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Short Communication

Antibacterial Activity of Homoeopathic Medicine Hepar Sulphuricum 200 C & 1 M against Lung Pathogens - *In Vitro*

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A B S T R A C T

Introduction: Antibiotic resistance is one of the most significant global health problems. It occurs when bacteria adapt to the use of antibiotics, leading to resistance. Certain bacterial strains have developed resistance to nearly all available medications, necessitating the discovery of new antibacterial drugs.

Objective: To evaluate the antibacterial activity of homoeopathic medicines using zone of inhibition (ZOI) analysis against specific bacterial strains

Methods: The study involved the preparation of broth cultures of the targeted organisms, allowing colonies to form. The ZOI and minimum inhibitory concentration were assessed for each sample against *Staphylococcus aureus* and *Klebsiella pneumoniae*. *Hepar sulphuricum* 200 C and 1 M were tested for their antibacterial activity. Dispensing alcohol was used as a vehicle control.

Results: Hepar sulphuricum 200 C and 1 M exhibited significant antibacterial activity against both *Staphylococcus aureus* and *Klebsiella pneumoniae.* The ZOI was measured, confirming the antibacterial effect of the homoeopathic medicine.

Conclusion: The study demonstrated that the homoeopathic medicine *Hepar sulphuricum* 200 C and 1 M has an effective antibacterial effect against *Staphylococcus aureus* and *Klebsiella pneumoniae*, providing a potential alternative in combating resistant bacterial strains.

Keywords: Hepar sulphuricum, homoeopathy, antibacterial activity, *Staphylococcus aureus*, *Klebsiella pneumoniae*, zone of inhibition, short communication

Introduction

When bacteria, fungi, and other microbes develop resistance to the effects of drugs intended to remove them, this is known as antimicrobial resistance. Treatment for infections that are resistant to treatment can be difficult or perhaps impossible. Antimicrobial resistance, which was linked to at least 1.27 million deaths worldwide and about 5 million deaths in 2019, is an urgent threat to public health everywhere. Approximately 2.8 million antibiotic-resistant diseases occur in the US each year. According to the CDC's 2019 Antibiotic Resistance (AR) Threats Report, over 35,000 people die as a result.[1]

Gram-negative, encapsulated, and non-motile, *Klebsiella pneumoniae* is a member of the *Enterobacteriaceae* family. Gram-positive *Staphylococcus* bacteria are ovoid or spherical, non-motile, non-sporing, and grouped in clusters that resemble grapes. Both are linked to a significant global illness burden and resistance to certain drugs.[2] These bacteria cause a wide range of illnesses, including nosocomial infections, surgical infections, shock from septicaemia, arthritis caused by septicaemia, and bacteraemia. It is also the most frequent aetiological factor responsible for infective endocarditis. There are two types of pneumonia brought on by *K. pneumoniae*: pneumonia acquired in a hospital or pneumonia acquired in the community. Severe pneumonia (lobular or lobar), enteritis, UTIs, and various septic diseases such as sinusitis, meningitis, and otitis are caused by *K. pneumoniae*.[3] According to Dr Zhang, the WHO's Medical Officer, homoeopathy is becoming more and more popular and can be a suitable alternative option with lower side effects and more cost-effectiveness. The safety of homoeopathic medicinal ingredients, which are very diluted for use in traditional medicine, was confirmed in 1994 at the International Homoeopathic Medical Congress. However, the mechanism of action of homoeopathy has not been confirmed using a contemporary evidence-based system. Here, homoeopathic medicine *Hepar sulphur* 200 C and 1 M has been chosen on the basis of repertorisation by Kent repertory (repertory is a tool to find medicines according to the symptom similarity). It is frequently used for the treatment of symptoms that are similar to those produced by *S. aureus* and *K. pneumoniae*. Through the current study, the antibacterial effect of this medicine in 200 C and 1 M potencies has been assessed and evaluated.

Materials and Methods

Type of Study

In vitro experimental study.

Design

Laboratory-based antimicrobial susceptibility testing using standard microbiological techniques.

Methods Used

Kirby–Bauer disc diffusion method to assess zones of inhibition (ZOI).

Broth dilution method to determine Minimum Inhibitory Concentration (MIC).

