

Research Article

Anti-Bacterial Activity of Homoeopathic Nosodes *Anthracinum*, *Pyrogenium* and *Variolinum* in 30 C, 200 C and 1 M Potencies Against Methicillin Resistant *Staphylococcus Aureus* - An *In Vitro* Study

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A B S T R A C T

Introduction: The homoeopathic system of medicine is still considered as pseudoscience by the scientific community due to the lack of material substance in highly diluted medicinal formulations, despite clinical results. On the other hand, biomedicine is threatened by antimicrobial resistance against various pathogens. Methicillin-Resistant *Staphylococcus aureus* (MRSA), a virulent strain of the bacteria *Staphylococcus aureus*, is resistant to several antibiotics like methicillin, nafcillin, oxacillin, and cephalosporins. In this study, homoeopathic nosodes also called biotherapeutic preparations, which are homoeopathic preparations made from pure microbiological cultures isolated from sick tissue and clinical samples like secretions, discharges, etc., were tested against MRSA to understand its antibacterial effects.

Method: Homoeopathic nosodes *Anthracinum*, *Pyrogenium* and *Variolinum* each in three different potencies namely 30 C, 200 C and 1 M were tested against MRSA by standardised Kirby-Bauer disk diffusion technique. Antibiotic vancomycin and dispensing alcohol were used as positive and negative control respectively.

Result: After the study, it was found that homoeopathic nosode *Pyrogenium* 200 C, *Pyrogenium* 1 M and *Anthracinum* 200 C showed maximum inhibition zones of 11 mm, 10 mm and 10 mm respectively.

Conclusion: The results of this study indicate the efficacy of highly diluted homoeopathic medicines against the most virulent and threatening MRSA.

Keywords: Homoeopathy, Methicillin-Resistant *Staphylococcus aureus* (MRSA), Nosodes, *Anthracinum*, *Pyrogenium*, and *Variolinum*, Kirby-Bauer Disk Diffusion

Introduction

Dr Christian Fredrick Samuel Hahnemann discovered the homoeopathic medical system more than 200 years ago, since then, it has spread its roots around the globe. Due to the lack of material substance in highly diluted medicinal formulations, it is still considered as pseudoscience despite clinical results.¹ On the other hand, in this era of biomedicine² resistance to antibiotics is dangerously increasing all over the world. As a result, the treatment of numerous infections like pneumonia, food poisoning, septicemia, tuberculosis and so on remains questionable.³

Staphylococcus aureus is a gram-positive bacteria found in the environment and also an inhabitant of the nasal mucosa and skin of healthy human beings (30%).⁴ This does not cause infection on healthy skin, but if allowed to reach the circulation or internal tissues, may result in a number of potentially harmful infections. Methicillin-Resistant *Staphylococcus aureus* (MRSA), a virulent strain of the bacteria *Staphylococcus aureus* is resistant to several antibiotics like methicillin, nafcillin, oxacillin, and cephalosporins.⁵ The most prevalent diseases caused by MRSA are skin and subcutaneous tissue infections namely diabetic foot ulcers, cellulitis and necrotising fasciitis. Deep-seated infections including empyema, lung abscess, pneumonia, meningitis, and osteomyelitis, which pose a threat to modern biomedicine can also be caused.⁶ In this study, nosodes also known as biotherapeutic preparations, which are homoeopathic preparations made from pure microbiological cultures isolated from sick tissue and clinical samples like secretions, discharges, etc. are tested against MRSA to understand its antibacterial effects.⁷ *Anthraxinum* is prepared from the spleen of cattle infected with anthrax disease, *Pyrogenium* is prepared by allowing the lean beef to decompose and drying up in the sun for two weeks and *Variolinum* is prepared from the lymph from cowpox/smallpox pustule.⁸

Materials & Methods

Selection of Samples

- The homoeopathic nosodes *Anthraxinum*, *Pyrogenium* and *Variolinum* each in three potencies 30 C, 200 C and 1 M, used in the study were procured from the Reckeweg brand, which is the number one German homoeopathic brand available in India.
- Vancomycin 500 mg diluted in 1000 ml water and dispensing alcohol were used as the positive control (PC) and negative control (NC), respectively.
- MRSA strain was obtained from a patient sample from PRS hospital, Trivandrum.
- Mueller-Hinton agar culture medium purchased from

high media and plain Whatman Filter Paper disc of 6 mm diameter.

