

In-Vitro Study of Antimicrobial Activity of Homoeopathic Preparations against *Staphylococcus aureus*

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ABSTRACT

There has been rapid emergence of antibacterial resistance in the past two decades showing rapid shift in epidemiology of *Staphylococcus aureus* infection. The current study aimed at screening of various homoeopathic dilutions for their antimicrobial activity against *S. aureus*. The antibacterial activity of homoeopathic high dilutions Staphylococcinum in 30C, 200C and 1M potency and Lachesis and Echinacea each in 6C, 12C, 30C, 200C and 1M potencies was assessed on clinical isolates of *S. aureus* using 96 well plate assay. Statistically significant reduction in the growth of *S.aureus* was observed by Lachesis and Staphylococcinum nosode in all of the selected potencies as compared to 90% alcohol ($p<0.05$). However, Echinacea did not show statistically significant result. Further studies may be appropriate for more confirmatory activity of Staphylococcinum nosode and Lachesis against *S.aureus* using in-vivo models.

Keywords- Anti-bacterial, in-vitro, Homoeopathic Medicines, Staphylococcinum, Lachesis, Echinacea, *Staphylococcus aureus*.

INTRODUCTION

The increasing global burden of infectious diseases is a major cause of mortality and morbidity causing significant impact on health of communities across the world. The imprudent use of the antibiotics leading to the rapidly developing resistance makes the bacterial infections a threat throughout the world. The antibiotic resistant infections cause significant burden on the countries with already overburdened healthcare system. The modern therapy strategies have been rendered precarious as well as expensive by the resistant organism. [1,2]

Staphylococcus genus is among the most antibiotic resistant bacteria. *S. aureus* is an ovoid or spherical, non-motile, non-

spring Gram positive bacterium arranged in grape like clusters. [3] It is a common commensal as well as pathogenic bacteria, and is colonising approximately 30% of healthy human population. The virulence of *S. aureus* is very high and it the most common factor responsible for nosocomial infections. It is indicated that its prevalence will continuously rise because of increasing antibiotic resistance. Its resistance to multiple antibiotics is associated with vast disease burden worldwide. *S.aureus* is responsible for various clinical infections and can involve any organ or system in the human body. This pathogen is the most common etiological factor responsible for bacteraemia as well as infective endocarditis, there is vast range of

infections caused by it like suppurative affections, surgical infection, septic shock, septic arthritis, nosocomial infection. The mortality rate associated with *S.aureus* is particularly high among the vulnerable patient group. [4-6]

Considering the great virulence of *S.aureus*, its broad spectrum of infections and ability to cause great morbidity and mortality, the biggest challenge in front of the medical community is to have strong novel strategies to deal with this threat as the conventional therapies are alarmingly failing. Homoeopathy is holistic way of healing based on principle "like cures like". There is widespread increase in use of homoeopathy and it can serve as good alternative option with greater cost effectiveness and lesser side effects as quoted by Dr. Zhang (WHO's medical officer) for traditional medicine at an international homoeopathic medical congress 1994, the homoeopathic medical substances are extremely diluted so there is no doubt regarding their safety, yet there have not been verification of mode of action of homoeopathy in terms of modern evidence based system. There are several proposals by WHO for the improvement of the homoeopathic science out of which development of basic research on the homoeopathic medicines is one way for the establishment of possible mode of action and scientific proving of the effect of homoeopathic medicines. [7,8]

In this study homoeopathic medicines Staphylococcinum nosode, Lachesis and Echinaecea have been selected on the basis of repertorisation (synthesis Repertory 9.1 volume). These medicines are used for treatment of symptomatology similar to those produced by *S.aureus*. Through the present study, antibacterial effect of these medicines in different potencies has been assessed.

MATERIALS AND METHOD

Media and chemicals- All media and reagents were procured from Hi Media, Mumbai, India.

Homoeopathic medicines- Homoeopathic medicines Lachesis and Echinaecea each in 6C, 12C, 30C, 200C and 1M dilution and Staphylococcinum nosode in 30C, 200C and 1M dilution were obtained from GMP certified homoeopathic manufacturing unit.

Procurement of test Organism- 30 different Clinical isolates of *S.aureus* were obtained from Bharati charitable hospital, Pune and were maintained on MHA slants in refrigerator.

