods, namely, the dilution method and the gradient method. To determine the MIC values, both methods utilise either a Mueller-Hinton agar medium (MHA) or broth (MHB). Depending on the bacteria, Mueller Hinton medium is supplemented with 5% lysed horse or sheep blood or other compounds for fastidious bacteria such as *S. pneumoniae* (Sawada, Katayama *et al*. 2021: 1).

2.17 CONCLUSION

The literature review reveals a substantial body of evidence supporting the application of homoeopathic nosodes across various disease contexts. The resurgence of traditional nosodes and the introduction of new nosodes through modern scientific methods highlight their significant therapeutic potential. Overall, while the evidence supporting the efficacy of homoeopathic nosodes continues to grow, there remains a need for further *in vitro* and *in vivo* research to solidify these findings and address existing gaps. *In vitro* studies are crucial for enhancing the understanding and potential applications of homoeopathic treatments in modern medicine.

CHAPTER 3: RESEARCH METHODOLOGY

3.1 INTRODUCTION

This chapter describes the methodology utilised in this study. In addition, it presents the research design, research setting, and data collection and data analysis process.

3.2 STUDY DESIGN

This quantitative *in vitro* control study was conducted at the National Health Laboratory Service (NHLS), Department of Medical Microbiology, Inkosi Albert Luthuli Hospital, with authorisation (Appendix 3).

3.3 STREPTOCOCCUS PNEUMONIAE

S. pneumoniae ATCC 49619 was obtained from the culture collection of the Department of Medical Microbiology. The bacterial cultures were plated onto Mueller-Hinton agar plates supplemented with 5% sheep blood (Mueller-Hinton agar w/ 5% sheep blood) and grown overnight at 35 °C. The study was conducted under the supervision of a qualified medical microbiologist according to the Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute [CLSI] 2023: 149).

3.4 PREPARATION OF *STREPTOCOCCUS PNEUMONIAE* NOSODES

The test substance, *S. pneumoniae nosode* 6CH (20%), was obtained from Comed Health, a commercial supplier in Pretoria (Waltloo), South Africa. It was made following the German Homoeopathic Pharmacopoeia method 44 (GHP method 44) (Bibliothek 2005: H5.4.4). The respective potency was prepared from the *S. pneumoniae* bacteria. The researcher prepared the desired potencies (9CH, 30CH, and 200CH) from *S. pneumoniae* nosode 6H, batch order number 2229660 according to the GHP method 44 (Bibliothek 2005: H5.4.4).

3.4.1 PREPARATION OF *S. PNEUMONIAE* 6CH

S. pneumoniae mother tincture was prepared following the GHP method 44. This involved making a mother tincture (Ø) from a killed culture of *S. pneumoniae*.

S. pneumoniae cultures were adjusted to contain 10^7 colony-forming units (CFU) per gram before undergoing heat treatment at 133 °C. One part of the heat-treatment raw material was mixed and succussed with 9 parts glycerol 85% and the mixture was left to stand for not less than 5 days. After 5 days, the mixture was filtered using a muslin cloth and the filtrate obtained was the mother tincture, corresponding to the 1st decimal dilution (Ø = D1) (Bibliothek 2005: H5.4.4).

To prepare the first centesimal dilution (1CH), 10 parts of the mother tincture (D1) were combined with 90 parts of ethanol 30% (m/m) and succussed 10 times, then labelled as 1CH. The second centesimal dilution (2CH) was made by mixing one part of the 1st centesimal dilution (1CH) with 99 parts of ethanol 43% (m/m) and succussing 10 times, then labelled as 2CH. This method was repeated for subsequent dilutions until 6CH was reached (Bibliothek 2005: H5.4.4).

3.4.2 PREPARATION OF *STREPTOCOCCUS PNEUMONIAE* NOSODE POTENCIES (9CH, 30CH, AND 200CH)

The researcher prepared the potencies from *S. pneumoniae* nosode 6CH according to the GHP method 44 to potentise and reach the desired end test potencies which are 9CH, 30CH, and 200CH (Bibliothek 2005: H5.4.4). The seventh centesimal dilution (7CH) was prepared by measuring 0.2 ml of 6CH and adding 19.8 ml of 96% ethanol in a 30ml Amber glass screw-top bottle, followed by 100 succussions, and labelled as 7CH. The eighth centesimal dilution (8CH) was prepared by measuring 0.2 ml of 7CH, adding 19.8 ml of 96% ethanol in a 30 ml Amber glass screw-top bottle, and succussing 100 times, then labelled as 8CH. The nineth centesimal dilution (9CH) was prepared by measuring 0.2 ml of 8CH, adding 19.8 ml of 20% ethanol in a 30 ml Amber glass screw-top bottle, and succussed 100 times, then labelled as 9CH. Subsequent dilutions were made in the same manner until the desired potencies (30CH, and 200CH) were reached (Bibliothek 2005: H5.4.4).

3.5 MEDIA

The Mueller Hinton Agar w/ 5% sheep blood plates were obtained from the Department of Medical Microbiology Laboratory, University of KwaZulu-Natal, National Health Laboratory Service (Clinical and Laboratory Standards Institute 2023: 10).

3.6 PREPARATION OF GROWTH CULTURE

S. pneumoniae ATCC49619 colonies were inoculated into Mueller Hinton agar w/ 5% sheep blood using a sterile transfer loop. Media plates were streaked according to the streak quadrant method (Katz 2008: 4; Mahato, Sah *et al.* 2019: 300). Prepared plates were incubated at optimum temperature, usually at 37 °C, in an inverted position for 20-24 hours (Rosario, Johnson 2021: 4).

3.7 PREPARATION OF THE SALINE TEST CULTURES

Prior to the preparation of saline test cultures, 9 g of sodium chloride was weighed and added to a beaker. 1000 ml of distilled water was added into the beaker and mixed to dissolve the sodium chloride.10 ml of the saline was distributed into each of the falcon tubes using a pipette. The tubes were closed and labelled. The tubes were autoclaved for sterilisation for 15 minutes at 15 psi and 121 °C. Once the solution had cooled down, it was transferred to 3 small tubes (Zampieri, Machado *et al*. 2021: 828).

3.8 PREPARATION OF THE INOCULUM

S. pneumoniae colonies from the overnight Mueller Hinton agar w/ 5% sheep blood cultures of *S. pneumoniae* were mixed into a 10 ml sterile solution (0.9g/l). The solution was calibrated to 0.5 McFarland Equivalence Turbidity Standard (Jeon, Shin *et al.* 2021: 560; Clinical and Laboratory Standards Institute 2023: 10)

3.9 PREPARATION OF FILTER PAPER DISCS

Sterile Whatman® filter paper was utilised. It was punched into 5 mm discs. The discs were wrapped in aluminium foil and autoclaved at 15 lbs pressure for 15 min for sterilisation. The discs were transferred to sterile petri dishes using sterile needles. Each disc was impregnated with an appropriate test substance or negative control. The first impregnation stage involved 20 µl of the appropriate substance, followed by a second impregnation of 10 µl. After each impregnation stage, the discs were dried at 37 °C with the petri dish lids closed.

3.10 PREPARATION OF *S. PNEUMONIAE* NOSODE 6CH, 9CH, 30CH AND 200CH DRY DISCS

Each disc was carefully placed on a sterile petri dish utilising sterile needles, with one disc per dish. Using a calibrated micropipette, 20 µl of the respective *S. pneumoniae* nosode potencies were pipetted onto each disc. The plates were organised into groups from 1-12, prepared in triplicate (Plates 1-3: *S. pneumoniae* nosode 6CH dry discs, Plates 4-6: *S. pneumoniae* nosode 9CH dry discs, Plates 7-9: *S. pneumoniae* nosode 30CH dry discs, Plates 10-12: *S. pneumoniae* nosode 200CH dry discs). The plates were incubated at 37 °C to dry. Once dry, a further 10 µl of respective *S. pneumoniae* nosode potencies were measured onto each disc before the plates were incubated at 37 °C for further drying (Invernizzi 2002: 17).

3.11 PREPARATION OF *S. PNEUMONIAE* NOSODE 6CH, 9CH, 30CH AND 200CH WET-DISCS

Each disc was placed in a sterile plate utilising a sterile needle, with each plate containing one disc. 20 µl of respective *S. pneumoniae* nosode potencies were measured onto each disc utilising a calibrated micropipette. The plates were grouped from 1-12, prepared in triplicate, with one impregnated disc each (Plates 1-3: *S. pneumoniae* nosode 6CH wet discs, Plates 4-6: *S. pneumoniae* nosode 9CH wet discs, Plates 7-9: *S. pneumoniae* nosode 30CH wet discs, Plates 10-12: *S. pneumoniae* nosode 200CH wet discs). The discs were allowed to dry for 5 min (Invernizzi 2002: 17). The discs were applied to the media plates, and each disc was placed upon the centre of the inoculated media plate using a sterile needle, for each media plate contained one disc. A further 10 µl of respective *S. pneumoniae* nosode potencies were measured onto each disc, the disc was applied to the media plate and then incubated at 37 °C for 24 to 48 hours.

3.12 PREPARATION OF 20% ETHANOL DRY DISCS

Each disc was placed on the petri dish utilising sterile needles, with one disc each. Using a calibrated micropipette, 20 µl of ethanol 20% were measured into each disc. Plates were prepared in triplicate, followed by incubation at 37 ℃ to dry. Another 10 µl of 20% ethanol were added to each disc. The discs were incubated at 37 °C for further drying (Invernizzi 2002: 18).

3.13 PREPARATION OF 20% ETHANOL WET-DISCS

Each disc was placed on the petri dish utilising a sterile needle. Using a calibrated micropipette, 20 microlitres of 20% ethanol were added to each disc. The plates were prepared in triplicate and dried for 5 minutes (Invernizzi 2002: 18). The discs were applied to the media plates, and each disc was placed upon the centre of the inoculated media plate using a sterile needle, for each media plate contained one disc. Additionally, 10 µl of respective ethanol 20% were measured onto each disc, the disc was applied to the media plate and then incubated at 37 °C for 24 to 48 hours.

3.14 CEFTRIAXONE

Ceftriaxone (30 µg) discs were obtained from JVL Lab Engineering and General Supplies Close Corporation, South Africa. The discs were stored at 2 °C to 5 °C until they were used.

3.15 PREPARATION OF CEFTRIAXONE E-TEST SCRIPTS

Ceftriaxone E-test scripts were obtained from the NHLS, Department of Medical Microbiology, Inkosi Albert Luthuli Hospital. The e-test scripts were stored at 2 °C until they were used.

3.16 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC of ceftriaxone was determined using the Epsilometer (E-test) method, following the manufacturer's instructions (Biomeriuex, South Africa). The test was conducted in triplicate to ensure optimal and reliable findings. A cotton swab was immersed in a saline test culture and utilised to streak the entire dried surface of Mueller Hinton agar w/ 5% sheep blood plates. After streaking, the swab was discarded in an appropriate container. The lid ajar was left for three to five minutes, for excess moisture to be absorbed before applying the E-test strips. The strips were applied on the agar surface using forceps and then incubated at 37 °C for 24-48 hours (Mandal, Preethi *et al*. 2012: 674; Yin, Guo *et al* . 2021: 1152).

3.17 DISC DIFFUSION ASSAY

The experiment was conducted in triplicate to ensure optimal and reliable results. The cotton swab was immersed in a saline test culture and utilised to streak the entire dried

surface of Mueller Hinton agar w/ 5% sheep blood plates. The lid of the jar was left slightly ajar for three to five minutes for excess moisture to evaporate before applying the drug-impregnated discs. Each agar plates were labelled on the undersurface to indicate which test substance or control disc was applied (Bauer, Kirby *et al*. 1966).

The plates were labelled as follows; 3 plates: *S. pneumoniae* nosode 6CH dry discs; 3 plates: *S. pneumoniae* nosode 9CH dry discs; 3 plates: *S. pneumoniae* nosode 30CH dry discs; 3 plates: *S. pneumoniae* nosode 200CH dry discs; 3 plates: 20% ethanol dry discs; 3 plates: *S. pneumoniae* nosode 6CH wet discs; 3 plates: *S. pneumoniae* nosode 9CH wet discs; 3 plates: *S. pneumoniae* nosode 30CH wet discs; 3 plates: *S. pneumoniae* nosode 200CH wet discs; 3 plates: 20% Ethanol wet discs (CLSI, 2020: 149).

3.17.1 APPLICATION OF DRY MEDICATED DISCS

The impregnated discs (*S. pneumoniae* nosode 6CH, 9CH, 30CH, 200CH, and 20% ethanol) and antibiotic (ceftriaxone discs) were taken from the freezer and brought to room temperature use. The discs were distributed with sterile needles over the agar surface, placing one disc at the centre per plate. The plates were placed in the incubator for 24 to 48 hours at 37 °C.

3.17.2 APPLICATION OF WET MEDICATED DISCS

The sterile non impregnated discs were impregnated with test substances. The discs were distributed with sterile needles over the agar surface, placing one disc at the centre per plate. After applying the discs, a further 10µl of respective *S. pneumoniae* nosode potencies and 20% ethanol were pipetted onto discs as described in 3.11 and 3.13 (preparation of 20% ethanol wet discs). The plates were incubated for 24 to 48 hours at 37 °C within 15 minutes (Prasad, Aathavan *et al*. 2023: 74).

