

# Anti-Bacterial Activity of Homoeopathic Medicine Sulphanilamide against *Staphylococcus epidermidis* in-vitro

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## ABSTRACT

Recently nosocomial infections by *Staphylococcus epidermidis* have gained much attention. *S. epidermidis* was invariably present on the skin and generally known as an opportunistic pathogen. It also causes endocarditis, cystitis, any defective valvular heart diseases and hospital born sepsis. Most commonly occurs on intravenous catheterisation and on medical prosthesis. This study was aimed to screen the ability of different potencies of Homoeopathic Medicine Sulphanilamide to inhibit *S. epidermidis*. Different potencies of Sulphanilamide 6C, 12C, 30C, 200C, 1M were screened for antimicrobial potential by Agar well diffusion method against *S. epidermidis*. Where 12C potency showed 0.7cm and in 30C potency showed 0.8cm inhibition zone. Whereas Sulphanilamide 30C is found to be the Minimum Inhibitory Concentration, bactericidal activity showed higher amount of dead cells present in the death phase.

**Keywords:** Anti-bacterial, in-vitro, Homoeopathic Medicines, *Staphylococcus epidermidis*, Sulphanilamide.

## INTRODUCTION

Anton Julius F Rosenbach distinguished *Staphylococcus epidermidis* from *Staphylococcus aureus* in 1884, and it's initially naming *S. epidermidis* as *Staphylococcus albus*.<sup>[1]</sup> A. J. F. Rosenbach chooses *aureus* and *albus* since the bacteria formed yellow and white colonies. *S. epidermidis* is a very hardy microorganism that was non-motile and it arranged like cluster in grapes. It has no haemolytic action

on blood agar.<sup>[2]</sup> It is a catalase-positive and coagulase-negative micro-organism; which can grow by fermentation and aerobic respiration.<sup>[3]</sup> The major pathogenic factor for *S. epidermidis* is plastic devices capable of creating biofilms.<sup>[4]</sup> One of the most likely causes is surface protein that binds to blood and extracellular matrix proteins. It produces extracellular material known as PIA or Polysaccharide Intracellular Adhesion and is made from sulphated

polysaccharides. Enables several bacteria to bind to an existing biofilm and create a multilayer biofilm. Some biofilms reduces the metabolism of the bacteria and decrease the metabolism of impaired antibiotic diffusion. [2] These pathogens are part of human micro-flora and have a positive relationship with the host for this reason, but *S. epidermidis* was shown to be a pathogen creates various diseases. [6] The staphylococci are a diverse group of bacteriaemia from mild skin infections to life-threatening. Despite extensive efforts to control their spread, they remain a key cause of infection developed worldwide by hospitals and populations. In hospital, *S. epidermidis* is responsible for over than one million serious infections per year. [7] *S. epidermidis* is found in acne vulgaris pores, which are normally the sole residents of cutibacterium acnes. [8] *S. epidermidis* is a generalised infection it produces endocarditis that can lead to granulomas of the hepatic fibre ring. [9] The *S. epidermidis* as a recurrent and often unrecognized pathogen in patients with deep granulocytopenia tend to be significant predisposing factors for mucosal damage and concomitant colonisation. [10] These infections are difficult to cure by antibiotics likes Rifamycin, Clindamycin Fluoroquinolones, Gentamicin, Tetracycline, and Sulfonamide and also it is a multi-drug resistant in ophthalmic problem. [4,5] To find antimicrobial potential of various substances become the research of interest. [11,17,18] Homoeopathy is a unique and classical therapeutics of science. Sulphanilamide is a homoeopathic drug which has antibacterial activities in human being as mentioned in Synthesis Repertory 9.1 Volume [12] based on the symptoms of *Staphylococcus* on GENERAL LABORATORY findings. The Homoeopathic medicine in 6C, 12C, 30C, 200C, 1M potencies is made available from standard manufacturer in this work.

## MATERIAL AND METHOD

**Media and Chemicals-** All media and chemicals were procured from HI-MEDIA Lab, Mumbai.

**Homoeopathic Medicines-** Homoeopathic medicine Sulphanilamide 6C, 12C, 30C, 200C, 1M dilution were brought from GMP approved standard homoeopathic manufacturing unit.

Control-Vehicle Control used Dispensing alcohol (Ethanol 90% according to HPI Volume 1) [13] and as a positive control drug from Modern Medicine used Amikacin is widely accepted as a drug of choice in Modern Medicine for *Staphylococcus epidermidis*.

**Procurement of culture-**The live culture of *Staphylococcus epidermidis* (Accession no.- NCIM 2493) procured from National Collection of Industrial Microorganisms (NCIM), Pune and maintained as per given instruction.

**Agar Well-Diffusion Assay:** Validated agar plates were swabbed with 24 hours old culture (1.5 AOD). After swabbing the plate were kept for incubation for 15 min at 37°C. After incubation, wells were prepared with the help of sterilized borer. In each well 40µL of different liquid potencies of homoeopathic medicine, Amikacin (1mg/ml) as, distilled water and dispensing alcohol (ethanol 90% according to HPI Vol-1) were used as positive control and vehicle control respectively.

**Minimum Inhibitory Concentration:** MIC was determined by checking the growth of pathogen after adding 500 µL of different drug dilutions in tubes contains 500 µL of broth and 500 µL of pathogen. There were different tubes preparation for Positive control (Amikacin), Vehicle control (Dispensing alcohol), Culture control (broth + culture) and Negative control (Nutrient Broth). Readings were taken at 0, 3, 24hour to calculate minimum inhibitory concentrations values.