Study Setting: Sarada Krishna Homoeopathic Medical College Research Laboratory, Kulasekharam

Study Design: Experimental *in vitro* study

Method of Collection of Data: Standardised Kirby-Bauer disk-diffusion method

Study Duration: The study was conducted from June 8, 2023 to June 9, 2023.

The approval for the study was obtained from the institution's research department.

Methodology

Preparation of Media

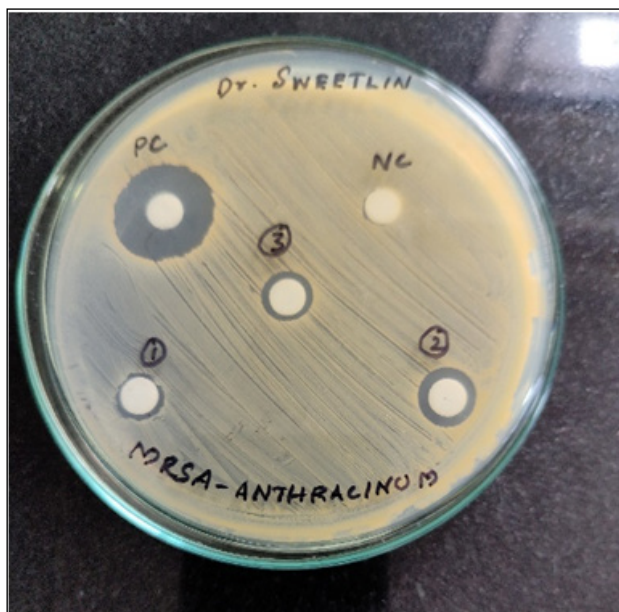
38 g of Mueller Hinton Agar Medium purchased from Hi Media was dissolved in 1 L of water and was autoclaved at 121 °C for 15 min. After cooling down, 25 mL of the media was poured into each Petri plate.

Inoculation of the MH Plate Using a sterile swab dipped in the MRSA inoculum tube, three MH plates were streaked with MRSA in a back-and-forth motion. Five Whatman Filter Paper discs of 6 mm diameter were placed in each of the three Petri plates named plates 1, 2 and 3. Vancomycin used as positive control (PC) was prepared in the concentration of 5 mcg/μL of distilled water. 10 μL of vancomycin (PC) and 10 μL of dispensing alcohol used as negative control (NC) were loaded in the respective filter paper in each of the three plates.

In plate 1, 10 μL of homoeopathic medicines *Anthraxinum* in 30 C, 200 C, and 1 M were loaded in the remaining three discs. In plate 2, 10 μL of homoeopathic medicines *Pyrogenium* in 30 C, 200 C, and 1 M were loaded in the remaining three discs and in plate 3, 10 μL of homoeopathic medicines *Variolinum* in 30 C, 200 C, and 1 M were loaded in the remaining three discs. After this, all three plates were placed in an incubator, and the temperature was set at 37 °C for a period of 24 hours. After incubation, all three plates were examined for zones of inhibition around each disc, their diameters were measured using a transparent ruler in millimetres.

