

In-Vitro Evaluation of Antibacterial Activity of Homoeopathic Preparations on *Klebsiella pneumoniae*

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ABSTRACT

Increasing resistance to present antibiotics and rising virulence of microorganisms is the real concern of medical fraternity these days. *Klebsiella pneumoniae* is very notorious microorganism with remarkable mortality rate and shows high antibiotic resistance. What else can be the solution for present problem then to find the alternative ways of treatment. This study was aimed to detect the antibacterial activity of selected homoeopathic medicines like Sulphur, Senega, Lobelia Inflata and *Klebsiella Pneumoniae* nosode. These medicines were used in different potencies like 6C, 12C, 30C, 200C and 1M to find the antimicrobial activity of these homoeopathic medicines against *Klebsiella pneumoniae* using 96well microtiter plate.

Keywords- Antimicrobial activity, Homoeopathic medicine, Sulphur, Senega, Lobelia inflata, *Klebsiella pneumoniae* nosode, *Klebsiella pneumoniae*.

INTRODUCTION

Community is facing big threat of infectious diseases these days. Infectious disease is the invasion of organism body tissue by the disease causing agent, their multiplication and the reaction of the host tissue to the infectious agent and toxins they produce. According to WHO, 43% of global burden of diseases is due to infectious diseases. [1] It is caused by different microorganisms and “*Klebsiella pneumoniae*” is one of the biggest contributors of such infectious diseases.

Klebsiella pneumoniae is a gram negative, lactose fermenting, encapsulated, non-motile, rod shaped, facultative anaerobic bacterium. [8] It is responsible for wide range of infections including pneumonia, bacteraemia, UTI and liver abscess. [8] It mostly affects immunocompromised individuals and

causes nosocomial infections. Increasing number of infections and lack of effective treatment is the reason of recent notoriety that *Klebsiella pneumoniae* gained. Additional genetic traits and hypervirulent or antibiotic resistance acquired by *Klebsiella pneumoniae* strains lead to present critical circumstances. [2] Data evaluated in 2014 says that carbapenems, the last store antibiotic class, has lost its effect on 57% of *Klebsiella pneumoniae* strains in India. [1]

Range of mortality rate associated with *Klebsiella pneumoniae* is 3 to 42%. 55% of high mortality rate is shown for community acquired pneumoniae associated with bacteraemia. [3] Not just high mortality rate but resistance gained by *Klebsiella pneumoniae* against antibiotics is also a big threat. According to several studies conducted, near by 80% of *klebsiella*

isolates has already resistant to first line antibiotics. [4,10] Usually third generation antibiotics are been used against *Klebsiella pneumoniae* infections like cephalosporin, carbapenems, quinolones and aminoglycosides. *Klebsiella pneumoniae* carbapenems confer broad resistance and are associated with a higher mortality rate (50%). [5,10]

These conditions left physician with very less treatment options and raises an urgent need to screen new drugs from natural sources or to modifying existing drugs. There is another approach that is to use alternative medicine such as homoeopathy. WHO has reported that homoeopathy is gaining popularity among people and become the 2nd largest therapeutic system, in practice. Its charm among population is also due to lesser side effects of homoeopathic medicines. Dr.Zhang (WHO's medical officer) said that use of extreme dilution as homoeopathic drug omit all the doubts regarding the safety issue. [6] Homoeopathy has significant role in treating such infectious conditions including pneumonia and UTI.

Recently there have been many research papers published on the antibacterial activity of homoeopathic medicines. There is list of medicines used in infectious diseases like pneumonia, UTI etc. according to synthesis homoeopathic repertory SULPHUR, LOBELIA INFLATA, SENEGA are the medicine showing similar symptoms as produced in pneumonia. Borland pneumonia suggest above medicines in conditions like chronic bronchitis, and broncho-pneumoniae. [9] There is another nosode made from *klebsiella pneumoniae* only, named as *klebsiella pneumoniae* nosode, raising the curiosity in physicians mind regarding its effect in such conditions but there is not a single study available which can clarify the doubt regarding the mechanism of action. Thus, it is needed to find out the effect of these homoeopathic medicines for management of *klebsiella* infections. Present study was conducted to find the

antimicrobial effect of above homoeopathic medicines against *Klebsiella pneumoniae* by using in-vitro study methods.

MATERIAL AND METHOD

Media and chemicals- All media will be procured from Hi Media, Mumbai, India. All the reagents and chemicals to be used will be of AR grade and procured from MERK India.

Homoeopathic drugs

Homoeopathic medicines Sulphur, Lobelia inflata and Senega in 6C, 12C, 30C, 200C, 1M liquid dilutions and *Klebsiella pneumoniae* nosode in 30C, 200C, 1M potencies were purchased from GMP certified standard homoeopathic pharmacy.

Organism-

30 different clinical isolates were collected from "Bharti Vidyapeeth charitable hospital, Pune. The cultures were maintained in MHA slant and incubated at 37°C for 24hr. Later, slants were stored in refrigerator.

Antibiotic sensitivity of clinical isolate-

Antibiotic activity of clinical isolate was checked by using agar diffusion assay. Culture of *Klebsiella pneumoniae* was first spread well over an agar, poured and settled in different petri dishes. Later, the hexa disc for gram negative bacteria and antibiotic disc of cephalexin(30µg) were kept in the middle of the different petri dishes. All of them were kept in the incubator for 24hr and zone of inhibition were measured in mm using scale.