3.18 MEASUREMENTS

The plates were observed at 18, 24, and 48 hours for signs of zone inhibition around the discs. The inhibition zones for the tested substances, control, and antibiotic discs were measured in millimetres of vernier callipers for precision (Sandhya, Vickram 2023: 807). The average diameter of the inhibition zones for each group was recorded in a table.

3.19 TEST COMBINATIONS

The researcher applied the disc diffusion method using the following test combinations; *S. pneumoniae* nosode on Mueller-Hinton agar at dilutions of 6CH, 9CH, 30CH, and 200CH, evaluated against *S. pneumoniae*. Ceftriaxone (positive control), and 20% ethanol (negative control) served as controls. Additionally, the MIC of ceftriaxone was determined by E-test on *S. pneumoniae.*

3.20 ETHICAL APPROVAL

Ethical approval was obtained from the Institutional Research Ethics Committee (IREC), IREC 068/23 (Appendix 1). The gatekeeper permission was obtained from the National Health Laboratory Service Manager (Appendix 3). This study was conducted at the NHLS, Department of Medical Microbiology, Inkosi Albert Luthuli Hospital. The study was done under the supervision of a qualified Medical Microbiologist, Dr Yesholata Mahabeer, with permission from Professor K. Swe Swe Han who is the Head of the Department of Medical Microbiology.

3.21 DATA ANALYSIS

In this microbial study employing a quantitative experimental design method, inhibition zones were measured using Vernier callipers after 24 hours. The analysis focused on comparing the inhibition zones of ceftriaxone, the positive control, with those of various nosode potencies, assessed through *S. pneumoniae* impregnated discs. Data was systematically presented using tables and figures to illustrate the study findings. Statistical analyses using descriptive statistics were performed to evaluate differences in antimicrobial efficacy among the treatments, with the ultimate goal of delineating the relative performance of nosode potencies against the positive control.

CHAPTER 4: RESULTS

4.1 INTRODUCTION

This chapter outlines the findings generated from testing *S. pneumoniae* growth with exposure to *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH. The following information will report on the test results from the experiments as described in Chapter 3, the MIC of ceftriaxone (positive control) to inhibit the growth of *S. pneumoniae,* and the disc diffusion method to assess the potential effect of the nosode on the bacteria.

The study results demonstrated that the homoeopathic remedy *S. pneumoniae* nosode displayed no restrictive effects against *S. pneumoniae* culture. Ceftriaxone, the positive control, displayed a significant restrictive impact (24 mm) against *S. pneumoniae*, while the negative management (20% ethanol) did not show a restrictive impact. Figure 4.1 illustrates the inhibition zones after 18, 24, and 48 hours of incubation at 37 °C for dry medicated discs

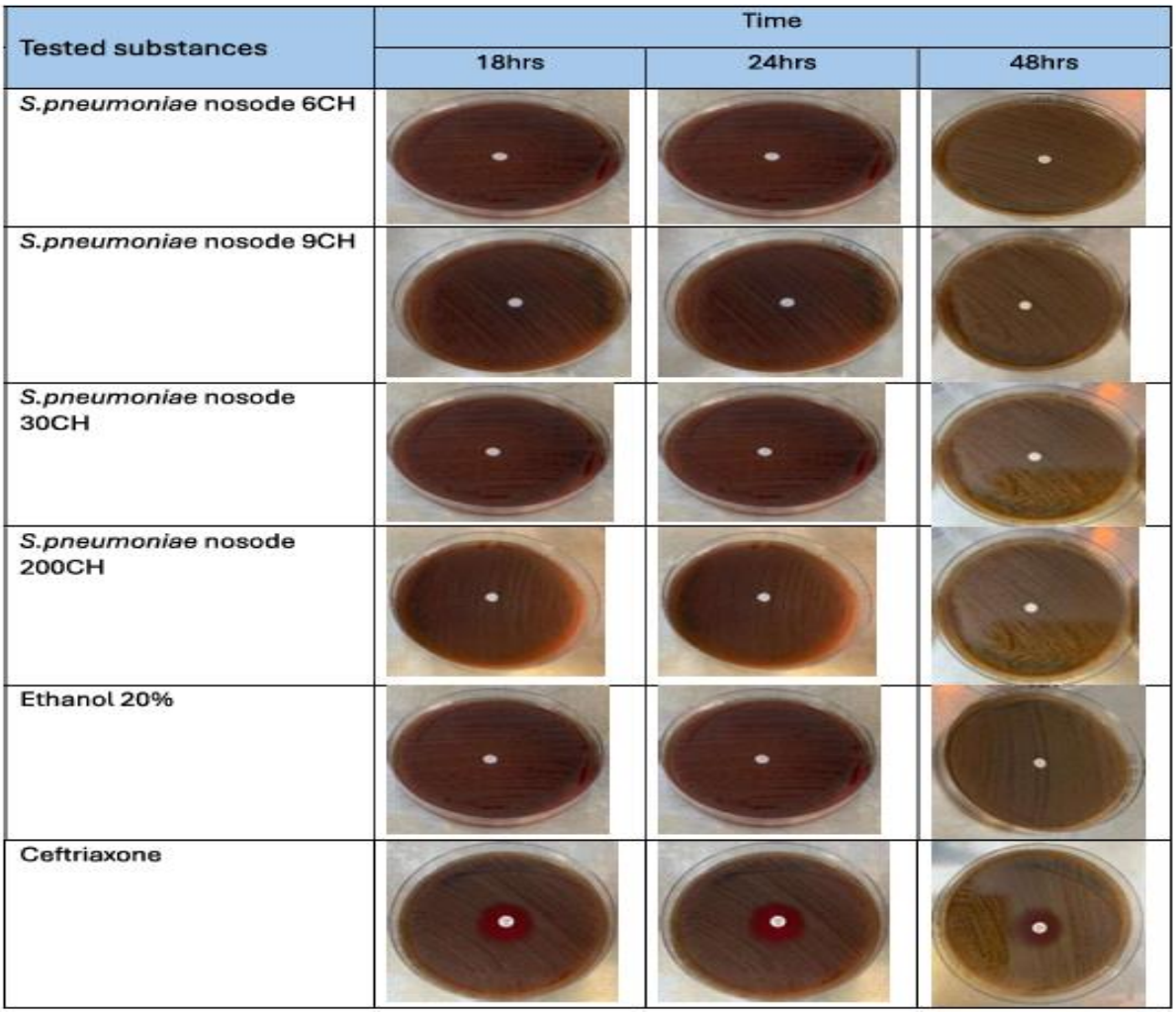


Figure 4.1: Antibacterial assay of *S. pneumoniae* nosode and controls by Kirby Bauer method in *S. pneumoniae-medicated* dry discs

The disc diffusion assay findings demonstrated that the *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH impregnated dry discs displayed no inhibitory activity against the *S. pneumoniae* evaluated. 20% ethanol dry-impregnated discs did not exhibit an inhibitory effect against *S. pneumoniae*. This was interpreted as resistance. In contrast, ceftriaxone (positive control) exhibited a significant inhibitory effect against *S. pneumoniae* with a clear zone of inhibition. Figure 4.2 shows the inhibition zones for wet discs after incubation at 37 °C.

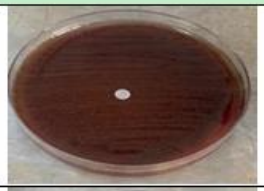
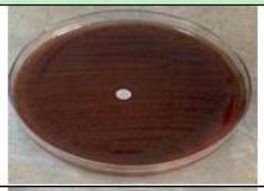
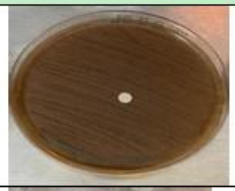
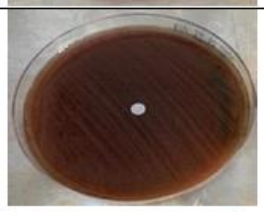
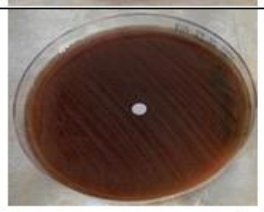
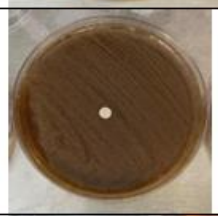
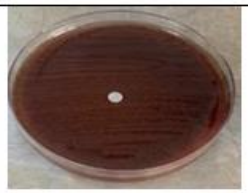
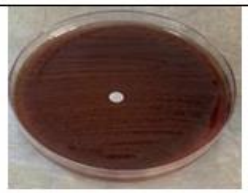
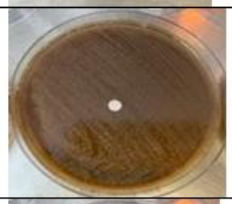
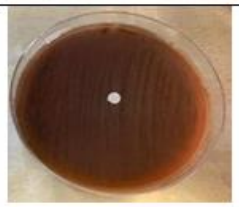
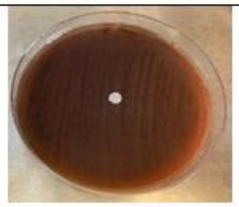
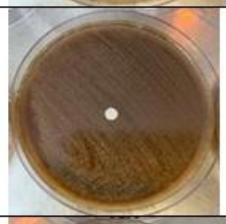
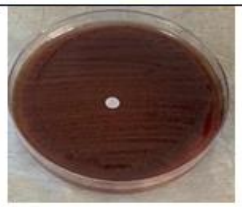
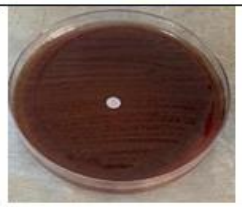
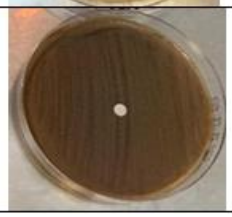
Tested substances	Time		
	18hrs	24hrs	48hrs
S.pneumoniae nosode 6CH Wet disc			
S.pneumoniae nosode 9CH Wet disc			
S.pneumoniae nosode 30CH Wet disc			
S.pneumoniae nosode 200CH Wet disc			
Ethanol 20% Wet disc			

Figure 4.2: Antibacterial assay of *S. pneumoniae* nosode and controls using the Kirby Bauer method in *S. pneumoniae* (medicated wet discs)

Table 4.1 illustrates the inhibition zones for all tested substances after 18, 24, and 48 hours of incubation at 37 °C.

Table 4.1: *S. pneumoniae* growth inhibition zones

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 as (mm).

The Minimum Inhibitory Concentration assay findings demonstrated that the MIC of ceftriaxone on *S. pneumoniae* is 2 µg/mL as shown in Figure 4.4.

Figure 4.3: MIC results of ceftriaxone on *S. pneumoniae* using the e-test method

4.2 CONCLUSION

Based on the results obtained from the study, the findings are that the antimicrobial susceptibility test demonstrated that *S. pneumonia*e was resistant to *S. pneumoniae* nosode 6CH, 9CH, 30CH, 200CH, and 20% ethanol, as there was an absence of a zone of inhibition. The antimicrobial susceptibility test also demonstrated that *S. pneumoniae* was sensitive to ceftriaxone 30 µg, with a zone diameter of 24 mm which

was within the expected zone diameter. Additionally, the MIC obtained for ceftriaxone was 2 µg/mL.

CHAPTER 5: DISCUSSION

5.1 INTRODUCTION

While antibiotics have saved many lives, the rise of bacterial resistance threatens to undermine progress in both the clinical and agricultural sectors. Additionally, it could lead to life-threatening conditions even for infections that were previously treatable (Ahmed, Hussein *et al*. 2024: 1). The ongoing search for new and other antimicrobials is critical in the fight against bacterial infections. The *S. pneumoniae* nosode is used by homoeopaths in their clinical setting, and is considered effective (Khan, Nayak *et al*. 2020: 19). Although several *in vitro* studies have been conducted on homoeopathic nosodes and other homoeopathic remedies, exhibiting positive results (Khan, Nayak *et al*. 2020: 19) there is an absence of *in vitro* or *in vivo* studies conducted on *S. pneumoniae* nosode antimicrobial efficacy.

5.2 *S. PNEUMONIAE* NOSODE 6CH

The study findings demonstrated that *S. pneumoniae* nosode 6CH in 20% v/v ethanol, had no bacterial effect on *S. pneumoniae* when assessed through disc-diffusion assay. No inhibition was observed, as indicated by the absence of inhibition zones at 18, 24, and 48 hours. This suggests that *S. pneumoniae* was completely resistant to *S. pneumoniae* nosode 6CH when tested using dry discs and wet discs. The stability of the *S. pneumoniae* nosode may have been compromised by the storage and environmental conditions during the study. Furthermore, there was no difference between the findings of the nosode and the 20% ethanol in both dry and wet-impregnated discs.

5.3 *S. PNEUMONIAE* NOSODE 9CH

The study findings demonstrate that *S. pneumoniae* nosode 9CH in 20% v/v ethanol exhibited no antibacterial effect on *S. pneumoniae* when examined through disc-diffusion assay. The sample showed no inhibition, with no zones observed at 18, 24,

and 48 hours, which means that *S. pneumoniae* was completely resistant to *S. pneumoniae* nosode 9CH when evaluated using dry discs and wet discs. The stability of *S. pneumoniae* nosode may have been affected by the storage and environmental conditions. Moreover, there was no difference between the results of the nosode and 20% ethanol in both dry discs and wet discs.

