

Study the Anti-Bacterial Activity of Homoeopathic Medicines against *Staphylococcus epidermidis* in-vitro

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ABSTRACT

The *Staphylococcus epidermidis* is a facultative anaerobic gram-positive bacterium and is a part of normal human flora which most commonly causes diseases like prosthetic valve disease, endocarditis, and hospital acquired infections particularly seen in people with catheter or other surgical implants. This study is aimed to screen Homoeopathic Medicines such as Antimonium crudum, Arsenic album, Hepar sulphur, Silicea, and Kali bichromicum with potencies of 6C, 12C, 30C, 200C, 1M, 10M for antibacterial activity against *Staphylococcus epidermidis*. Medicines were screened by Agar well diffusion method, MIC assay and Bactericidal activity. Where in Arsenic album 10M (1.06 ± 0.05)cm, Hepar Sulphur 30C (1.1 ± 0.1)cm, 200C (1.03 ± 0.20)cm, 10M (1 ± 0.26)cm, Silicea 10M (0.93 ± 0.28)cm, 1M (0.9 ± 0.17)cm, Kali bichromicum 1M (1.06 ± 0.25)cm shows the Zone of inhibition in Agar well diffusion assay. Where in MIC assay in each potency of four Homoeopathic Medicines among the five Homoeopathic Medicines such as in Arsenic album 6C, Antimonium crudum 200C, Hepar sulphur 200C, Silicea 12C. In Bactericidal study there were more dead cells as compared to live cells.

Keywords- Anti-bacterial, Homeopathic Medicines, In-vitro, *Staphylococcus epidermidis*

INTRODUCTION

Friedrich Jullis Rosenbach distinguishes *Staphylococcus epidermidis* from *Staphylococcus aureus* in 1884, initially naming *Staphylococcus epidermidis* as *Staphylococcus Albus*. It looks like “bunches of grapes” Commonly cause of surgical and skin infections. [1] Most commonly it is causative factor for prosthetic valve diseases and acne vulgaris. [2] Most of the time nosocomial infections are cause due to *Staphylococcus epidermidis*. Protein and exopolysaccharide

aggregation substance both are responsible for biofilm formation on efficient *Staphylococcus epidermidis*. [3] Antibiotic resistance has become a serious and widespread problem in developing countries, (both in hospitals and the community), causing high mortality each year. [4] Inappropriate usage of antibiotics is the most influential factor for antibiotic resistance and the global emergence of multi-drug resistant bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure.

[4,5] Strain of staphylococcus epidermidis are resistant to antibiotics including rifamycin, fluoroquinolone, gentamicin, tetracycline and sulfonamides, where Methicillin resistance is particularly widespread. [3] Now a day's antibiotic resistance results in reduced efficacy of antibacterial drugs, making the treatment of patients difficult, costly, or even impossible. The impact on particularly vulnerable patients is most obvious, resulting in prolonged illness and increased mortality. [4] Various in vitro studies were performed in Homeopathy to rule out the efficacy of Homeopathic Medicines. [6-8] Since last few decades, Homeopathy as a system of treatment has progressed remarkably. It is system a medicine par excellence. The Homeopathic medicines namely Antimonium crudum, Arsenicum album, Hepar sulphur, Kali bichromicum, Silicea have been reported for an anti-bacterial activity in humans according to Murphy's Repertory in chapter diseases → Staphylococcus, infection. [9] Hence, among the various potencies available in the market 6C, 12C, 30C, 200C, 1M and 10M were selected from each of the above said medicines for my research work. The above Homeopathic Medicines in 6C, 12C, 30C, 200C, 1M and 10M potencies were made available from standard manufacturer to perform this research work.

MATERIALS AND METHODS

Procurement of chemicals and methods

All media and chemicals such as Agar-Agar type 1, Nutrient Agar were procured from Hi-Media, Mumbai, and Amikacin from SRL, Mumbai, Dispensing alcohol (ethanol 90%, according to HPI vol-I) [10] used as a Vehicle control.

Procurement of Microorganism

Staphylococcus epidermidis (Accession no. NCIM 2493) procured from National Collection of Industrial Microorganisms (NCIM), Pune, and maintained as per instructions. [11]

Homeopathic Medicines

Homeopathic Medicines such as Arsenicum album, Antimonium crudum, Hepar sulphur,

Silicea and Kalibichromicum 6C, 12C, 30C, 200C, 1M and 10M in liquid potencies were brought from GMP approved Standard Homeopathic Manufacturing unit.