Each culture was tested for its purity before each experiment by using gram staining method and assessed under compound microscope.

96 well microtiter plate assay-

Antibacterial activity was found out of selected medicines by using 96well microtiter plate with lid. Total liquid adjusted was 250µl with Mueller-Hinton broth (130µl), various potencies of homoeopathic medicine(100µl) and culture of *Klebsiella Pneumoniae*(20µl). The experiment was conducted along with negative control (Mueller-Hinton Broth +culture), and ethanol (90%- vehicle

control). The O.D. was recorded using spectrophotometer at 600nm wavelength, after 48 hour's. [7,11]

Statistical analysis of the obtained data is done using (graph pad software) PRISM VIRSION 5

RESULT

Antibiotic sensitivity test-

Modern medicine, especially antibiotics are largely in use but with time this mode of treatment is becoming ineffective. Largely developed antibiotic resistance by *Klebsiella pneumoniae* is confirmed by many studies and above

antibiotic sensitivity test re-establish that fact only.

An organism is considered as highly sensitive if the zone of inhibition is measured ≥ 19 mm, considered intermediate if zone of inhibition is measured from 15-18mm and resistant if diameter was less than 13mm. [12]

No inhibitory zone was shown by modern medicine "cephalexin" (fig- A). experiment done with Hexa disc also showed the high resistance of *Klebsiella pneumoniae* isolate with only tobramycin showing inhibitory zone of 15mm and co-trimoxazole- 16mm (fig- B), considered as intermediately sensitive.

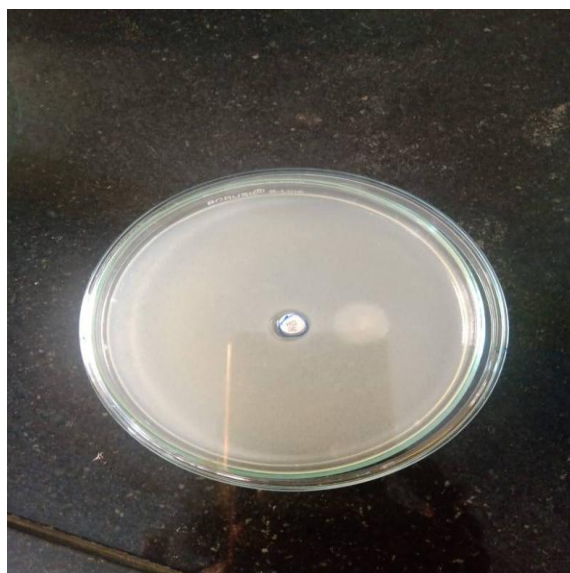


Fig-A
a) Antibiotic sensitivity of cephalexin



Fig-B
b) antibiotic sensitivity of hexa disc

96 well plate assay-

This study was conducted to evaluate the antibacterial activity of homoeopathic medicines against *Klebsiella pneumoniae*. Different homoeopathic medicines were selected according to their symptoms.

Thirty different clinical isolates were collected and study was conducted with use of 96well microtiter plates and optical densities of different potencies on clinical isolates were measured through spectrophotometer at 600nm.

Sulphur, Senega, Lobelia inflata and *Klebsiella pneumoniae* nosode in different potencies (6ch, 12ch, 30ch, 200ch, 1M), were the medicines that were assessed for their effect on *Klebsiella pneumoniae* isolates.

Results were measured and assessed through statistical analysis and it was found that Sulphur, Senega and Lobelia inflata gave statistically significant result with P value $< 0.05\%$. But no statistically significant result was obtained with *Klebsiella pneumoniae* nosode.

Minimum inhibitory concentration-

Selected homoeopathic medicines showed different inhibitory concentration against klebsiella pneumoniae isolates. Best results were shown by 6CH potencies for all medicines. Percentage of inhibition was measured using suitable methods and M.I.C was calculated, shown in table below-

TABLE- M.I.C value for selected homoeopathic medicines shown statistically significant results.

MEDICINE	M.I.C
Sulphur	6CH
Senega	6CH
Lobelia inflata	6CH

DISCUSSION

Effect of homoeopathic dilution is in debate since many years. There is speculation about its placebo effects due to its very small concentration of active principle, lesser than Avogadro number but understanding the nanotechnology could open the door of knowledge. Present study was aimed to assess the antimicrobial activity of selected homoeopathic medicines against *Klebsiella pneumoniae*. Homoeopathic medicines were selected by using synthesis repertory and reference of Borland's pneumonia. *Klebsiella pneumoniae* nosode was selected out of curiosity to check the effect of nosode of same bacteria over it.

After evaluating the whole data collected through present study, using ANOVA as statistical test, it was concluded that Sulphur, Senega and lobelia inflata showed inhibitory effect against clinical isolates of *Klebsiella pneumoniae* with P value- 0.00%. There was no statistically significant results shown by klebsiella pneumoniae nosode.

This in vitro study was framed to find, if selected medicines possess any antimicrobial activity against *Klebsiella pneumoniae* or not and result obtained can be further verified through in-vivo and clinical studies

CONCLUSION

Through present study we can conclude that homoeopathic medicines have

antibacterial activity against "*Klebsiella pneumoniae*". Different potencies of Senega, Sulphur and Lobelia Inflata can be effectively used in treating klebsiella infections but further study is needed. Verification of present results through in vivo and clinical trials is necessary for clearing the concept of mode of action.

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