5.4 *S. PNEUMONIAE* NOSODE 30CH

The findings of this study indicate that *S. pneumoniae* nosode 30CH in 20%v/v ethanol exhibited no antibacterial effect on *S. pneumoniae* when assessed utilising the disc diffusion assay. The sample showed no inhibition, with no zones detected at 18, 24, and 48 hours. This suggests that *S. pneumoniae* was entirely resistant to *S. pneumoniae* nosode 30CH when tested with both dry and wet discs. The stability of the *S. pneumoniae* nosode may have been affected by the storage and environmental conditions under which the study was conducted. Additionally, there was no difference between the results of the tested sample and the 20% ethanol in both dry and wet-impregnated discs.

5.5 *S. PNEUMONIA*E NOSODE 200CH

The results of this study show that *S. pneumoniae* nosode 200CH in 20%v/v ethanol displayed no antibacterial effect on *S. pneumoniae* when assessed utilising the disc diffusion assay. The sample demonstrated no inhibition, with no zones of inhibition observed at 18, 24, and 48 hours. This indicates that *S. pneumoniae* was completely resistant to *S. pneumoniae* nosode 30CH when tested with both dry and wet discs. The stability of *S. pneumoniae* nosode may have been compromised by the storage and environmental conditions during the study. Furthermore, there was no difference between the results of the tested sample and the 20% ethanol in both dry and wet-impregnated discs.

5.6 20% ETHANOL

The findings of this study demonstrate that 20% ethanol had no antibacterial effect on *S. pneumoniae* when assessed using the disc-diffusion assay. No zones were observed at 18, 24, and 48 hours, suggesting that *S. pneumoniae* was completely

resistant to 20% ethanol in both dry and wet discs. The stability of the 20% ethanol may have been compromised by the storage and environmental conditions during the study. Additionally, there was no difference between the results of the *S. pneumoniae* nosode and the 20% ethanol in either the dry or wet-impregnated discs.

5.7 CEFTRIAXONE

The study findings demonstrate that the positive control, ceftriaxone, exhibited an antibacterial effect on *S. pneumoniae* when examined using the disc-diffusion assay. Clear zones of inhibition were observed at 18 hours, measuring 20 mm in diameter. After 24 and 48 hours of incubation, the zone of inhibition increased to 24 mm in diameter. This indicates that *S. pneumoniae* was susceptible to ceftriaxone under the test conditions. Ceftriaxone showed statistically significant results compared to *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH.

5.8 GENERAL DISCUSSION

Given the constituents of *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH, one would expect *S. pneumoniae* nosode to exhibit significantly pronounced antibacterial activity compared to 20% ethanol. A synergistic antibacterial effect between the constituents of *S. pneumoniae* nosode and 20% ethanol was anticipated.

Several reasons can be postulated for the absence of antibacterial activity in *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH. Firstly, there may have been an error in identifying the source material during the preparation of *S. pneumoniae* mother tincture. Secondly, the concentration of *S. pneumoniae* nosode potencies (6CH, 9CH, 30CH, and 200CH) was probably high as the *S. pneumoniae* mother tincture (lowest dilution) was not tested. Thirdly, the stability of the components in *S. pneumoniae* nosode (6CH, 9CH, 30CH, and 200CH) may have been compromised by the storage and environmental conditions during the study.

The absence of a positive antimicrobial response of *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH against *S. pneumoniae* contradicts the findings of the study conducted by Simi *et al.* (2024: 3) on the antimicrobial effectiveness of certain nosode on bacterial microorganisms. Their study results showed *Pyrogenium* 200CH, 1M, and

Anthracinum 200CH to have a significant antimicrobial effect against methicillin-resistant *Staphylococcus aureus* (MRSA).

Munshi *et al.* (2022: 1) evaluated the efficacy of polyvalent nosodes, including *C. albicans* polyvalent nosode, *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *K. pneumoniae, E. coli* polyvalent nosode and *S. typhi* polyvalent nosode using the MIC method. According to the results, *C. albicans* polyvalent nosode and *N. gonorrhoeae nosode* inhibited the growth of *C. albicans* species. *K. pneumoniae* and *E. coli* polyvalent nosodes inhibited the growth of *K. pneumoniae. E. coli* polyvalent nosode inhibited the growth of *E. coli*. *S. typhi* polyvalent nosode inhibited the growth of *S. typhi* (Munshi, Talele *et al*. 2022: 1). A similar study conducted by Sinha *et al*. (2020: 173) showed that *Lachesis* (6CH, 12CH, 30CH, 200CH, and 1M) and *Staphylococcinum* nosode (30CH, 200CH, and 1M) had an antimicrobial effect on *Staphylococcus aureus (S. aureus)*.

The absence of growth inhibition of *S. pneumoniae* as a result of the application of *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH is consistent with the findings of Pareek *et al*. (2020: 179). Their 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, *Sulphur*, *Senega*, and *Lobelia inflata* inhibited the growth of *K. pneumoniae*. However, there were no statistically significant results demonstrating *Klebsiella pneumoniae* nosode to have an antibacterial effect on *K. pneumoniae* (Pareek, Jadhav 2020: 179).

Rissato *et al*. (2016: 321) conducted a study on the verification of the antimicrobial activity of *S. sclerotiorum* nosode and *Sulphur* on the mycelial growth of *S. sclerotiorum*. The study results indicated that neither *S. sclerotiorum* nosode nor *Sulphur* reduced the growth of *S. sclerotiorum* (Rissati, Stangarlin *et al*. 2016: 321).

An *in vitro* study conducted to analyse the micro-sclerotia and mycelial growth of fungi showed that the *M. phaseolina* nosode did not reduce the micro-sclerotia and mycelial growth (Lorenzetti, Stangarlin *et al*. 2016: 3412).

Meneses (2016: 2) examined the antimicrobial activity of *Streptococcinum* nosode 6CH, 30CH, 200CH, and 0/6, 0/30 and 0/60 LM potencies. The author found that *Streptococcinum* nosode in infinitesimal dilutions did not exhibit antimicrobial activity in either the broth dilution method or the disc diffusion method, however, it did exhibit promicrobial activity in both methods.

The 20% ethanol used as a negative control in the current study did not exhibit any antibacterial effect against *S. pneumoniae*. According to Sauerbrei (2020: 3) effective bactericidal concentrations of ethanol range from 60% to 85%, with required exposure times between 0.5 to 5 min. Ethanol concentrations between 30% and 50% demonstrate significantly lower bactericidal activity, and the exposure times tested (5–30 min) to achieve a meaningful bactericidal effect (Sauerbrei 2020: 3; Fallica, Leonardi *et al*. 2021: 1). Thus, 20% ethanol is considered too low to produce a significant antibacterial effect. The Centers for Disease Control and Prevention recommends ethanol concentrations of 60% and 90% for disinfection (Yip, Bixler e*t al.* 2020: 1074; Centers for Disease Control and Prevention 2023: 5).

In a study evaluating the effectiveness of *Calendula officinalis* tincture 60% (v/v) against *Pseudomonas aeruginosa*, no significant difference was observed between the activity of *Calendula officinalis* tincture made from 60% ethanol and 60% ethanol alone on *in vitro P. aeruginosa*. Since the mean activities were 6.88 mm and 6.69 mm, respectively, it is reasonable to infer that the antibacterial properties of *Calendula officinalis* tincture 60% (v/v) ethanol are primarily due to the 60% ethanol present in the tincture (Mabuza 2002: 11).

It is crucial to note that *in vitro* conditions differ significantly from *in vivo* environments, which means findings from *in vitro* studies may not necessarily align with those from *in vivo* research (Jiang, Wang *et al*. 2020: 3852). Homoeopathy operates on the principles of stimulating the body's vital force, aiming to induce recovery by influencing the body's overall vital energy rather than targeting specific drug receptors. According to homoeopathic theory, this vital force drives physical changes that lead to healing from both acute and chronic conditions (Hahnemann 1991: 156).

This vital force is considered an immaterial dynamic that animates human organisms in health and disease. According to this theory, biological processes cannot be fully explained by the laws governing inert substances alone, as living organisms are distinguished from non-living matter by this immaterial force (Hahnemann 1991: 156; Bell 2004: 123). In this study, bacteria were cultured under ideal conditions for replication, but homoeopathic remedies may not affect these organisms in the absence of a disease host. This lack of response from healthy bacteria might help explain our findings. Further research and improvements of *in vitro* models are crucial to better understand how homoeopathic treatments impact bacteria. This lack of response from non-challenged organisms may partly explain our results. Thus, additional research and refinement of *in vitro* models are needed to comprehend the direct effects of homoeopathy on bacteria (Hahnemann 1991: 156)

5.9 CONCLUSION

This chapter discussed the results obtained from the study and compared them with findings from other *in vitro* studies. The absence of growth inhibition observed in *S. pneumoniae* treated with nosode 6CH, 9CH, 30CH, and 200CH was consistent with findings from other studies on homoeopathic nosodes. Additionally, the results were analysed in the context of homoeopathic principles, providing further insight into their implications within the framework of homoeopathy.

CHAPTER 6: CONCLUSION

6.1 INTRODUCTION

In the previous chapter, the researcher examined the study's findings and explored why the *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH could not inhibit the growth of *S. pneumoniae*. This chapter presents the conclusion, summarises the study, addresses its limitations, and offers recommendations based on the research.

6.2 CONCLUSION OF THE STUDY

The study aimed to evaluate the effectiveness of *S. pneumoniae* nosode at potencies of 6CH, 9CH, 30CH, and 200CH on the growth of *S. pneumoniae* using a disc diffusion assay. This research is unique as it represents the first *in vitro* investigation into the potential antibacterial effect of *S. pneumoniae* nosode on *S. pneumoniae* strains.

The results revealed that the negative control, 20% ethanol, did not exhibit significant antibacterial activity against *S. pneumoniae*. Conversely, ceftriaxone, the positive control, demonstrated substantial antibacterial, confirming its efficacy. The *S. pneumonia*e nosode did not exhibit a statistically significant difference in antibacterial activity compared to the negative control group. Minimum inhibitory concentration (MIC) testing further confirmed that *S. pneumoniae* was sensitive to ceftriaxone at the concentration of 2 µg/mL.

Overall, the study found that *S. pneumoniae* nosode 6CH, 9CH, 30CH, and 200CH, prepared in 20% ethanol, was ineffective in inhibiting the growth of *S. pneumoniae* when assessed using the disc diffusion method. These findings suggest that, under the tested conditions, the nosode did not exert a measurable antibacterial effect, highlighting the need for further research to explore its potential efficacy under different experimental conditions or *in vivo*.

6.3 LIMITATIONS OF THE STUDY

The limitations of the study were as follows: Firstly the research was confined to a single bacterial species, *S. pneumoniae*. Secondly, it focused exclusively on a specific strain of *S. pneumoniae*, namely serotype 19F (ATCC49619). Finally, since the study was conducted *in vitro*, the results necessitate validation through *in vivo* experiments to establish their applicability and relevance in a biological context.

6.4 RECOMMENDATIONS

Given the lack of statistically significant positive results from this experiment, a thorough review of the methodology was conducted, informed by the relevant literature, to identify potential shortcomings. The review identified several key considerations for researchers undertaking similar experiments.

1. The bacteria utilized for preparing the *S. pneumoniae* mother tincture and 6CH nosode must be accurately identified to prevent the utilisation of similar sub-species from the same family instead of the intended *S. pneumoniae* strain. The process of manufacturing the nosode, encompassing the transition from bacterial culture to mother tincture and the preparation of the desired potencies, is essential for maintaining control over the factors influencing the final product. This approach is preferable to purchasing a pre-manufactured *S. pneumoniae* nosode, as it allows for precise control over sample material identification, selection of the appropriate extractant, and adjustment of the final nosode concentration to align with the specific experimental methodology.
2. The potential antimicrobial effect of *S. pneumoniae* nosode should be evaluated against additional bacterial strains.
3. Explore additional testing variations, such as agar dilution tests.
4. Use specifically manufactured assay discs with consistent thickness and diameter rather than discs from filter paper. The zones can be significantly affected by the thickness and the filter paper's composition utilized to fabricate the discs (Prasad, Aathavan *et al*. 2023: 4).
5. The antibacterial effectiveness of *S. pneumoniae* nosode should be assessed *in vivo* through a controlled clinical trial.

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APPENDICES

Appendix 1: University ethics clearance

Institutional Research Ethics Committee
Research and Postgraduate Support Directorate
2nd Floor, Berwyn Court
Gate 1, Steve Biko Campus
Durban University of Technology

P O Box 1334, Durban, South Africa, 4001

Tel: 031 373 2375
Email: lavishad@dut.ac.za
http://www.dut.ac.za/research/institutional_research_ethics

www.dut.ac.za

23 August 2023

Ms N Zulu
P.O. Box 5066
Sundumbili
4491

Dear Ms Zulu

Evaluation of the effectiveness of *Streptococcus pneumoniae nosode* (6CH, 9CH, 30CH and 200CH) on the growth of *S. pneumoniae*
Ethical Clearance number IREC 068/23

The DUT-Institutional Research Ethics Committee acknowledges receipt of your gatekeeper permission letter.

Please note that FULL APPROVAL is granted to your research proposal. You may proceed with data collection.

Any adverse events [serious or minor] which occur in connection with this study and/or which may alter its ethical consideration must be reported to the DUT-IREC according to the DUT-IREC Standard Operating Procedures (SOP's).

Please note that any deviations from the approved proposal require the approval of the DUT-IREC as outlined in the DUT-IREC SOP's.

It is compulsory for a student or researcher to apply for recertification on an annual basis. The failure to do so will result in withdrawal of ethics clearance. It is the responsibility of the researcher and the supervisor to apply for recertification.