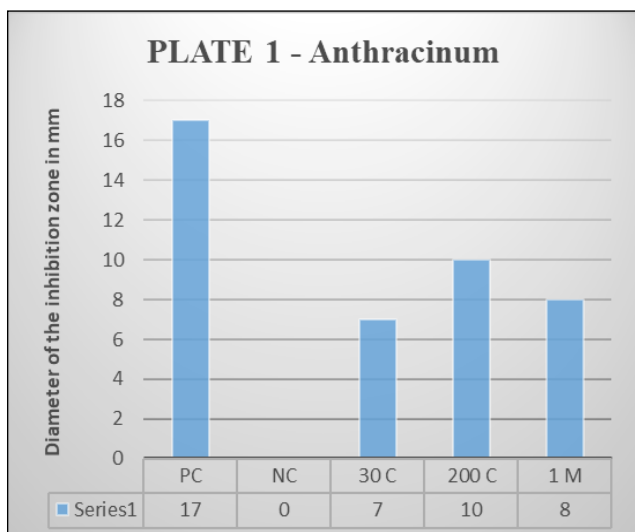
Results

In plate 1, all three potencies of *Anthraxinum* 30 C, 200 C, and 1 M showed antibiotic activity with zones of inhibition of around 7 mm, 10 mm, and 8 mm respectively. The activity was high in 200 C potency when compared with others. Positive control showed 17 mm and negative control showed no activity (Figures 1 and 2).



PC: Vancomycin, NC: Dispensing Alcohol, 1: Anthracinum 30 C, 2: Anthracinum 200 C, 3: Anthracinum 1 M

Figure 1. Plate 1 - Anthracinum



PC: Vancomycin, NC: Dispensing Alcohol

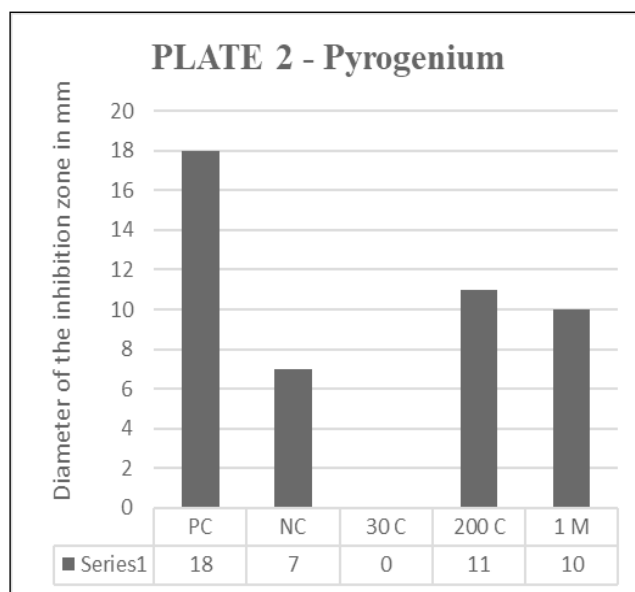
Figure 2. Diameter of the Inhibition Zone (mm) in Plate 1 - Anthracinum

In plate 2, *Pyrogenium* 200 C and 1 M potency showed antibiotic activity with zones of inhibition of 11 mm and 10 mm, respectively, whereas 30 C potency showed no activity. Positive control showed 18 mm and negative control showed 7 mm (Figures 3 and 4).



PC: Vancomycin, NC: Dispensing Alcohol, 1: *Pyrogenium* 30 C, 2: *Pyrogenium* 200 C, 3: *Pyrogenium* 1 M