96 well microtiter plate assay [9,10]

Antibacterial activity of selected homoeopathic medicines was determined using 96 well plate assay, each well containing total volume of 250µl, Mueller Hinton broth and culture as negative control and dispensing alcohol (ethanol 90%) as vehicle control were used. Using micropipettes, various potencies of the homoeopathic dilutions (100µl), bacterial culture (20 µl) and Mueller Hinton broth media (130µl) were poured down in first five wells. In one well of each row, 230µl of media and 20µl of culture was taken. For vehicle control, volume adjusted was 130µl of media, 20µl of culture and 10µl of dispensing alcohol. One well of each row was poured with 150µl of media and 100µl of dispensing alcohol. Each experiment was conducted in replicates. Each plate was incubated for 48hrs at 37°C and after the incubation period, the O.D of bacterial growth was measured by Elisa Reader (BIORAD) at 600nm wavelength to assess the antibacterial activity of the homoeopathic dilutions. Statistical analysis of the obtained data was done by using Graphpad software, Prism Version 5.

Antibiotic sensitivity testing-

Antibiotic sensitivity testing was done using cephalixin antibiotic disc (30µg) on petri plates prepared using agar- agar and Mueller Hinton broth and *S.aureus* culture. Zone of inhibition were measured in mm using scale after the incubation of the petri plate for 24 hours at 37°C.

RESULT

Antibiotic sensitivity testing –

The zone of inhibition of cephalixin antibiotic disc against *S.aureus* was found to be 17 mm in diameter. As per the standard chart for zone of inhibition, for Cephalixin disc content of 30µg, all test organisms are considered to be resistant at GIZ ≤14, intermediate at 15-17 and sensitive at GIZ ≥18 (Zone diameter nearest whole mm). [11]

96 well plate assay-

In this study, the effects of Staphylococcinum, Lachesis and Echinacea were observed on the bacterial growth inhibition. Statistically significant result (p-value < 0.05) was obtained with Lachesis in 6C, 12C, 30C, 200C and 1 M potency and Staphylococcinum in 30C, 200 C and 1M potency. The percent growth inhibition of the cells exposed to the medicines in all the potencies as compared to ethanol control (90%) was calculated. [12]

No statistically significant result was obtained with Echinacea. This study was aimed at assessing whether the selected homoeopathic dilutions have any antimicrobial activity against *S.aureus* using in -vitro model.

Minimum Inhibitory concentration –

Significant inhibitory results were obtained with Staphylococcinum nosode and Lachesis in all potencies.

Table 1-MIC of the Homoeopathic Medicines-

MEDICINE	MIC value of Homoeopathic potency
Staphylococcinum	200C
Lachesis	12C

DISCUSSION

The extreme injudicious use of antibiotics has led to the drastic change in the prevalence of the infectious disease. The modern therapy is more expensive and its effectiveness is being threatened because of the multidrug resistant strains of the microbes. Especially in the context of the developing nations, the more expensive antibiotics are not easily available to all the classes. The frequency of resistance has risen due to increased use of the antimicrobial drugs easily available over the counter. *Staphylococcus aureus* is colonizing humans as a natural reservoir, both asymptotically and infectious

colonization with a vast clinical manifestation and has been included under high priority in the WHO priority pathogen list for the research and development of new antibiotics. [13] This study focuses on the basic research in Homoeopathy that can be a potential alternative in the treatment of vast medical conditions caused by the pathogen, and is an attempt to make the holistic science of homoeopathy more evidence-based. This study was aimed at screening of antimicrobial activity of homoeopathic medicines against *S.aureus* using 96- wellled plate assay and showed the effectiveness of homoeopathic medicines in inhibiting the bacterial growth.

CONCLUSION

Homoeopathy is the science of holistic healing and has immense scope as the alternative treatment option for the various medical conditions. In this study it was observed that homoeopathic medicines have antibacterial activity. Statistically significant result (p-value <0.05) was obtained with all the potencies of Staphylococcinum nosode and Lachesis, however the best antibacterial activity was observed by the homoeopathic medicine Staphylococcinum in 200C potency and Lachesis in 12 C potency. The medicines can be a good alternative of antibiotics for the pathogen *S.aureus*. Further confirmatory studies through in vivo models are suggested.

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