Please note that you are required to submit a Notification of Completion of Study form together with an abstract to the DUT-IREC office on completion of your study.

Yours Sincerely

Prof J K Adam
Chairperson: DUT-IREC

Appendix 2: Letter of permission to use the Department of Medical Microbiology laboratory

P.O. Box 4873
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Department of Medical Microbiology
University of KwaZulu-Natal and National Health Laboratory Service
School of Laboratory Medicine and Medical Sciences
Inkosi Albert LuthuliCentral Hospital Academic Complex
Durban, 4000

Dear Prof. Khine Swe Swe- Han

Request Letter to use the Medical Microbiology Laboratory and Technician assistance

My name is Nokwanda Zulu (21703712), currently registered for MHSc: Homoeopathy at Durban University of Technology. I am requesting a gatekeeper permission to use the Medical Microbiology Laboratory, to access *Streptococcus pneumoniae* and Technician assistance. I would also like to request a one-day training to familiarize myself with the laboratory equipment. My Supervisor is Dr SBN Krishna from the Department of Biomedical and Clinical Technology, Co-Supervisor is Dr SF Majola, from the Department of Homoeopathy. The title of my study is "Evaluation of the effectiveness of *Streptococcus pneumoniae* nosode(6CH, 9CH, 30CH, and 200CH) on the growth of *S. pneumoniae"*.

Outline of procedures: The experimental study will take approximately a week, of which only a few hours will be used each day. Namely, making pure cultures of bacterial strain, medicated discs, performing minimum inhibitory concentrations, and disc diffusion assays.

Specific resources that will be required in addition to the laboratory include a laminar flow air cabinet, spectrophotometer, incubator, autoclave, analytical balance (weighing scale), Bunsen burner, flask, pipette, densitometer, test tubes, petri dish, and vortex mixer. The media and other consumables that will be required from UKZN will be billed to DUT. Everything will be done under the supervision of the lab technician.

If you require any further information, please do not hesitate to contact me. I will appreciate your time and consideration in this matter.

Yours Sincerely,
Nokwanda Zulu
Department of Homoeopathy
Durban University of Technology

Appendix 3: Approval letter from National Health Laboratory Service

NATIONAL HEALTH LABORATORY SERVICE

Academic Affairs and Research
1 Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 555 0367/0406
Email: babatyi.kgokong@nhls.ac.za
academic.research@nhls.ac.za
Web: www.nhls.ac.za

22 November 2023

Applicant: Nokwanda Zulu
Institution: Durban University of Technology
E-mail Address: 21703712@dut4life.ac.za
Cell: 078 626 8539

Project Title: EVALUATION OF THE EFFECTIVENESS OF STREPTOCOCCUS PNEUMONIAE NOSODE (6CH, 9CH, 30CH, AND 200CH) ON THE GROWTH OF S. PNEUMONIAE
Reference Number: PR2343029

Research Application Type(s):
1. Permission to use NHLS Facilities

RE: APPROVAL LETTER: REQUEST TO ACCESS NHLS RESOURCES FOR RESEARCH PURPOSES

This letter serves to advise that the application requesting permission to conduct the above-mentioned research using the listed NHLS resources has been reviewed and **"Approved"**. Please note that the approval is granted on the condition that you comply with the NHLS Research Material and Data Access Policy and requirements stated below.

1. All material and data requested shall be used as per the research protocol submitted to the NHLS and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to the NHLS material and/or data shall be limited to the minimum required for successful completion of the approved study and shall be made available ***without patient names and other patient identifiers (including, but not limited to, national identity numbers, hospital/clinic file numbers, addresses and telephone numbers)***.
3. Confidentiality shall be maintained at the participant and institutional level and there shall be no disclosure of personal information or confidential information.
4. Data and/or material shall not be shared with other parties unless approved by the NHLS
5. The material and/or data obtained from the NHLS shall be anonymised and not, for any reason, be used to track or recruit patients as no pre-approval/consent is obtained from patients.
6. Processes shall be discussed with the relevant NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, Operations Office, etc.) and agreed upon.
7. Any amendments to the study requirements, including the use of the material and/or data for purposes not initially disclosed to the NHLS) shall be cleared by an approved HREC and submitted to the NHLS for approval via the AARMS system – https://aarms.nhls.ac.za.
8. The NHLS shall be acknowledged as a source of material and/or data in any output, such as abstracts and journal articles, emanating from the project.
9. A final report of the research study and any published output resulting from this study shall be submitted to the NHLS via AARMS

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. The NHLS entities tasked with providing the material and/data may have additional requirements for access. Sample related queries shall be directed to the relevant business manager.

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix 4: Permission application letter to use laminar air flow unit

P.O. Box 4873
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Faculty of Health Sciences
Department of Homoeopathy
Head of Department
Durban
4000

Dear Prof. A Ross

Permission Application Letter to use the Laminar air flow unit

My name is Nokwanda Zulu (21703712). I am currently registered for MHSc: Homoeopathy and I am requesting to use the Laminar air flow unit at Ritson. My Co-Supervisor is Dr SF Majola from your Department. The title of my study is, "Evaluation of the effectiveness of *Streptococcus pneumoniae* nosode(6CH, 9CH, 30CH, and 200CH) on the growth of *S. pneumoniae"*.

Outline of procedures: The experimental study will take approximately a week to be conducted of which only 5-7 hours will be used each day in the laboratory under the supervision of Dr Sirpal (laboratory technician). Namely, to make potencies from 6CH potency prepared from *S. pneumoniae*, 6CH potency will be purchased from Comed Health Company. The materials that will be provided by the Department include a pipette and labels. The materials that will be provided by the researcher include ethanol, bottles, *S. pneumoniae* nosode 6CH, and marker.

Yours Sincerely,
Nokwanda Zulu

Appendix 5a: Preparation of *Streptococcus pneumoniae* Nosode by German Pharmacopoeia Homoeopathic Method

1. Mother tinctures and liquid dilutions

Mother tinctures made by method 44 are made from killed cultures of microorganisms, decomposition products of animal organs, or body fluids containing pathogens or products of disease processes. Adjust microorganism cultures to a content of 10^7 microorganisms (CFU) per gram prior to heat treatment at 133°C. To prepare the mother tincture, mix and succuss 1 part of the heat-treated raw material with 9 parts glycerol 85%. Leave to stand for not less than 5 days, after 5days filter using muslin cloth. The filtrate is the mother tincture (Bibliothek 2005: H 5.4.4; Xaba 2018; Mpangase 2020)

2. Potentization

The mother tincture corresponds to the 1st decimal dilution (Ø=D1)
The 2nd decimal dilution (D2) is made from:

- 1 part of the mother tincture (D1) and
- 9 parts of ethanol 30% (*m/m*)

The 3rd decimal dilution (D3) is made from:

- 1 part of the 2nd decimal dilution and
- 9 parts of ethanol 43% (*m/m*)

Subsequent dilutions are prepared accordingly:

The first centesimal dilution(1CH) is made with

- 10 parts of mother tincture and
- 90 parts of ethanol 30% (*m/m*),

- Succuss 10 times then label as 1CH.

The 2nd centesimal dilution (2CH) with

- 1 part of the 1st centesimal dilution (1CH) and
- 99 parts of ethanol 43% (*m/m*).
- Succus 10 times than label as 2CH.

Subsequent dilutions will be made this way until the desired potency is reached, which is 6CH

Reference

Bibliothek, D. 2005. Method 43-Preparation of nosode. *German Homoeopathic Pharmacopoeia*. 2: 34-36

Mpangase, S. 2020. An in vitro study of the antimicrobial effect of Indigofera daleiodes plant tinctures using Disc Diffusion and Well Diffusion Assay. Master's dissertation, Durban University of Technology, Durban, South Africa.

Xaba, N. 2018. A controlled in vitro study of the antimicrobial effectiveness of *Colibacillinum* against *E. coli*. Master's dissertation, Durban University of Technology, Durban, South Africa.

Appendix 5b: Preparation of *Streptococcus pneumoniae* nosode potencies (9CH, 30CH, and 200CH) from *Streptococcus pneumoniae* nosode 6CH

1.*S.pneumoniae* nosode 9CH, 30CH, and 200CH will be prepared from *S. pneumoniae* nosode 6CH according to the GHP method 44 (Bibliothek 2005: H5.4.4).

2. Potentization

The seventh centesimal dilution (7CH) will be prepared by

- Measuring 0.2ml of 6CH by means of a micropipette
- Adding 19.8ml of 96% ethanol in a 30ml amber glass screw-top bottle
- Succus 100 times.

The eighth centesimal dilution (8CH) will be prepared by

- Measuring 0.2ml of 7CH by means of a micropipette
- Adding 19.8ml of 20% ethanol in a 30ml Amber glass screw-top bottle
- Succuss 100 times.

The ninth centesimal dilution (9CH) will be prepared by

- Measuring 0.2ml of 8CH by means of a micropipette
- Adding 19.8ml of 20% ethanol in a 30ml Amber glass screw-top bottle
- Succuss 100 times.

The tenth centesimal dilution (10CH) will be prepared by

- Measuring 3.8ml of 96% ethanol
- Adding 2 drops of 9CH in a 7ml clear glass bottle
- Succuss 100 times.

The eleventh centesimal dilution (11CH) will be prepared by

- Measuring 4.6ml of 96% ethanol
- Adding 4 drops of 10CH in a 7ml clear glass bottle
- Succuss 100 times.

Subsequent dilutions will be prepared accordingly until 28CH potency is reached.

The twenty-eighth centesimal dilution (28CH) will be prepared by

- Measuring 0.2ml of 27CH by means of a micropipette
- Adding 19.8ml of 96% ethanol in a 30ml amber glass screw-top bottle
- Succus 100 times.

The twenty-ninth centesimal dilution (29CH) will be prepared by

- Measuring 0.2ml of 28CH by means of a micropipette
- Adding 19.8ml of 20% ethanol in a 30ml amber glass screw-top bottle
- Succus 100 times.

The thirty centesimal dilution (30CH) will be prepared by measuring

- Measuring 0.2ml of 29CH by means of a micropipette
- Adding 19.8ml of 20% ethanol in a 30ml amber glass screw-top bottle
- Succus 100 times.

The thirty-one centesimal dilution (31CH) will be prepared by measuring

- Measuring 3.8ml of 96% ethanol
- Add 2 drops of 30CH in a 7ml Clear glass bottle
- Succuss 100 times.

The thirty- two centesimal dilution (32CH) will be prepared by measuring

- Measuring 4.6ml of 96% ethanol
- Add 4 drops of 31CH in a 7ml Clear glass bottle

- Succuss 100 times.

Subsequent dilutions will be prepared accordingly until 198CH potency is reached.

The one hundred and ninety-eight centesimal dilution (198CH) will be prepared by measuring

- Measuring 0.2ml of 197CH by means of a micropipette
- Adding 19.8ml of 96% ethanol in a 30ml amber glass screw-top bottle
- Succus 100 times.

The one hundred and ninety-nine centesimal dilution (199CH) will be prepared by

- Measuring 0.2ml of 198CH by means of a micropipette
- Adding 19.8ml of 20% ethanol in a 30ml amber glass screw-top bottle
- Succus 100 times.

The two hundred centesimal dilution (200CH) will be prepared by

- Measuring 0.2ml of 199CH by means of a micropipette
- Adding 19.8ml of 20% ethanol in a 30ml amber glass screw-top bottle
- Succus 100 times.

References

Bibliothek, D. 2005. Method 43-Preparation of nosode. *German Homoeopathic Pharmacopoeia*. 2: 34-36

Appendix 6: Preparation of saline and inoculum

1. Preparation of growth culture

Single colonies will be obtained from the University of Kwazulu Natal, Department of Medical Microbiology laboratory. Stock cultures of *S. pneumoniae* ATCC49619 to be tested will be used to inoculate separate Mueller Hinton agar supplemented with 5% sheep blood plates. Mueller Hinton Agar supplemented with 5% sheep blood plates will be inoculated. A few colonies will be suspended from an overnight Mueller Hinton agar culture of *S. pneumoniae* using a sterile transfer loop. Media plates will be streaked according to the streak quadrant method (Katz. 2008: 4; Mahato, Sah *et al*. 2019: 300). Prepared plates will be incubated at optimum temperature, usually at 37 °C, in an inverted position for 20-24 hours (Rosario, Johnson. 2021: 4).

2. Preparation of the saline test cultures

Prior to the preparation of saline test cultures, 9g of sodium chloride will be weighed and added to a beaker. 1000 ml of distilled water will be added into the beaker and mixed to dissolve the sodium chloride.10 ml of the saline will be distributed into each of the falcon tubes using a pipette. The tubes will be closed and labelled. The tubes will be autoclaved for sterilisation for 15 minutes at 15psi and 121°C. Once the solution has cooled down, it will be transferred to 3 small tubes (Zampieri, Machado *et al*.2021: 828).