Test Organisms

Standard reference strains of Staphylococcus aureus and Klebsiella pneumoniae.

Interventions

Homoeopathic preparations Hepar sulphuricum 200 C and 1 M (procured from Schwabe India).

Control

Dispensing alcohol (vehicle control).

Sample Size

The study used standard laboratory strains of two bacterial species:

Staphylococcus aureus (1 strain)

Klebsiella pneumoniae (1 strain)

No human or animal subjects were involved.

Bacterial Strains

Standard strains of *S. aureus* and *K. pneumoniae* were used for this study. The cultures were purchased from NABL-accredited Lab-Vivek Laboratories, Nagercoil, Kanniyakumari District, Tamil Nadu.

Homoeopathic Medicine

In this study, homoeopathic medicine *Hepar sulph* 200 C and 1 M was purchased from Schwabe India.

Replicates

While the exact number of replicates per test is not explicitly stated, standard microbiological practice (implied by use of ANOVA with df = 27 within groups) suggests at least triplicate testing for each condition (potency and concentration), totaling 30 observations (as indicated by total df = 29 in ANOVA table).

Study Duration

The manuscript does not specify the exact months and years of the study conduct. However, based on standard academic timelines and the absence of future-dated references, it is reasonable to infer the study was likely conducted shortly before manuscript submission (possibly in 2024 or early 2025).

Preparation of Disc

A plain, sterile disc was bought from Himedia, immersed in extracts at all concentrations, and allowed to air dry for

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six hours at room temperature. The disc paper was then marked and put to use in the investigation.

Mueller–Hinton Agar (MHA)

The broth culture of *S. aureus* and *K. pneumoniae* was prepared in nutrient broth. After that, a bacterial lawn was prepared in a Petri dish using Muller–Hinton agar media. The colonies were allowed to be formed, following which, the changes surrounding medium and the bacterial growth were assessed. After the formation of colonies at this juncture, these colonies were administered homoeopathic medicine *Hepar sulph* 200 C and 1 M.

The Petri dish with bacterial colonies and the medicated disc of different potencies with alcoholic control were incubated at 37 °C for 24 hours. The zone of inhibition's dimensions, including the disc, were expressed in millimetres (mm). It was believed that no activity was present in the absence of a zone of inhibition. The activities were classified as follows: sensitive if the zone of inhibition was greater than 11 mm, moderate if it was between 8 and 10 mm, and resistant if it was less than 7 mm.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was assessed through the broth dilution assay. Here, 0.2 mL of pathogens was cultured in various concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mL) of *Hepar sulph* 200 C and 1 M. It was then incubated for 24 hours, after which the optical density was measured at 630 nm using a UV-Vis spectrophotometer.

Statistical Tools Used for Analysis

- **Primary Statistical Method**: One-way Analysis of Variance (ANOVA).
- **Purpose**: To compare the mean optical density (OD) values across different treatment groups (200 C, 1 M, and control) and assess significance of differences in MIC.
- **Significance Threshold**: $p < 0.05$.
- **Reported Result**: $F = 0.414$, $p = 0.005$ (statistically significant)..

Ethical Approval

Not required, as the study was purely in vitro and used standard, commercially available bacterial strains (from NABL-accredited Vivek Laboratories). No human participants, animals, or clinical samples were involved.

Institutional Permission

The study was conducted at Sarada Krishna Homoeopathy Medical College, Kulasekharam, Tamil Nadu, India. While not explicitly stated, institutional approval for laboratory research is typically implied for postgraduate work.

Biosafety Compliance

Use of standard pathogens (S. aureus and K. pneumoniae) likely required BSL-2 laboratory practices, though this is not documented in the protocol. No funding or external regulatory permissions (e.g., IEC, IACUC) were needed or reported.

Results

From this antibacterial study, *Hepar sulph* 200 C and 1 M were able to produce zones of inhibition of about 9 mm and 12 mm against *Staphylococcus aureus*, as shown in Figure 1. Against *Klebsiella pneumoniae*, zones of inhibition of 7 mm and 8 mm were formed in Figure 2. The details of these zones are documented in Table 1.