**Bactericidal study:** Culture was inoculated in Nutrient broth and kept it at 37°C for 24 hours. Then 1 ml of culture was added with 1 ml of homoeopathic medicine at its best potency. After that, it was kept for 24 hours

for incubation. After 24 hours 10 µL of the culture and 10 µL of the Trypan Blue were added and the culture was loaded into

Haemocytometer then WBC chamber to calculated bacteriolytic activity.

## RESULT

### Agar Well-Diffusion Assay:

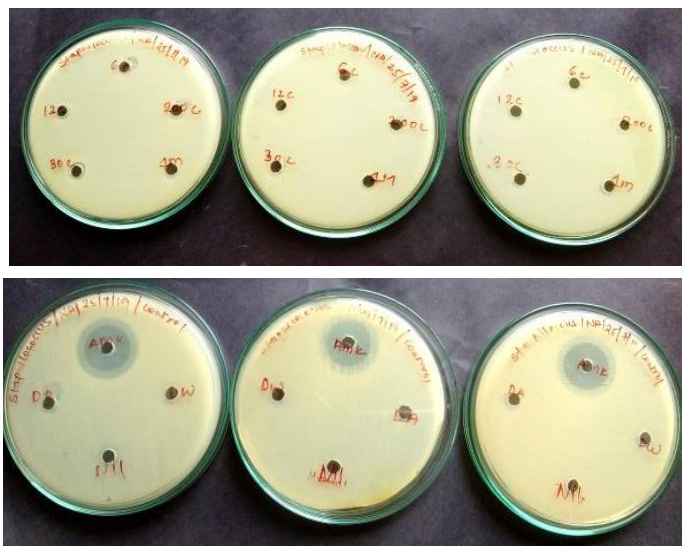


Figure 1:- Agar Well-Diffusion Assay (Sulphanilamide-6C, 12C, 30C, 200C, 1M, DA-Dispensing Alcohol, AMK-Amikacin, DW- Distilled Water, Nil)

Table 1:- Agar Well-Diffusion Assay (Mean and Standard Deviation)

Medicine and Control Names and its Potencies	Mean ±Standard Deviation in c.m.
Sulphanilamide 6C	0.0 ±0.0
Sulphanilamide12C	0.6 ±0.1
Sulphanilamide30C	0.7 ±0.1
Sulphanilamide 200C	0.0 ±0.0
Sulphanilamide 1M	0.0 ±0.0
Amikacin 1mg/ml	2.7 ±0.0
Dispensing Alcohol 90% according to HPI volume-1	0.0 ±0.0
Distilled Water	0.0 ±0.0

Nearby all potencies are showed antibacterial activity. Effectiveness of Homoeopathic medicine was seen by observing the zone of inhibition, which range from 0.6 to 0.8 mm for different potencies.

### Minimum Inhibitory Concentration:-

All potencies of Sulphanilamide and all control groups (Positive Control, Vehicle Control, Culture control, and Negative control) are showed minimum inhibitory result but Sulphanilamide 30C showed the best result among all potencies and control group.

### Bactericidal activity:-



Figure 2:- Bactericidal activity

In bactericidal activity after 24 hours, the treatment with the medicine, the growth of the culture is seen in the death phase. More dead cells are present rather than live cells.

## DISCUSSION

Very few researches have been performed against *S. epidermidis* with Homoeopathic medicines. In this research work, Homoeopathic medicine showed very good qualitative effect against *S. epidermidis*.

In present-day, the main type of infection by *S.epidermidis* is nosocomial infections. [14] It is identified in 1998 as one of the most commonly isolated bacterial pathogens in hospitals in general and the primary pathogen implicated in nosocomial fluke, heart infection and infection of the eye, ear, mouth, nose and throat. [15] *S. epidermidis* very major infective agent in compromised patients such as drug abuser and immune compressive patients. The intravascular catheter is the main reason for this infection. [4, 16] This pathogen is part of human micro-flora and has a positive relationship with the host. *S. epidermidis* was shown to be a pathogen and which creates various diseases like endocarditis, cystitis, hepatic fibrin ring granulomas and in patients with deep granulocytopenia tend to be significant predisposing factors for mucosal damage and concomitant colonisation. [6,9,10] It is a multi-drug resistant bacterium in ophthalmic problem. [5] In now day's in-vitro study is showing positive result in *S. epidermidis* against homoeopathic medicine. In this study, the purpose was to inhibition of the *S. epidermidis* by the antibacterial activity of Homoeopathic medicine. The in-vitro analysis result was very favourable. It is shown that the homoeopathic medicine Sulphanilamide has specific inhibitory activity and bactericidal activity against *S. epidermidis* which was selected on the basis of Synthesis Repertory volume 9.1. Some modern medicine has resistant in staphylococcus epidermidis and it may sometimes have created many side effects. Whereas homoeopathic medicines have no side effect as far as known and it has inhibitory activity against the bacteria. It has a cost-effective and preventive and safe mode of the treatment in this reason the

homoeopathic medicine was the effective choice of treatment. The results of the experiment prove that the homoeopathic ultra-diluted medicines are effective in in-vitro antibacterial study as evidence-based medicine, it's not placebo therapy.

## CONCLUSION

In present-day Homoeopathy is the great system of the alternative system of medicine. In this study, we saw that the inhibitory activity and bactericidal activity of *Staphylococcus epidermidis* against homoeopathic medicine was proving that the homoeopathic system was evidence-based. All the potencies were found to have inhibitory activity but Sulphanilamide 30C showed the best result in 3hour and 24 hour. Amikacin also gives good result in 3 hour and 24 hour. In bactericidal activity study after 24 hours, the treatment with the medicine the growth of the culture is seen in the death phase. More dead cells are present rather than a live cell.

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