3. Preparation of the inoculums

A few individual colonies from the overnight MHA supplemented with 5% Sheep blood cultures of *S. pneumoniae* will be suspended in 10ml sterile solution (0.9g/l). The solution will be adjusted to 0.5 McFarland Equivalence Turbidity Standard (Jeon, Shin *et al.*20121: 560; Mack 2022: 17)

References

Rosario, Y., Johnson, M.D. 2021. Media Matters, Examining Historical and Modern *Streptococcus pneumoniae* Growth Media and the Experiments They Affect. *Frontiers in Cellular and Infection Microbiology*.11: 1-15

Zampieri, F.G., Machado, F.R., Biondi, R.S., Freitas, F.G.R., Velga, V.C., Figueiredo, R.C. 2021. Effect of Intravenous Fluid Treatment with a Balanced Solution vs 0.9% Saline Solution on Mortality in Critically ill Patients*. Jama*. 326(9): 818-829

Jeon, S., Shin, J.H., Lim, H.J., Choi, M.J., Byun, S.A., Lee, D., Lee, S.Y., Won, E.J., Kim, S.H., Shin, M.G. 2021. Disk Diffusion Susceptibility Testing for the Rapid Detection of Fluconazole Resistance in Candida Isolates. *Clinical Microbiology*. 41(6): 559-567

Appendix 7: Preparation of dry medicated discs

1. Preparation of *S.pneumoniae* nosode 6CH, 9CH, 30CH and 200CH dry discs

Sterile 5mm Whatman® filter paper number 4 discs, will be evenly spaced upon the bottom of a sterile petri dish using a pair of sterile forceps so that each petri dish contains one disc. 20 microlitres of respective *S. pneumoniae* nosode potencies will be pipetted into each disc using a calibrated micropipette. Plates will be grouped from 1-12, prepared in triplicate, with one impregnated disc each (Plate 1-3: *Streptococcus pneumoniae* nosode 6CH dry discs, Plate 4-6: *Streptococcus pneumoniae* nosode 9CH dry discs, Plate7-9: *Streptococcus pneumoniae* nosode 30CH dry discs, Plate10-12: *Streptococcus pneumoniae* nosode 200CH dry discs). The Petri dishes will be placed in a dark incubator at 37℃ to be allowed to dry. Once dry, a further 10 microlitres of respective *S. pneumoniae* nosode potencies will be pipetted onto each disc, before being returned to the darkened incubator at 37℃, allowing the discs to dry again. The dry discs will be stored in sterile jars until used (Invernizzi. 2002: 19).

2. Preparation of 20% ethanol dry discs

Sterile 5mm Whatman® filter paper number 4 discs, will be evenly spaced upon the bottom of a sterile petri dish using a pair of sterile forceps so that each petri dish contains one disc. 20 microlitres of respective 20% ethanol will be pipetted into each disc using a calibrated micropipette. Plates will be prepared in triplicate. The Petri dishes will be placed in a dark incubator at 37℃ to dry. Once dry, a further 10 microlitres of respective *S. pneumoniae* nosode potencies will be pipetted onto each disc, before being returned to the darkened incubator at 37℃, allowing the discs to dry again. The dry discs will be stored in sterile jars until used (Invernizzi. 2002: 18).

Reference

Invernizzi. 2002. A controlled in vitro study of the effectiveness of Tulbagia violacea in herbal tincture and homoeopathic dilution (1X and 6X) against gram-positive and gram-negative bacteria. Master's dissertation, Durban University of Technology, Durban, South Africa.

Appendix 8: Preparation of wet medicated discs

1. Preparation of *S. pneumoniae* nosode 6CH, 9CH, 30CH and 200CH wet-discs

Sterile 5mm Whatman® filter paper number 4 discs, each disc will be placed on the sterile petri dish using a sterile needle, for each plate contains one disc. 20 microlitres of respective *S. pneumoniae* nosode potencies will be pipetted into each disc using a calibrated micropipette. The plates will be grouped from 1-12, prepared in triplicate, with one impregnated disc each (Plate 1-3: *Streptococcus pneumoniae* nosode 6CH wet discs, Plate 4-6: *Streptococcus pneumoniae* nosode 9CH wet discs, Plate7-9: *Streptococcus pneumoniae* nosode 30CH wet discs, Plate10-12: *Streptococcus pneumoniae* nosode 200CH wet discs). The discs will be allowed to dry for 5 minutes (Invernizzi. 2002: 18). The discs will be applied to the media plates, and each disc will be placed upon the centre of the inoculated media plate using a sterile needle, for each media plate contains one disc. a further 10 microlitres of respective *S. pneumoniae* nosode potencies will be pipetted onto each disc, before being placed in the dark incubator at 37℃ for 24 to 48 hours.

2. Preparation of 20% ethanol wet-discs

Sterile 5mm Whatman® filter paper number 4 discs, each disc will be placed on the sterile petri dish using a sterile needle, for each plate contains one disc. 20 microlitres of respective 20% ethanol will be pipetted into each disc using a calibrated micropipette. The plates will be prepared in triplicate, with one impregnated disc each. The discs will be allowed to dry for 5 minutes (Invernizzi. 2002: 18). The discs will be then applied to the media plates, and each disc will be placed upon the centre of the inoculated media plate using a sterile needle, for each media plate contains one disc. a further 10 microlitres of respective 20% ethanol will be pipetted onto each disc, before being placed in the dark incubator at 37℃ for 24 to 48 hours.

References

Invernizzi. 2002. A controlled in vitro study of the effectiveness of Tulbagia violacea in herbal tincture and homoeopathic dilution (1X and 6X) against gram-positive and gram-negative bacteria. Master's dissertation, Durban University of Technology, Durban, South Africa.

Appendix 9: Minimum inhibitory concentration

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of ceftriaxone will be determined using the Epsilometer (E-test) method, following the manufacturer's instructions (Biomeriuex, South Africa). The test will be conducted in triplicate to obtain optimal and reliable results. A sterile cotton swab will be immersed in a well-mixed saline test culture and utilized to streak the entire dried surface of Mueller Hinton agar supplemented with 5% sheep blood plates. After streaking, the swab will be discarded in an appropriate container. The lid ajar will be left for three to five minutes, to allow any excess surface moisture to be absorbed before applying the E-test strips. The strips will be applied on the agar surface using forceps. The plates will be then incubated in an inverted position at 37°C for 18-24 hours.

Appendix 10: Disc diffusion assay

Disc diffusion assay

The experiment will be conducted in triplicate to ensure optimal and reliable results. The sterile cotton swab will be dipped into a well-mixed saline test culture and utilized to streak the entire dried surface of Mueller Hinton agar supplemented with 5% sheep blood plates. The lid ajar will be left for three to five minutes, to allow any excess surface moisture to be absorbed before applying the drug-impregnated discs. A marker pen will be used to label the undersurface of each agar plate to denote which test substance or control disc was applied (Bauer, Kirby *et al*.1966).

The plates will be labelled as follows; (3 plates *S.pneumoniae* nosode 6CH dry discs; 3 plates: *S.pneumoniae* nosode 9CH dry discs; 3 plates: *S.pneumoniae* nosode 30CH dry discs; 3 plates: *S.pneumoniae* nosode 200CH dry discs; 3 plates: : 20% Ethanol dry discs; 3 plates: *S.pneumoniae* nosode 6CH wet discs; 3 plates: *S.pneumoniae* nosode 9CH wet discs; 3 plates: *S.pneumoniae* nosode 30CH wet discs; 3 plates: *S.pneumoniae* nosode 200CH wet discs; 3 plates: : 20% Ethanol wet discs(CLSI, 2020: 149).

References

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M.1966. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology*. 45(4): 493-496

Clinical and Laboratory Standards for Antimicrobial Susceptibility Testing. 2020. Performance Standards for Antimicrobial Susceptibility Testing. *Clinical and Laboratory Standards for Antimicrobial Susceptibility Testing*. M100, 30th edition. 1-23

Appendix 11: Application Letter for increase of research budget

70 Steve Biko Road
Gate 7
Durban
4000

Director: Research and Postgraduate Support
Tromso Annex, 1st Floor
Gate 1, Steve Biko Campus
P.O. BOX 1334
Durban

Dear Dr Linganiso

Application Letter for increase of research budget

Thank you for reading this letter. My name is Miss Nokwanda Zulu (21703712). I am currently registered for MHSc: Homoeopathy. I am writing to request an additional **R5000** to my allocated research budget. Most of my research budget is for consumables and editorial services. The title of my study is, "Evaluation of the effectiveness of *S. pneumoniae* nosode (6CH, 9CH, 30CH, and 200CH) on the growth of *S.pneumoniae"*.

Outline of the Procedures: The experimental study will be conducted at the National Health Laboratory Service (NHLS), Department of Medical Microbiology, located at Inkosi Albert Luthuli Hospital. The procedures are expected to span approximately one week, with only a few hours of work required each day. The tasks include culturing bacteria, preparing medicated discs, and performing minimum inhibitory concentration and disc diffusion tests. All work will be done under the supervision of a lab technician.

Yours faithfully.

Miss N.D Zulu(21703712)-Researcher 0786268539 (21703712@dut4life.ac.za)

Dr. S.B.N Krishna (Supervisor) – 0798459515 (sureshk@dut.ac.za)

Dr. S.F Majola (Co-supervisor) - 0624814432 (drsindilemajola@gmail.com)

Appendix 12: Quotations for research consumables from Shalom Laboratory Supplies CC, Dalgen Packaging CC, Comed Health Pty Ltd, and JVL Lab Engineering and General Supplies CC

SHALOM LABORATORY SUPPLIES c.c.

TEL: 00 27 31 3053573 • FAX: 00 27 31 3053572
FAX: 0866327302 • EMAIL: sales@shalomlabs.co.za
WEBSITE: www.shalomlabs.co.za

P.O. BOX 51030
MUSGRAVE ROAD,
DURBAN 4062

Suppliers of Quality Glassware, Chemicals, Consumables and Instrumentation

132 COMMERCIAL ROAD
(DR A.B. XUMA ST)
INTERNATIONAL PLAZA
DURBAN 4001

Reference: IN/SM/005115

Date : 03-10-2022

Durban University of Technology

FOR THE ATTENTION OF: Ms Nokwanda

Dear Mam

Re: Labware

We thank you for your valued enquiry and have pleasure in submitting our quote as follow:

No	Description	Unit Price
1	Ethanol, Food Grade,99.9% ,2.5 Litre	R 1 550-00
	SUBTOTAL EXCLUDING VAT	R 1 550-00
	VAT	R 232-50
	TOTAL INCLUDING VAT	R1 782-50

Tel. 031-5694288
Fax. 031-5694294
Reg. No. 1998/047125/23
Vat Reg. No.4880182623

Dalgen Packaging CC

P O Box 201228
Durban North
4016

11 Malone Road
Maxmead
Pinetown

Quotation

Date	11/10/2022
Page	1
Document No	QU210502

CASH SALE - OCTOBER 2022

Deliver to

DUT Homeopathy Clinic
Research: N.D Zulu
11 Ritson Rd, Gate 6 Entrance
Berea, Durban
21703712@dut4life.ac.za

Account	Order No.	VAT Exempt	Customer VAT No	Sales Code	Expiry	
OCT22		N		002	11/10/2022	Exclusive

Code	Description	Quantity	Unit	Unit Price	Disc%	Vat	Nett Price
STO-DROPPER3	30ML AMBER DROPPER BOTTLE	15.00	168	2.32		15.00%	34.80
STO-DROPPER8	WHITE FAST FLOW CAP 15 units	15.00	6000	1.10		15.00%	16.50
TRANSPORT3	TRANSPORT - DW	1.00		160.00		15.00%	160.00

No Returns:
Due to health & safety reasons
We cannot accept returns

Trading Hours:
Mon - Thurs: 7:30 to 16:00
Friday: 7:30 to 15:30
www.dalgen.co.za

Banking Details
NEDBANK - HAYFIELD
BRANCH CODE - 134025
ACCOUNT - 1340475812

Received in good order by :
(Name) _____

Signed_____ Date_____

Sub Total		211.30
Discount @	0.00%	0.00
Amount Excl VAT		211.30
VAT @ 15%		31.70
Total		243.00

Comed Health Pty Ltd
313 Kuit Street
Waltloo
Tax Registration 4010 25 3583
Telephone 012 813 9400
Fax 012 813 9496

Quotation

Date: 09/09/2022
Account NAT13
Quotation number:: SOQ019645

Customer VAT number: 4080199559

To:

NAT13
DURBAN UNIVERSITY OF TECHNOLOGY

HOMOEOPATHY CLINIC RESEARCH: N.D. ZULU
11 RITSON ROAD,GATE 6 ENTRANCE
BEREA
DURBAN

Item Code	Item Description	Qty	Price (Ex)	Disc %	Tax	Total (Excl VAT)
09SI96L0025	STREPTOCOCCUS PNEUMONIAE NOS. 6CH h i	1.00000	92.78	20.00	11.13	74.22
POSCOU	Delivery Charge - 48 Hour Courier	1.00000	84.00		12.60	84.00

Total (Excl)	158.22
Tax	23.73
Total (Incl)	**181.95**
Discount	0.00
Total (Incl)	**181.95**

BANK DETAILS:

Account Name: Comed Health
Bank Name Nedbank
Bank Account 1497218365
Branch Code 149745
PAYMENT REFERENCE: SURNAME AND/OR SOQ NR

Proof of payments to be sent to:
monica@comedhealth.co.za/

Pro-Forma

Date 19/02/24
Page 1

IO100370

JVL LAB ENGINEERING &GENERAL SUPPLIES CC
P.O BOX 72095
MOBENI
4060
TEL:031-301-6829

38 PIET RETIEF ROAD
UNIT12
QUEENSMEAD INDUSTRIAL
4093
VAT REG NO:4920257054
CK:1992/008339/23

Bill To: CASH
CASH

Ship To:
RESEARCH STUDENT

Due By
Sales Code
Payment Terms 0 days

Your Reference
Tax Reference
Tax Exempt N

Incl/Excl
Exclusive

Code	Description	Qty	Unit	Unit Price	Disc%	Tax	Nett Price
CT0417B	CEFTRIAXONE ANTIMICROBIAL SUSCEPTIBILITY	1.00	50	340.00		15.00%	340.00
COURIERCHAR	COURIER CHARGES	1.00	EACH	300.00		15.00%	300.00