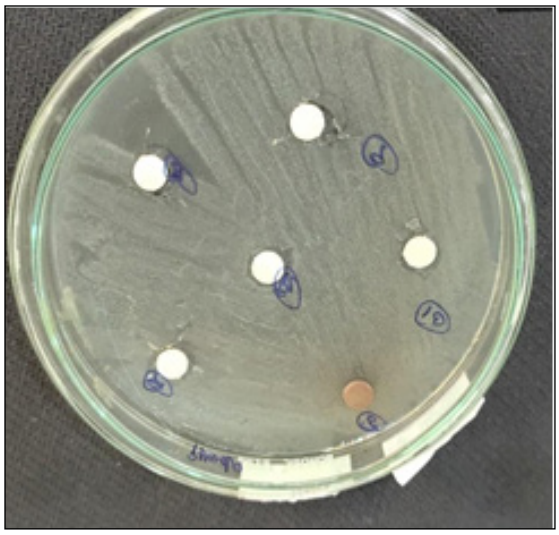

Figure 1.Antibacterial Assay of Homoeopathic Medicine and Control by the Kirby–Bauer Method in *Staphylococcus aureus*

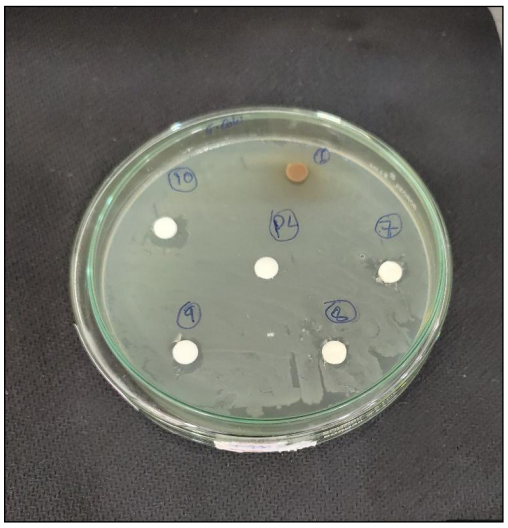

Figure 2.Antibacterial Assay of Homoeopathic Medicine and Control by Kirby–Bauer Method in *Klebsiella pneumoniae*

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Minimum Inhibitory Concentration

Hepar sulph 200 C and 1 M were able to produce mild to moderate minimum inhibitory changes in the broth dilution assay. The value below 0.1 indicated the MIC of *Hepar sulphuricum*, which was more significant in 1 M potency than in 200 C, especially against *Staphylococcus aureus* shown in Table 2. No significant changes were observed against *Klebsiella pneumoniae* shown in Table 3.

This shows (Table 4) that the bacterial strain has an influence on the MIC.

Table 1.Zones of Inhibition of Hepar sulph and Control by Kirby–Bauer Method

Bacterial Strain	Potency	Inhibition Zone (mm)
Staphylococcus aureus	200 C	9
	1 M	12
	PL (Dispensing alcohol)	6
Klebsiella pneumoniae	200 C	7
	1 M	8
	PL (Dispensing alcohol)	7

Table 2.Minimum Inhibitory Concentration in Different Concentrations of *Staphylococcus aureus*

Hepar sulph	0.2 mL	0.4 mL	0.6 mL	0.8 mL	1.0 mL
200 C	0.47520	0.37800	0.21630	0.15100	0.12100
1 M	0.35610	0.32120	0.21180	0.18550	0.01542

Table 3.Minimum Inhibitory Concentration in Different Concentrations of *Klebsiella pneumoniae*

Hepar sulph	0.2 mL	0.4 mL	0.6 mL	0.8 mL	1.0 mL
200 C	0.4891	0.3340	0.2021	0.1500	0.1100
1 M	0.3580	0.3001	0.2005	0.1860	0.0162

Table 4.Analysis of Minimum Inhibitory Concentration using One-Way ANOVA

Across/ Within Groups	Sum of Squares	df	Mean Square	F	Sig.
Across groups	0.018	2	0.009	0.414	0.005*
Within groups	0.577	27	0.021	-	-
Total	0.595	29	-	-	-