Agar Well Diffusion Method

Antibacterial activities of Homeopathic Medicines were determined by Agar well diffusion method. Validated agar plates were swabbed with 24 hours old culture. After swabbing the plate, were kept for incubation at 37 °C for 15 min. Then incubation wells were prepared with the help of borer. In each well 40µl of Homeopathic Medicines Arsenicum album, Antimonium crudum, Hepar sulphur, Silicea and Kali bichromicum were added (different potencies in each well), and also different tubes for Amikacin (1mg/ml) as positive control, distilled water and dispensing alcohol (ethanol 90%, according to HPI vol-I) used as vehicle control was added. Then plates were kept for incubation at 37°C for 24 hours. After 24 hours zone of inhibition was measured in cm.

MIC Assay (Minimum Inhibitory Concentration)

Cultures were taken which was 24 hours old and O. D. has measured. Then Homeopathic Medicines (such as Arsenicum album, Antimonium Crudum, Hepar sulphur, Silicea, Kali bichromicum), Nutrient Broth, Culture were added in equal concentration into tubes (500 µL Medicines + 500 µL Nutrient Broth + 500 µL Culture). Each tube was prepared with Medicine and its potencies. There were different tubes preparation for Positive control (Amikacin), Vehicle control (Dispensing alcohol), Culture control (Broth+control) and Negative control. Then OD was taken at 0, 3 and 24 hours.

Bactericidal Study

Among the five Homeopathic Medicines four medicines were carry forward for further assays, from that best potency from each medicine were selected. Culture was inoculated and incubated at 37°C for 24 hours, 1ml of culture and 1 ml of Arsenicum album 6C, Antimony crude 200C, Hepar sulphur 200C, Silicea 12C

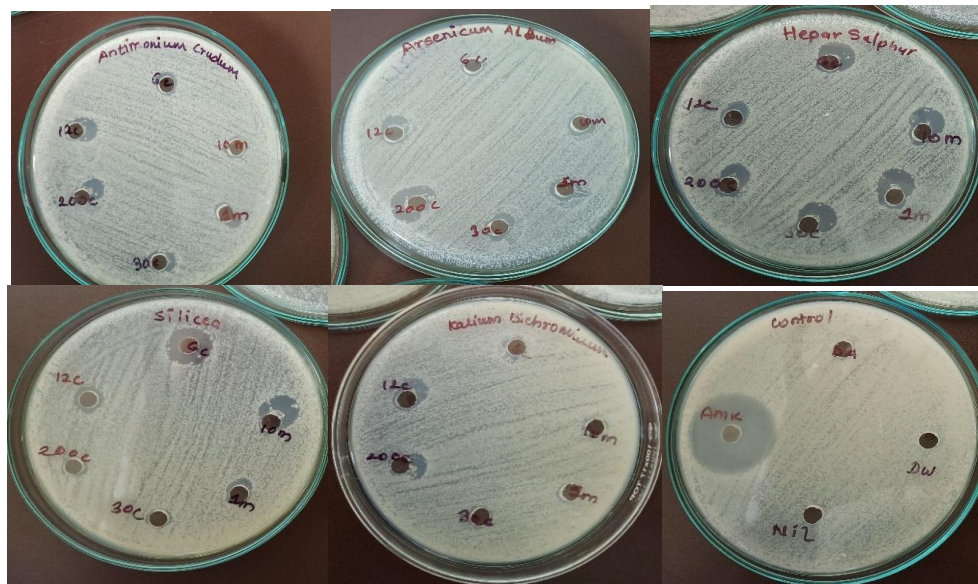
added in the screw cap tube (each medicine in different tube) and Kept it for 24 hours for incubation. After 24 hours 10 µl of Trypan blue and 10 µl culture were added and the culture was loaded into Haematometer and WBC chamber was

counted, dead cells take up the stain while live cells are seen in transparent colour.

Statistical Analysis

All experiments carried out in Triplicates. Mean and Standard deviation were calculated.

RESULTS



(Figure 1- Zone of inhibition against *Staphylococcus epidermidis* with Homoeopathic Medicines)

(Table no: -1 Agar Well diffusion assay of Homoeopathic Medicines against *Staphylococcus epidermidis*)