Sub Total	640.00
Discount @ 0.00%	0.00
Amount Excl Tax	640.00
Tax	96.00
Total	**736.00**

Appendix 13: Published manuscript

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RESEARCH ARTICLE:

The Use of Homoeopathic Nosodes: Consideration for Human Health

Nokwanda Zulu[1], Sindile Majola[2] and Suresh Krishna Naidu[3]

Received: 02 December 2022 | **Revised**: 06 November 2023 | **Published**: 16 November 2023

Reviewing Editor: Dr. Francis Fabian Akpa-Inyang, Durban University of Technology

Abstract

Homoeopathy is a system of medicine based on the law of similars 'let likes be cured by likes'. This means that any substance with the capacity for producing disease in its crude state has the capacity to treat a similar disease if taken in a very small dose. Homeopathic nosodes are homoeopathic remedies sourced from diseased materials and organisms. Homoeopathic nosodes may be used to treat and prevent infectious and non-infectious diseases. The incidence of infectious disease epidemics is now occurring more often around the world. This common public threat includes the emergence of antibiotic resistance which is an increasingly global issue. Contributing factors of this emergence are linked to underlying biological and environmental issues, lifestyle changes, and the misuse and overprescribing of antibiotics. Despite strengthening health infrastructure, every epidemic and multi-drug resistance bacterium poses a challenge to the government, policymakers, health professionals, and the whole population. This article will review data available on homoeopathic nosodes as well as present evidence that is available to support the use of homoeopathic nosodes in disease prevention and treatment. It also highlights several clinical trials and in vitro studies on the use of homoeopathy to target health issues that have the potential to harm public health and those that could possibly assist in reducing healthcare costs.

Keywords: *homoeopathy; nosode; antibiotic resistance; human health*

Introduction

Homoeopathy is an alternative holistic form of complementary medicine that was developed and implemented by German physician, Dr. Samuel Hahnemann (Bala, 2020: 215). This medical system treats a disease through the administration of small doses of a remedy (highly diluted between succussions) that would in its crude form produce similar symptoms to those of the disease in a healthy individual. This principle of homoeopathy is called the Law of similars, "likes cures likes" (Khuda-Bukhsh, 2018: 2; Elavarasan *et al*., 2023: 5120). Succussion is the process of vigorous shaking and is used to increase the effectiveness of a remedy (Ullman, 2021: 2). Homeopathic medicines are prepared from natural sources (plants, minerals, animals, diseased products, healthy tissues) (Kumari, 2022: 355). The word 'nosode' is derived from the Greek term '*nosos*' referring to the disease. Nosodes are homoeopathic remedies that consist of dilutions of pathogenic organisms' causative agents, such as fungi, parasites, viruses or bacteria, and disease products (Varanasi and Nayak, 2020: 130). Homoeopathic nosodes may be used to treat and prevent non-infectious as well as infectious diseases. More than 60 homoeopathic nodoses have been used by homoeopathic practitioners, playing a significant role in clinical practice. Nosodes can be prepared from bacteria (*Diphtherinum, Streptococcinum*), viruses (*Variolinum* for smallpox and *Morbillnum* for measles), parasites (*Psorinum* for scabies), and diseased tissues (*Carcinosin* from infected breast cancer). As a result of their diverseity, the individual evaluation of new nosodes for safety assessment is always recommended and required (Munshi *et al*., 2022: 43).

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Antibiotics are conventional treatment drugs that are used for the prevention and treatment of bacterial infections. Thus the use of antibiotics has saved millions of lives worldwide (Ribeiro da Cunha *et al.*, 2019: 1; Uddin *et al.*, 2021: 1750). In addition, antibiotics have provided a platform for the implementation of surgical interventions, organ transplants, and cancer chemotherapy (Gajdács and Albericio, 2019: 1). Albeit, antibiotics have existed for decades and due to exposure to many antibiotics and their adaptation, some disease strains become multi-resistant to treatments employed (Nogueira, 2019: 86). Overtime, the overuse, and overprescribing of antibiotic due to delays in accurate diagnoses has caused a reduction in effective antibiotics available to treat infections (Morris, 2020: 3). The total number of infections occurring because of multi-drug resistance is increasing worldwide and the threat of untreatable infections is increasing (Gajdács and Albericio, 2019: 1; Uddin *et al.*, 2021: 1752). Antimicrobial resistance presents a significant issue to healthcare systems around the globe. Microorganisms constantly evolves to avoid being killed by antibiotics, hence, antimicrobial resistance cannot be avoided (Verstraete *et al.*, 2022: 466). Infectious pathogens can develop resistances over time if they are exposed to newly developed drugs (Chokshi *et al.*, 2019: 36). The main contributing factors behind the development of antimicrobial resistance is the overuse and the misuse of antimicrobial agents both in humans and veterinary medicine (mismanagement of antibiotics in the form of interruption of therapy, self-medication, and being unrestriced in access to high rates of antimicrobial prescriptions), as well as in agricultural settings (Ribeiro da Cunha *et al.*, 2019: 2). This antimicrobial resistance challenge has become worse as nosocomial infections have become a leading cause of morbidity and mortality, resulting in longer hospital stays. This has resulted in a significant increase in healthcare costs (McFee, 2009: 422; Geta and Kibret, 2021: 1).

In addition, more than 15 percent of nosocomial infections are already occurring due to multidrug-resistant pathogens (Theuretzbacher, 2012: 296 and Ribeiro da Cunha *et al.*, 2019: 2). This includes some ineffective antimicrobials, for some of which there are no effective antimicrobials (Frieri *et al.*, 2017: 370; Ribeiro da Cunha *et al.*, 2019: 2). Future perspectives are not looking promising as a government-commissioned study, undertaken in the United Kingdom, estimated that antimicrobial-resistant infections will lead to nearly 10 million deaths each year by 2050 and a total GDP loss of $100.2 trillion by 2050 if appropriate actions are not taken (Nieuwlaat *et al.*, 2021: 1657). A systematic review done by Sekyere and Mensah in 2019 documented the increasing prevalence of antibiotic resistance in gram-positive bacteria in Africa. For instance, changing antibiotic resistances caused by *S. aureus* in the population has been recorded in bloodstream infections in Gambia, Ghana, Burkina Faso, and Niger (Bernabe *et al.*, 2017: 630; Sekyere and Mensah, 2020: 29). *Staphylococcus aureus* has been recorded as the bacteria responsible for causing pneumonia and septicemia (infection of bloodstream) with resistance rates of 90 percent, 29 percent, and 20 percent to gentamycin, ampicillin, and cloxacillin, respectively, among children in Africa (Workneh *et al.*, 2017: 3; Sekyere and Mensah, 2020: 30). Additionally, Founou reported 100 percent and 93.8 percent multidrug resistance (MDR) rates respectively in *S. aureus* and *Enterococcus Spp* (Williams *et al.*, 2018: 2; Sekyere and Mensah, 2020: 30). The emergence of tuberculosis drug resistance in South Africa is also increasing. There are more than 322 000 new cases of tuberculosis reported annually in South Africa, 4.4 percent of cases are drug resistant (Daftary *et al.*, 2021: 480). A study was conducted in KwaZulu-Natal on patients with drug-resistant tuberculosis to identify Resistance-Associated Variants (RAVs) and to evaluate the extent of the bedaquiline and clofazimine cross-reference. The results revealed that a Bedaquiline and clofazimine cross-reference is emerging repeatedly in Southern Africa. Onward transmission was greatly increased due to Rv0678 mutations in M tuberculosis. Bedequiline and clofazimine roll-out treatment in the setting of limited drug susceptibility testing has the potential to allow for the further spread of resistance (Nimmo *et al.*, 2020: 165).

Classification of Homoeopathic Nosodes

As aforementioned, homoeopathic nosodes can be prepared from biological material including diseased tissues and cultures (bacteria, viruses, parasites, and fungi) from the decomposed products of humans and animals. There are more than 60 nosodes that have been incorporated in homoeopathy treatments in clinical settings since 1830 with claimed clinical benefits. However, a small number of nosodes have been scientifically tested for their effectiveness, particularly using *in vitro* models (Daftary *et al.*, 2016: 3; Khuda-Bukhsh, 2018: 2). In the Homoeopathic Pharmacopoeia of India (HPI), the preparations are categorised into four groups depending on the nature of the substance, whether the organism has the capability of producing endotoxins or exotoxins. These groups are N- Ⅰ, N- Ⅱ, N-Ⅲ, and N-Ⅳ (Sankar *et al.*, 2017: 158 and Nayak *et al.*, 2020: 130). The four groups are demonstrated in figure 1 below.

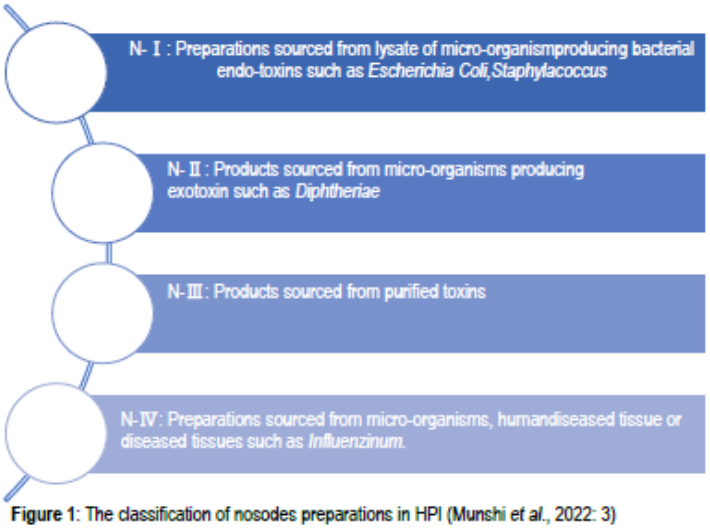

Figure 1: The classification of nosodes preparations in HPI (Munshi *et al.*, 2022: 3)

Nosodes are classified into basic, exanthem, isopathic, autogenous, intestinal, and commonly used (well-proven). Basic nosodes include *Psorinum, Carcinosinum, Medorrhinum, Syphilinum*, and *Bacilinum*. All these major nosodes were prepared before 1901. *Psorinum* (sourced from sero-purulent discharge from scabies vesicle) was made and proven by Hering in 1828. *Medorrhinum* was developed by Swan before 1890 from a urethral discharge of a Gonorrhea infected patient. *Syphilinum* was developed by Swan during the 1880s and was sourced from Syphilis. *Bacillinum* was developed by Burnet and prepared from tuberculosis sputum during 1885s (Shah, 2017: 203). James Compton Burnett was the first to develop the nosode from infected cancer breast tissue known as *Carcinosinum* (Shah and Talele, 2019: 27).

Bowel nosodes are remedies made from cultures of the non-lactose fermenting bacterial flora of the intestinal tract. They were developed by Dr. Bach, Dr. Wheeler, Dr. Dishngton, and Dr. Patterson with his wife Elizabeth Patterson. Dr. Bach and Patterson discovered many specific species of bacteria in the fecal matter of hospitalised patients. These bacteria were isolated and studied, followed by specific homoeopathic remedies being sourced from them known as nosodes (Nanda, 2020: 73). This includes *Morganella,* sourced from gram-negative bacteria causing acute infections such as pericarditis, central nervous system infection, urethritis, and sepsis. It is indicated to treat respiratory conditions such as asthma and pneumonia. *Proteus* is sourced from a group of negative gram bacteria responsible for inflammation (gastroenteritis, sepsis, meningitis). It is indicated to treat urological infections. *Bacillus No. 7* is another commonly used bowel nosode indicated to treat respiratory conditions (such as asthma) and low blood pressure (Nanda, 2020: 73). The classification of nosodes is demonstrated in table 1 below:

Table 1: Classifications of homeopathic nosode

Nosodes category	Nosodes
Basic	*Bacillinum, Carcinosinum, Medorrhinum, Psorinum, and Syphilinum*
Exanthem	*Anthracinum, Diphtherinum, Malandrinum, Influenzinum, Morbillinum, Parotidinum, Pertussinum, Vaccininum, and Variolinum*
Isopathic	*Malaria officinalis, Pyroginum. Staphylococcinum, and Streptococcinum*
dabbljazIntestinal (Dr. Bach and Dr. Wheeler)	*Bach nosodes or Bowel nosodes: Bacillus dysenteria coli, Bacillus faecalis, Bacillus Gaertner, Bacillus morgan, Bacillus mutabile, Bacillus No 7 and Bacillus proteus*
Commonly used (Well proven)	*Medorrhinum, Psorinum, Pyroginum Syphillinum, and Tuberculinum*
Other	*Ambra griesea, Anthracinum, Carcinosin, Cholestrinum, Eel serum, Eosinum, Influenzinum, Leuticum, Lyssin, Maladrinum, Malaria Officinalis, Morbillinum, Osteo arthritic, Parotidinum, Pertussin, Pneumococcinum, Scarlatina Scirrhinum, Staphylococcinum, Streptococcinum, Typhinum, Typhoidinum, Vaccinium, Variolinum*

Source: Galande, 2020: 101

Indications

Nosodes are considered to contribute to the regulation of the body's organs allowing them to function in a normal and healthy way, as well as to treat and cure patients holistically (Ijaz, 2020: 2). They are safe and effective when used in conjunction with conventional treatment. There exists the possible role of integrating homoeopathy with standard care to treat all types of infectious diseases, including those with high mortality and morbidity. This may result in reduced lengths of hospital stays and a lower cost of care, thus, reducing the health-care burden on hospitals. Nosodes may be incorporated when the use of antimicrobials (such as antibiotics or anti-viral drugs) is not yet evaluated for the new emerging disease (Nayak and Varanasi, 2020: 130). Nosodes are mostly used in clinical practice, such as *Psorinum* for ailments from a suppressed skin condition or pruritus, *Medorrhinum* for the history of suppressed gonorrhea, and *Syphilinum* for the history of syphilitic chancre suppression. Although *Psorinum* is sourced from a discharge containing scabies parasites, it does not only treat scabies but may also be beneficial against cancer, eczema, migraines, mental disorders (anxiety), and more (Munshi *et al*., 2022: 43). Conventionally, majorly used nosodes in homoeopathy are known as deep-acting medicines due to the virulent capacity of involving multiple organs and systems by the presumed organisms that are present (M. tuberculosis and Neisseria gonorrhoeae) in their making. Nosodes are utilised as a treatment for non- infectious and infectious diseases as well as homoeoprophylaxis during illness in epidemic outbreaks (Nayak and Varanasi, 2020: 130).