*p value < 0.05

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Discussion

In the current study, *Hepar sulph* 200 C and 1 M were evaluated for their antimicrobial properties. The medicine was tested against the most common lung pathogens, *Staphylococcus aureus* and *Klebsiella pneumoniae. Hepar sulph* 200 C was able to show zones of inhibition of about 9 mm and 7 mm against *Staphylococcus aureus* and *Klebsiella pneumoniae,* respectively. When a higher potency of 1 M was evaluated, zones of inhibition of about 12 mm and 8 mm were formed against *Staphylococcus aureus* and *Klebsiella pneumoniae*, respectively as in Table 1

When compared to a previous study by Pasalkar et al., "the lower potencies of *Hepar sulph* were able to form a mild to moderate zone of inhibition against selective lung pathogens, and it has been shown in previous studies that *Hepar sulph* 200 C was able to show very marked antibacterial properties against *Staphylococcus*".[4] Several other studies were done to substantiate the role of *Hepar sulph* against various microbes. With a zone of inhibition range of up to 31 mm against *Staphylococcus aureus* and up to 13 mm against *Klebsiella pneumoniae*, *Uva ursi* mother tincture exhibits the highest antibacterial activity.[5]

According to Rehman et al., almost all tested mother tinctures expressed antibacterial activity against *Staphylococcus aureus*, with maximum activity of *Eucalyptus globulus* (zone of inhibition 21.7 mm). Eight out of 10 mother tinctures expressed activity against *Escherichia coli*, with maximum activity of *Eucalyptus globulus* (zone of inhibition 17 mm). Hence, it was concluded that homoeopathic mother tinctures show antibacterial potential.[6]

According to Naik*, Echinacea angustifolia* and *Hepar sulph* have direct action on the pyogenic organism, i.e. *Staphylococcus aureus*. Potencies 6 C, 12 C, 30 C, and 200 C are equally efficient in inhibiting the growth of the bacteria.[7] The antibacterial effectiveness of the medicine was attributed to its antioxidant properties. It has been shown to be highly effective as a diaphoretic.[8,9] Additionally, the oil's hydrophobic characteristics enhance cell permeability, leading to leakage in bacterial cells.[10,11]

Limitations

- **Sample size:** The study had a limited sample size, which could affect the generalisability of the results. Larger, more diverse samples would provide more robust data.
- ***In vitro* study:** The research was conducted *in vitro*, meaning in a controlled laboratory environment. The results may not directly translate to *in vivo* conditions within a living organism, where various factors could influence the effectiveness of the treatment.
- **Limited strains tested:** Only two bacterial strains, *Staphylococcus aureus* and *Klebsiella pneumoniae*, were tested. Testing additional strains would provide a more comprehensive understanding of the antibacterial spectrum of *Hepar sulphuricum* 200 C and 1 M.
- **Lack of comparison with conventional antibiotics:** The study did not compare the efficacy of homoeopathic medicines with conventional antibiotics, which would provide a benchmark for understanding their relative effectiveness.
- **Mechanism of action:** The study did not investigate the underlying mechanisms by which *Hepar sulphuricum* 200 C and 1 M exert their antibacterial effects. Understanding the mechanism of action would be valuable for further development and application.

Conclusion

From this study, it is evident that *Hepar sulph* 200 C and 1 M show moderate antibacterial properties. The potencies 200 C and 1 M showed maximum zones of inhibition of about 9 mm and 12 mm, respectively, against *Staphylococcus aureus.* Minimal antibacterial property was noticed against *Klebsiella pneumoniae.* Homoeopathic medications do not cause drug resistance since they work by inducing a strong host response, which activates the body's natural self-regulating system. For homoeopathic medications to demonstrate their potential antibacterial properties, similar trials must be carried out in an efficient manner. This gave us the conclusion that when a drug's curative property resembles the pathogenetic effect of a bacterial and it is administered to the same bacteria *in vitro*, as a result of antigenic properties, the medication prevents the growth of bacteria..[12] In future, the researchers should explore the mechanism of action of *Hepar sulphuricum* 200 C and 1 M against antibiotic-resistant bacterial strains, including detailed studies on how these homoeopathic preparations interact with bacterial cell structures and processes.

Conflict of Interest: None

Source of Funding: None

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: None

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