Name of Medicine	Potency	Inhibition zone (Mean ±Standard Deviation) in cm
Arsenic album	6C	0.7 ± 0.1
	12C	0.8 ± 0.2
	30C	0.9 ± 0.3
	200C	0.9 ± 0.4
	1M	0.9 ± 0.1
	10M	1.0 ± 0.1
Antimonium Crudum	6C	0.7 ± 0.1
	12C	0.7 ± 0.1
	30C	0.7 ± 0.1
	200C	0.7 ± 0.1
	1M	0.7 ± 0.1
	10M	0.7 ± 0.1
Hepar Sulphur	6C	1.0 ± 0.3
	12C	0.7 ± 0.1
	30C	1.1 ± 0.1
	200C	1.0 ± 0.2
Hepar Sulphur	1M	0.9 ± 0.3
	10M	1.0 ± 0.3
Silicea	6C	0.8 ± 0.7
	12C	0.7 ± 0.1
	30C	0.8 ± 0.1
	200C	0.8 ± 0.1
	1M	0.9 ± 0.2
	10M	0.9 ± 0.3
Kali Bichromicum	6C	0.7 ± 0.1
	12C	0.7 ± 0.1
	30C	0.8 ± 0.2
	200C	0.8 ± 0.2
	1M	1.1 ± 0.2
	10M	0.8 ± 0.3
Chemical control (Amikacin)		2.5 ± 0.1
Dispensing alcohol	90%	0.0 ± 0.0
Distilled water		0.0 ± 0.0
Nil		0.0 ± 0.0

Agar Well Diffusion Assay

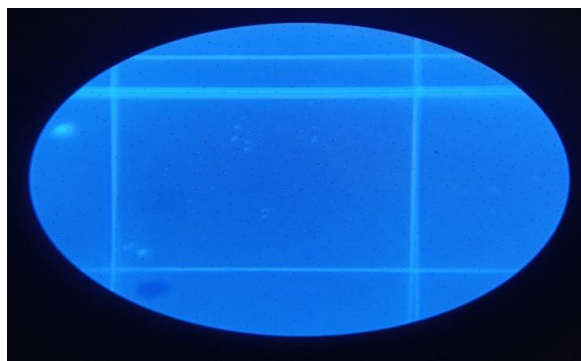
All Homoeopathic Medicines such as Arsenic album, Antimonium crudum, Hepar sulphur, Silicea, Kali bichromicum showed Antimicrobial activity. Effectiveness of Homoeopathic Medicines was seen by zone of inhibition which ranges from 0.6 to 1.5 cm for different potencies (**Figure No.1 and Table. No 1**).

Minimum Inhibitory Concentration

According to the present study the Homoeopathic Medicines such as Arsenic album, Antimony crudum, Hepar sulphur, Silicea shows good results in Minimum Inhibitory concentration as compared to positive, negative and vehicle control. The above medicine shows activity in various potencies such as Arsenic album 6C, Antimony crudum 200C, Hepar sulphur 200C, Silicea 12C. Results are seen in lower potencies as well as higher potency. But Kali bichromaticum does not show good anti-bacterial activity as compared to other Homoeopathic Medicines.

Bactericidal study

After 24 hours, the treatment with the Homoeopathic Medicine the growth of the culture was seen in the death phase. There were more dead cells are present rather than live cells.



(Figure 2: Microscopic view showing cell morphology by Trypan blue stains Neubauer's Hemocytometer)

DISCUSSION

According to the available literature only little work have been done in this field so far. *Staphylococcus epidermidis* most commonly cause of prosthetic valve diseases such as endocarditis, heart

infections ophthalmic infections, and also most commonly cause of acne vulgaris. [2]

These infections are more commonly seen in immune compromised patients. Homoeopathy is based on law similia similibus curenter that means like cures like. The Homoeopathic Medicines for this study was selected on the basis of Murphy's Homoeopathic Repertory. According to the results it is confirmed that Homoeopathic Medicines can inhibit the growth of *Staphylococcus epidermidis* as seen in Arsenic album 10M (1.06 ± 0.05)cm, Hepar Sulphur 30C (1.1 ± 0.1)cm, 200C (1.03 ± 0.20)cm, 10M (1 ± 0.26)cm, Silicea 10M (0.93 ± 0.28)cm, 1M (0.9 ± 0.17)cm, Kali bichromicum 1M (1.06 ± 0.25)cm in agar well diffusion method. In Minimum Inhibitory Concentration method, the results are seen in different potencies such as Arsenic album 6C, Antimonium crudum 200C, Hepar Sulphur 200C, Silicea 12C and bactericidal study by inhibiting the growth of *staphylococcus epidermidis* in which it is seen that there are more dead cells are more in number as compared to live cells.

CONCLUSION

According to present study it is confirmed that the Homoeopathic Medicines such as Arsenic album, Antimonium crudum, Hepar sulphur, Silicea has anti-bacterial activity as seen in Agar well diffusion assay, Minimum Inhibitory Concentration and bactericidal study. Homoeopathic Medicines can be used as alternative medicine for Amikacin and other modern medicines as Homoeopathic Medicines are cheaply available, cost effective and also does not have any side effects. According to results of my present study it is seen that Homoeopathic Medicines has anti-bacterial activity against *Staphylococcus epidermidis*.

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