A survey conducted by homoeopathic practitioners showed that 95 percent of homoeopathic practitioners consider nosodes to be crucial in their clinical practice (Nayak and Varanasi, 2020: 130; Tiwari, 2021: 193). An open-label study was designed by Herscu *et al*. to evaluate the safety and immune response of the coronavirus nosode BiosimCovex. The nosode was administered orally on three consecutive days to ten healthy volunteers (Herscu *et al*., 2022: 1). All forms of clinical examinations, immune parameters, and laboratory safety were performed. During the clinical trial, there were no fatal adverse events reported. The results showed an increase percentage of intetuken-6(IL-6) on day 17 in three participants. By day 34, ten participants showed elevated IL-6. A significant difference between IL-6 observations, calculated by repeated measures ANOVA, was found to be highly significant. The IL-6 values of nine participants were found to return to normal on day 60. An increase in CD4 number was noticed on day 60. This showed that Homoeopathic Pathogenic Trials (HPT) may extend into physiological changes in the immune response (Herscu *et al*., 2022: 1). *Oscillococcinum* is a homoeopathic remedy prepared from duck heart and liver. It is popularly used in influenza virus cases. The *Oscillococcinum* nosode has been successfully used in a double-blind study to treat patients with the influenza-like disease (Skripchenko *et al*., 2019: 93 and Munshi *et al*., 2022: 44). The leading country worldwide in developing homoeopathic nosodes and successfully implementing them in its public health is Cuba. Cuba has great experience in using nosodes as a stand-alone treatment or in combination with homoeopathic remedies that are not nosodes, such as prophylaxis. The country has used nosodes for different conditions including pneumonia, hepatitis, meningitis, dengue, chorale, leptospirosis, and other diseases (Bracho *et al*., 2010: 158 and Munshi *et al*., 2022: 44). Table 2, below, shows examples of homoeopathic nosodes that currently exist in clinical practice as well as with their biological functions in farm livestock (Munsh *et al*., 2022: 44).

Table 2: Treatment with homeopathic nosodes

Homoeopathic Nosode	Abbreviation	Source	Treatment	References
Anthracinum	Anthr.	Anthrax poison	Carbuncles, boils, septic inflammation, spleen diseases, malignant ulcers	Dabbir, 2017: 244; Shivadikar, 2020: 54
Bacillinum (Dr Burnett)	Bac.	Humans' tubercular sputum	Tuberculosis, Chronic catarrhal conditions	Jee *et al*. 1891: 14; Wadhwani and Chadha, 2022: 15
Borrelia burgdorferi (lyme nosode)		Bacterial species of the spirochete class	Lyme disease	Whitmont, 1998: 189; Greenspan, 2019: 5
Carcinosin	Carc.	Cancer-infected breast tissue	One of the greatest polychrest	Shah and Talele, 2019: 3
Diphtherinum	Diph.	*Corynebacterium diphtheria*	Immunity boosting for diphtheria	Bala, 2020: 216
Folliculinum	Foll.	Oestrogen	Crucial remedy for women with estrogen poisoning due to the use of birth control pills. Woman's infertility	Cooper, 1990: 102; Sharma and Singh, 2022: 89

			and regains ovulation cycle caused by the usage of birth control pills	
Histaminum hydrocholoricum		Histaminum hydrocloloricum	Allergies (reduces the amount of histamine released during allergic response hence reducing the effects of histamine)	Alexander, 2012: 3; Volinsky, 2020: 199
Influenzinum	Influ.	Prepared each year from the same influenza strain that is used in the flu vaccine for the year	Flu symptoms (stimulate the body's own immune system to resist the seasonal flu strains' onset. only homoeopathic remedy that is updated each year based on the flu strain reported by the World Health Organization (WHO)	Nayak and Varanasi, 2020: 131
Lathyrus Sativus		Lathyrus Sativus	Polio immune booster	Whatcott, 2022: 2
Lyssin (hydrophobinum)		Rabies	Indicated for patients who often feel teased, tormented, hypersensitive to noise, and light, react violently	Munshi *et al.* 2022: 43
Medorrhinum	Medh.	Gonorrhea	hormonal problems, mucus production, Children's behavioral problems, sleep disorders, and eating disorders.	Mercaldo, 1999: 69; Munshi *et al.* 2021: 43
Meningococcinum		prepared from a mix of cultures of different meningococcus bacteria.	Meningitis immune booster	Nayak and Varanasi, 2020: 133
Oscillococcinum	Oscillo.	Duck heart and liver	Treating and preventing influenza-like symptoms, decreasing the duration and intensity of fever, chills, body pains	Jacobs, 2018: 159
Parotidinum	Parot.	Mumps virus	Immune booster for mumps	Birch and Whatcott, 2013: 132
Pertussin	Pert.	*Bordetella pertussis*	Whooping cough	Nayak and Varanasi, 2020: 132
Psorinum	Psor.	Scabies discharge	Skin conditions (eczema, scabies), asthma	Munshi *et al.* 2022: 3
Rubella nosode		Rubella (German measles) virus	German measles immune booster	Loeb, 2018: 7423
Staphylococcinum	Staphycoc.	*Staphylococcus*	Diseases involving *staphylococci*	Sinha and Jadhav, 2020: 172
Streptococcinum	Streptoc.	*Streptococcus pyrogens*	History of *Streptococcus pyrogens* such as strep throat, Strep B infections in pregnant women	Seul, 2018: 34
Syphilinum	Syphil.	Syphilis	Chronic diseases such as chronic asthma, constipation, painful menses	Galande, 2020: 1; Munshi *et al.* 2022: 2
Varicella Zoster nosode		Varicella Zoster virus	Chickenpox immune booster	Shah and Talele, 2019: 28
Variolinum		Smallpox virus	Smallpox immune booster. Post-herpetic neuralgia after shingles	Whatcott, 2022: 1

Directions for Use

The rules for prescribing nosodes have been explained by a variety of stalwarts and experienced practitioners of homoeopathy (Nayak and Varanasi, 2020: 129). The following is the most documented ways of prescribing nosodes:

- If the mental, generals, and physicals including the particulars (PQRS), match the homoeopathic nosode indications, the nosode can be prescribed and function as a constitutional medicine. Constitutional remedies treat the patient as a whole, physically, emotionally, and mentally, including consideration of their past medical history (Sharma and Singh, 2021: 44)

- When treating a chronic disease, the simillimum (remedy corresponding to the totality of symptoms) is no longer effective, using the nosode in a healing process eliminates a blockage to the cure, enabling the simillimum to continue working (Deep, 2020: 385)
- Nosodes are used when the simillimum does not provide relief
- Nosodes can be incorporated when the case is unclear (lack symptoms)
- Nosodes can be prescribed as a miasmatic intercurrent remedy when treating a chronic disease. Miasm is an underlying predisposition to a persons inherited disease group. A miasmatic intercurrent remedy is the homeopathic remedy used to strengthen the body from a genetic weakness that causes predispositions to certain disease processes, given between doses of the remedy indicated by the patient's complaints to further the action of the indicated remedy (Murgod and Shah, 2021: 268)
- Nosodes can be used to prevent disease (homeoprophylaxis)
- Nosodes can be given as a *Genus Epidemicus*

Despite all the homoeopathic nosodes, it is crucial to prescribe them cautiously as they can be contradicted in a case (Nayak and Varanasi, 2020: 130). Nosodes must be prescribed cautiously in cases like that indicated in Figure 2:

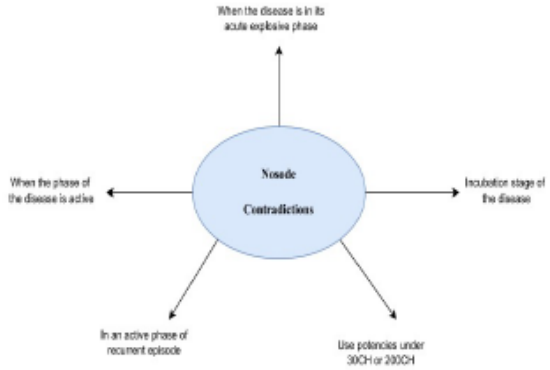

Figure 2: Nosodes must be prescribed cautiously in the following cases (Nayak and Varanasi, 2020: 131)

Preparation of Homoeopathic Nosode

Pharmacopoeia

Nosodes are prepared according to the standard prescribed in the official Pharmacopeia. Homoeopathic Pharmacopeia is an official book of standards consisting of authoritative information on homeopathic medicinal products and preparations, the main chemical properties used during identification, standards for strength, and the chemical tests used for determining purity and identity (Yapar and Ozdemirhan, 2020: 57). Most of the time, pharmacopoeias are published by an authority or government of any country. Currently, there are seven countries that have official homoeopathic pharmacopoeias. India (Homoeopathic Pharmacopeia of India, 1971-2016), Germany (German Homoeopathic Pharmacopoeia, 1825), the United Kingdom (British Homoeopathic Pharmacopoeia, 1st edition in 1870), the U.S.A. (Homoeopathic pharmacopoeia of the United States, 1897, revision 2016), France (French Homoeopathic pharmacopoeia,1897), Mexico (Mexican homoeopathic pharmacopoeia, 1998, 1st edition), and Brazil (Brazillian Homoeopathic pharmacopoeia, 2011, 3rd edition) (Valavan and Cesar, 2019: 233-235). Countries that do not have an official Pharmacopoeia are free to undertake manufacturing as per any of the recognised Pharmacopoeia from other countries (Máthé and Khan, 2022: 2). For instance, South Africa manufactures using the German Homeopathic Pharmacopoeia during the preparations of homeopathic medicines. The mentioned homeopathic pharmacopoeia differ according to the nosode preparation processes, such as number of succussions, concentration, etc. (Valavan and Cesar, 2019: 233-235) .

The only major difference is the continuous dilution of a mother tincture, according to homoeopathic laws. The centesimal method involves taking one drop of the previous potency (degree of dilution that the remedy has undergone during the preparation process) and diluting it with 99 diluent drops, then adding a drop of this into a clean vial and repeating this process many more times until the desired remedy potency is reached. At each stage, the remedy is potentised (a systematic and scientific process involving several dilutions with vigorous succussion, also known as shaking). This is vitally important and these dilutions are named potencies. Several products on public sale are 6Ch (meaning the remedy has been potentised six times, with a dilution of 1 in 100^6) or 30Ch (1 in 100^{30}). After the required homoeopathic nosode potency has been obtained, they are placed in the appropriate containers accompanied by the shelf-life package and recommended storage conditions (Frass *et al*., 2020: 1935; Dewanshu and Kumari, 2020: 156). Homoeopathic aggravation is the temporal or slight intensification of existing symptoms or the appearance of new symptoms after a dose of a homoeopathic remedy when the dose has been too large (Meena and Suman, 2020: 1). After the administration of a homoeopathic remedy sometimes symptoms may slightly worsen. This is known as homoeopathic aggravation. Aggravations are usually harmless, short-lived, and usually mild. A slight homoeopathic aggravation in the first hour after administration is considered a good indication that the acute disease or symptom will be cured. In cases where aggravations have persisted, this may be due to the patient proving the remedy, since the poorly matched remedy may bring out new symptoms. From these ideas, it is proven that homoeopathic aggravations are part of the treatment of homoeopathy and such a concept do not exist in another medical system (Sperl *et al*., 2020: 232).

Regarding chronic diseases, according to the application of Hahnemann's advanced methods, as described in the sixth edition of the Organon, the intensification of the original symptoms only must appear at the last stage of treatment when the cure is almost completed, given the accurately chosen remedy was taken in gradually increased potency and properly small and modified doses (Meena and Suman, 2020: 11). When a cure is almost completed, the vital force no longer needs medicine to continue its curative reaction. If further medicine is administered, the medicine symptoms are called into play. However, if the patient experiences a homoeopathic aggravation in their first dose in chronic disease cases, and in the same way every repeated, modified dose, this points to the fact that the dose was too large (Meena and Suman, 2020: 51). Homoeopathic aggravation occurrs because the smallest possible dose will be easily overcome by the vital force and does not prevent the disease from being cured. It is often not perceptible in patients unless they are oversensitive (§156, 283 Organon and Schuett, 2015: 2; Meena and Suman, 2020: 51). The duration and intensity of similar aggravations provides an idea of the accuracy of the chosen remedy, the patient's vital force, the prognosis, and the management of the case (Meena and Suman, 2020: 51).