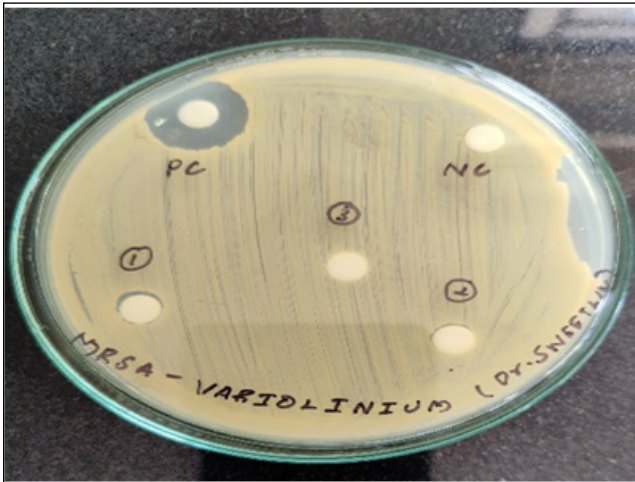
Figure 3. Plate 2 - *Pyrogenium*



PC: Vancomycin, NC: Dispensing Alcohol

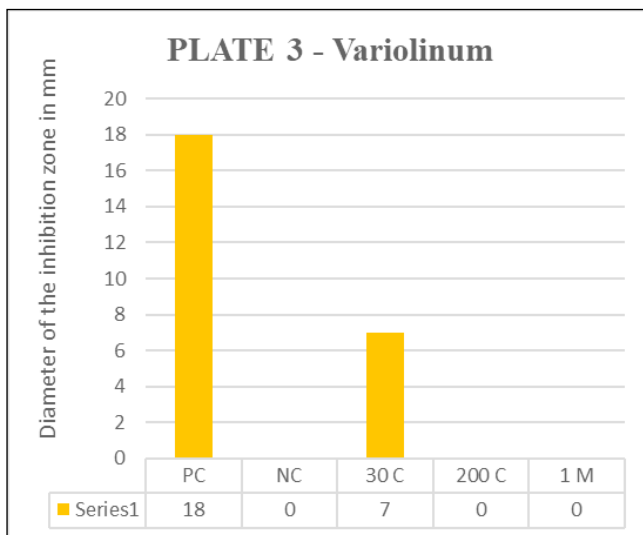
Figure 4. Diameter of the Inhibition Zone (mm) in Plate 2 - *Pyrogenium*

In plate 3, only *Variolinum* 30 C potency showed a minimal activity of 7 mm, and all the others showed no activity. Positive control showed 18 mm, and negative control showed no activity (Figures 5 and 6).



PC: Vancomycin, NC: Dispensing Alcohol, 1: *Variolinum* 30 C, 2: *Variolinum* 200 C, 3: *Variolinum* 1 M

Figure 5. Plate 3 - *Variolinum*



PC: Vancomycin, NC: Dispensing Alcohol

Figure 6. Diameter of the Inhibition Zone (mm) in Plate 3 - *Variolinum*

Discussion

MRSA is one of the most difficult-to-treat bacteria in biomedicine due to its wide variation in genomic strains and increasing resistance to common antibiotics. Vancomycin is the drug of choice against MRSA, but because of its haphazard use 'vancomycin-intermediate sensitive *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA)⁴ are evolving. In a study conducted using clinical specimens from south India, 6.08% of strains were resistant to vancomycin.⁴ The standard zone of diameter for vancomycin (30 mcg) sensitivity against MRSA is 12 mm, whereas moderate sensitivity is present when the diameter zone of inhibition is between 10–11 mm and 9 mm and below, which indicates antibiotic resistance.⁹ In this study, *Pyrogenium* 200, *Anthracinum* 200, and *Pyrogenium*

1 M potencies showed antibacterial activity with zones of inhibition of 11 mm, 10 mm, and 10 mm, respectively which comes in the range of moderate sensitivity of the antibiotic vancomycin. This indicates the efficacy of highly diluted homeopathic medicines against the most virulent and threatening MRSA.

Limitations

From this research, it is evident that homeopathic medicines are not placebos. However, the identification of active compounds present in the homeopathic formulations and how homeopathic medicines work to inhibit the growth of MRSA *in vitro* have not been evaluated in this study, which should be subjected to further investigations. The homeopathic system of medicine is based on certain laws and principles. Though this system is used by millions of people around the world, the scientific community still considers it a controversial topic. This is partly due to the inconsistency of its laws with fundamental scientific concepts and partly due to the absence of any detectable active ingredient in highly diluted preparations.¹⁰

Conclusion

Antibacterial activity of homeopathic nosodes *Pyrogenium* 200, *Anthracinum* 200, and *Pyrogenium* 1 M have been proved through this *in vitro* study. However, further research in experimental model organisms to substantiate the scientificity of homeopathic medicines is recommended.

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Conflict of Interest: None

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