Other stalwarts in homoeopathy have further investigated this event and observed that when a homoeopathic remedy is administrated, any one of the following responses is expected (Dipangkar, 2021: 451):

- Curative response – the improvement of symptoms
- Similar aggravation – At first the symptoms worsen, followed by improvement in symptoms.
- A dissimilar aggravation – New symptoms appear for a brief time while the old ones stay the same.
- Accessory symptoms – There is improvement in the symptoms while a new symptom appears in the process for a brief period.
- Return of old symptoms – There is improvement in the existing symptoms while the old symptoms from the past return for a brief period.
- An eruption appears for a brief time.
- Nothing happens

Use of Nosodes as Homoeoprophylaxis

Provided the difficulties in understanding how homoeopathy may work, researchers have concentrated on finding whether Homoeopathic nosodes are placebo treatments. Current evidence suggests that homoeopathy is not completely a placebo effect, however, more clinical trials and studies are required to strengthen this point of view (Raza, 2019: 1). A randomised, double-blind, placebo-controlled prophylaxis study was done in COVID-19 exposed participants in Mumbai, India. There were six groups and each group was treated with one of the following: Arsenicum album 30CH, Bryonia alba 30CH, a combination (consist of Arsenicum album 30CH, Bryonia alba 30CH, Gelsemium sempervirens 30Ch, and Influenzinum 30CH), coronavirus nosode CVN01 30CH, Camphor 1M, or placebo. The results demonstrated that good rates of recruitment and retention were achieved. Of the 4497

quarantined participants, 2233 participants completed the trial. Participants who were randomised to receive either the coronavirus nosode or Bryonia album, which showed a lower incidence of laboratory-confirmed COVID-19 and a shorter period of disease, along with fewer hospitalisation evidence than those who were administered a placebo. The three other groups (Arsenicum album 30CH, Camphora 30CH and a combination) did not show signs of efficacy (Talele *et al*., 2022: 2).

In March 2020, the COVID-19 outbreak was declared by the World Health Organization (WHO) as a pandemic. An open-label randomised study was done to assess the immune-boosting ability of a homoeopathic, therapeutic treatment including Tuberculinum 1M, Zincum metallicum, 6CH, Chininum arsenicosum 6CH, and Calcarea Phos 6XH in asymptomatic carrier of the coronavirus disease (COVID-19) in a group of high risk individuals. There were two groups. Group A (intervention group) participants were healthcare workers and their family members from the hospital at which the study was conducted (Father Muller Hospital). Group B was the control group which consisted of a high-risk participant in the age range from 14 to 60 with severe comorbidities (pregnant, end stage renal disease) (Dikshit *et al*., 2022: 85). The results showed significant effects observed in the strata of those aged 21 to 30 in the completion of treatment ($p<0.01$). The healthcare workers group showed statistically significant results in terms of other factors. In addition, the study used tests to positivity rate the methods used to monitor the testing efficacy and evaluate the disease activity status in the provided population (Centers for Disease control and Prevention (CDP)). It indicated that the treatment has potential antiviral activity (Dikshit *et al*., 2022: 85).

An observational study was undertaken between the 20th of January and the 20th of May 2021 on 1397 COVID-19 positive participants to determine the clinical-symptomatic profile of the vaccine breakthrough against COVID-19 infections and participants who were administered homoeopathic treatments.The observations were conducted against the data of participants treated with Homoeopathic medicines who confirmed the breakthrough infection standard, with a positive infection for more than or equal to 14 days after completion of both the suggested doses of an authorised COVID-19 vaccine. The study used IBM SPPSS statics 21.0 to analyse the descriptive data. Homoeopathic remedies that were found to be clinically effective in countering the adverse effects following vaccination included Acidum phosphoricum, Thuja occidentalis, Antimonium tartaricum, Pulsatilla nigricans, Aspidosperma, Sulphur, and nosodes (Typhoidinum, Influenzinum and Vaccinum) (Wadhwani and Chadha, 2022: 4). In addition, A total of 73 cases were found to be vaccine breakthrough infections out of the 1397 COVID-19 positive participants. The median recovery time observed in the data set was found to be approximately nine days. There were five participants that dropped out of the study. A total of 93.5 percent of patients responded well to the remedies. The percentage of patients that recovered completely with normal HRCT chest or serological markers was 75.34 percent. There were 29 (39.72) patients with mild clinical manifestations, 26 (35.61%) with moderate manifestations, 17 (23.28%) were severe, and one (1.36%) was critical. Ten homoeopathic medicines were prescribed to 73 participants that had adverse effects post vaccination. Many patients obtained an ORIDL score of four. A WHO clinical progression score of three was reported by the maximum number of participants (Wadhwani and Chadha , 2022: 11).

An experimental study done on nosodes prepared from the P. berghei parasite. Mice were infected with the P. berghei parasite. The study results demonstrated that the nosodes produced considerable activity. The mixture nosode exhibited an inhibition percentage of 71.42 percent (3D7) and 68.57 percent (RKL-9). The cell-free parasite nosode exhibited 62.85 percent (3D7) and 60 percent (RKL-9). The infected RBC'S nosode showed 60.61 percent inhibition (3D7) and 57.14 percent (RKL-9). On the mixture nosode-treated group, the biomarker levels of the liver and kidney were within the normal range. Cytokines showed an elevated IL-4 (interleukin 4) and IL-10, while there was a decline in IL-7 and IFN-Y in the group treated with a mixture nosode. As a result, it was concluded that the mixture nosode showed promising antimalarial activity in P. Falciparum and P. berghei (Suri *et al*., 2021: 121). An *invitro* study was performed by Munshi *et al*. in 2022 where the polyvalent nosode efficacy was tested using the minimum inhibitory concentration assay. The nosodes tested were Candida albicans (30CH, 100CH), Neisseria gonorrhoeae (35CH), Klebsiella pneumoniae (35CH,100CH), Escherichia coli (35CH, 100CH), and Salmonella typhi (30CH, 100CH) along with the positive and negative controls. Each nosode was tested against the infection it causes and cross-infections. The results revealed that the tested nosodes produced antibacterial potential against corresponding micro-organisms and against other selected organisms tested using this essay. On C. albicans species, the C. albicans polyvalent nosode (35CH. 100CH), N. gonorrhoeae nosode (35CH), and amphotericin B (positive control) exhibited growth inhibition. On K. pneumoniae species, K. pneumoniae (35CH), E, coli polyvalent nosode (100CH), and meropenem (positive control) showed inhibition of growth. However, this effect was not found on the three positive controls (ceftriaxone, ofloxacin, and amoxicillin antibiotics). The E. coli

polyvalent nosode (30CH) and positive controls (include ciprofloxacin, ofloxacin, and amoxicillin) exhibited inhibition of the growth of E. coli. The S. typhi polyvalent nosode 30CH and the positive controls (ciprofloxacin and ofloxacin) displayed inhibition of growth of S. typhi species for 24 hours. However, the effect did not last further than 48 hours. The N. gonorrhoeae polyvalent nosode could not produce inhibition on the growth of N. gonorrhoeae species. The positive controls (ceftriaxone and ofloxacin B) also could not exhibit inhibition on the growth of N. gonorrhoeae organisms. (Munshi *et al*., 2022: 42).

Worth noting is that a stringent biosafety-compliant environment is required when producing homoeopathic nosodes. Minimum handling and using sealed containers and disposable auto-tip pipettes is always recommended. The safety of nosodes in different potencies must be established according to sterility testing, as described in the Indian Pharmacopoeia or European Pharmacopoeia for aerobic and anaerobic organisms. Sterility testing results show the presence and the source organism growth in potencies such as 1C, 4C, and 6C. Any potency under 6X is not supposed to be dispensed, as well as potencies higher than 6X which must be sterile as per the Homoeopathic Pharmacopeia of India (Mashru *et al*., 2017:2585; Suri *et al*., 2021: 121). It is required to document the heat inactivation of pathogens if done. However, the Homoeopathic Pharmacopeia does not suggest inactivation (Mashru *et al*., 2017:2586). For cases where phlebotomy is performed on volunteers, they must be provided with an informed consent form stating the proposed use of the sample. According to ethical guidelines for biosafety, the viral safety of human blood plasma products, clinical research, and safety issues in the preparation of homoeopathic medicines, these process must be done accurately (World Health organization, 2009: 28). In 1964, the guidelines for biosafety in the form of the Laboratory Biosafety Manual and clinical research in the form of Declaration of Helsinki were developed by the World Health Organization (Mathur *et al*., 2019: 215).

The Right Dosage

Treating chronic illnesses (diseases that have been present for an extended period such as arthritis) with homoeopathic nosodes should be done with low potencies (such as 6x or 6C) and in acute conditions (new symptoms or diseases such as fever) treated with high potencies (such as 30CH-200CH). An exception includes cases where an accident is an etiology of the symptoms; the body might be given a kick start with a high potency dose, such as Arnica 200CH, at first followed by a lower potency remedy (Khatta and Srivastava, 2021: 36). The homoeopathic sensitivity of the patient is another factor that is required to be considered when choosing a remedy potency (hypersensitive patients take lower potencies). It is also crucial to consult with a homoeopath before taking a particular remedy, especially when in doubt. In cases where there is uncertainty in the remedy to use, a low potency, such as 6X, below 30CH or 30CH, should be used before a patient is given a high potency remedy and the patient should be observed. Then, in case the patient's symptoms improve but not fully, the remedy should be repeated but now in a higher potency (such as 200CH). In cases after using the low potency of a particular remedy where no improvement is observed or there are changes in the condition, the remedy used should be changed and a different remedy should be taken in low potency. High potencies, including 200CH and 1M, are normally taken in exceptionally low doses (typically once per week), whereas lower potencies (such as 6CH, and 30CH) can be taken every few hours for a few days (Khatta and Srivastava, 2021: 36).

Homoeopathic medicines are to be taken on a clean palate. All strong flavors including coffee, vicks, mint camphor, or toothpaste can interrupt the remedys' functions. Once relief from the symptoms is felt, use of the medication should cease. The patient can take the remedy again if the exact same set of symptoms flares up again and stop taking homoeopathic medicine once symptoms are cured. Only take it again if the same symptoms come back. If symptoms or conditions persist or worsen, consult a homoeopathic doctor (Khatta and Srivastava, 2021: 36). Homoeopathic nosodes and other non-nosode remedies are normally administrated in the form of pills, granules, globules, powders, and liquids, which are placed under the tongue. In low potencies, you can take two tablets every two hours for the first six doses, and then four times daily for up to five days. For some diseases, homoeopathic remedies are taken as an ointment, for example arnica cream which is applied directly to bruising. Avoid contact with the skin when taking the remedy in pill or granule form (including fingers to prevent contamination). A few granules or globules are dropped into the lid and poured directly into the mouth (Khatta and Srivastava, 2021: 36).

Conclusion

Towards the end of the 20th century, the use of homoeopathy lessened because of advancements in the conventional system. Despite this, in conjunction with conventional medicine, homoeopathy has been shown to

have more added advantages when it comes to the effective treatment of different diseases. Many different trial studies have revealed the effectiveness of this isopathy approach of homoeopathy, However, the absence of a standardisation of the source material and direct proof of effectiveness for human beings causes concerns and controversies around homoeopathy. The quantity of peer-reviewed homoeopathic nosode research is smaller than that of conventional medicinal research. Most significantly for global health, studies such as those presented in this article demonstrate that patients taking homoeopathic nosodes and following sensible disease avoidance measures may avoid infection or may be able to reduce reliance on conventional medication, including antibiotics, hence, reducing the prevalence of antibiotic resistances. In the meantime, the most important evidence still arises from practical clinical experience and from the successful treatment of millions of patients. A gap that needs to be filled in homoeopathic nosode research includes a high-quality study, such as multi-site research with multiple replications of the same approach for the treatment of the same medical conditions on a larger scale sample and with more practical applicability to clinical trials. This will improve publicity and reproducibility, build credibility in the healthcare system, and attract a large population of patients and professional practitioners toward this complementary treatment.

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Appendix 14: Proof of submission to Current Trends in Biotechnology and Pharmacy

Re: SUBJ: Submission Confirmation Inquiry

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Mon 2024/09/09 06:25

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Appendix 15: Editing certificate

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EDITING CERTIFICATE

Re: **Nokwanda Dudu Zulu (21703712)**
DUT master's dissertation: **Evaluation of the effectiveness Of *Streptococcus pneumoniae nosode* (6CH, 9CH, 30CH, and 200CH) on the growth of *Streptococcus pneumoniae***

I confirm that I have edited this dissertation and the references for clarity and language. I returned the document to the author with track changes so correct implementation of the changes and clarifications requested in the text and references is the responsibility of the author. I am a freelance editor specialising in proofreading and editing academic documents. My original tertiary degree which I obtained at the University of Cape Town was a B.A. with English as a major and I went on to complete an H.D.E. (P.G.) Sec. with English as my teaching subject. I was a part-time lecturer in the Department of Homoeopathy at the Durban University of Technology for 13 years and supervised many master's degree dissertations during that period.

Dr Richard Steele
26 September 2024
per email