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# Streptococcus Pneumoniae and Medicinal Plant Extraction: Experimental Research with Pneumonia Patients in Hospitals

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## Abstract

Pneumonia is a prevalent and often-seen illness in humans. This research investigates the antibacterial efficacy of pharmaceutical plant products contrary to Streptococcus pneumoniae (S.P.), a bacterium obtained from individual patients with pneumonia. The objective of the research is to separate antibiotic-resistant strains of Pneumococci from patients who are admitted to the hospital and then evaluate selected extracts from medicinal plants for their ability to inhibit the growth of Pneumococci by determining their minimum inhibitory concentration (MIC). Antibiotic susceptibility testing was conducted on clinical samples taken from 47 hospitalized patients. The analysis revealed that S. pneumoniae showed resistance to Ampicillin and Meropenem. Streptococcus pneumoniae is susceptible to Clarithromycin, Levofloxacin, Clindamycin, and Erythromycin. An experimental study was undertaken in the Jahangira area due to its diverse variety of medicinal plant species, such as Azadirachta indica, Nilotica, Allium Coriandrum, Allium sativum, Syzygium aromaticum, and Piper nigrum. These medicinal plants have not been previously used for the treatment of S.P. In this research, the antibacterial activity of traditional remedies from the Jahangira area was evaluated against S.P. The inhibitory zone was seen in numerous medicinal plants, such as Vachellia Nilotica, Syzygium aromaticum, and Allium sativum. The plant isolates have been diluted into a range of concentrations, namely 512g/ml, 256g/ml, 128g/ml, 64g/ml, 32g/ml, 16g/ml, 8g/ml, 4g/ml, 2g/ml, and 1g/ml. These dilutions were prepared in antiseptic nutritious broth and standard remedy and were placed on a 96-well microtiter plate. The purpose of this setup was to evaluate the Minimum Inhibitory Concentration (MIC). The amounts of MIC were measured at 8g/ml, 4g/ml, 2g/ml, and 1g/ml. The antibacterial efficacy of the specimen extracts was assessed in comparison to the conventional medications' vancomycin and penicillin, while it was shown that they exhibited resistance toward Streptococcus pneumoniae. Furthermore, the antibiotic's efficacy was assessed by ocular evaluation in hospitalized individuals by quantifying the area of inhibition. Policymakers are advised to prioritize the cultivation of therapeutic plant species, such as Azadirachta indica, Nilotica, and Achyranthesesaspera, which have shown beneficial in the S.P.

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## Introduction

Pneumonia is a respiratory disease characterized by an infection in the lungs, leading to difficulty in being able to breathe and reduced consumption of oxygen (Mani, 2018). It is a usual complication following a stroke, significantly increasing the risk of death and illness in stroke patients. Approximately 8.1 and 45.3 percent of stroke patients grow due to pneumonia (Bond et al., 2023; Qiu et al., 2023). Community-acquired pneumonia (CAP), a significant infectious disease, encompasses a high fatality rate. Various bacterial infections have been associated with this illness (Ticona et al., 2021).

Bacteria, the first form of life on Earth, exhibit remarkable diversity and abundance. Bacteria are a kind of microorganism consisting of single-celled organisms with a size of one micron. Bacterial infections are a significant cause of widespread illnesses globally and are considered a very critical issue in the medical field throughout history, now, and in the foreseeable future (Amyes, 2022). Bacteria are classified according to their phenotypic traits, which consist of their morphology, dimensions, pigmentation, and biochemical properties. One of these infections is caused by pathogenic bacteria, whereas the others are not. Microflora refers to the collection of nonpathogenic microorganisms. Certain microbial cells possess a high degree of infectivity, leading to the onset of sickness (Doloff, 2019).

Streptococcus pneumoniae is one of the most prevalent opportunistic diseases in humans. The bacteria often inhabit the nasal cavity of humans. Consequently, when it infiltrates regions of wound infections, it may result in the development of illnesses. Streptococcus pneumoniae is a frequently occurring cause of human diseases. The bacteria typically colonize the human nasopharynx, but when they infiltrate commonly sterile regions, it may lead to severe sickness. Pneumococcal invasive disease Pneumonia is a common complication in stroke patients, occurring in specific percent of cases (Weiser et al., 2018). Community-acquired pneumonia (CAP) is a common and serious chronic disease with a high mortality rate. Although many bacterial illnesses are associated with sickness (Cilloniz et al., 2023), conditions including sepsis, meningitis, and pneumonia cause the deaths of millions of individuals annually (Davis et al., 2023). The presence of antibiotic-resistant bacteria might diminish the range of effective therapies, making the diagnosis of some prevalent bacterial diseases challenging (Anderson & Feldman, 2023). The presence of antibiotic-resistant bacteria constitutes a well-recognized global health issue (Ladas et al., 2023).

## Literature Review

### Antibiotics

Antibiotics, sometimes referred to as antimicrobials, are prescribed for the treatment of bacterial illnesses. Antibiotics are a class of pharmaceuticals that effectively inhibit or impede the proliferation of bacteria (bactericidal agents). Alternatively, you might use bactericidal treatments to effectively eliminate them. The effectiveness of these compounds in limiting bacterial growth is due to their ability to block crucial biological processes in bacteria (Etebu & Arikekpar, 2016). The advent of advanced antibiotic therapy had a significant impact on our culture, revolutionizing pharmaceutical and medical practices. Antibacterial agents are used in the field of medical science to control illnesses, facilitate organ transplants and surgeries, and treat newborn infants. Additionally, they aid in promoting successful patient recuperation (Muteeb et al., 2023). Over the last several centuries, the use of medications has diminished the significance of bacterial infections as a health concern in Western nations. Microbial diseases, however, remain the primary cause of mortality in developing countries and the third biggest cause of death worldwide (Jamal, 2023). The presence of bacterial infection has posed challenges in effectively treating infectious disorders. The discovery of antibiotics occurred in Fleming's laboratory after the introduction of penicillin in 1928. This breakthrough coincided with a golden age of antimicrobial treatments in the 1950s, during which many new medicines and subcategories of antibiotics were developed to address the increasing prevalence of resistance (Uddin et al., 2021).

### Infection with Antimicrobial Resistance

The development of medication resistance in bacteria, which is used to treat diseases, leads to a reduction in the effectiveness and lifespan of antibiotics due to the natural process of acclimatization and antibacterial activity. The emergence of drug resistance has recently increased due to the widespread and inappropriate use of antibiotics, especially in pediatric patients. Consequently, a significant number of bacterial pathogens underwent hypermutation, leading to the proliferation of harmful strains (Dekker, 2023). The increasing levels of tolerance and toxicity are accompanied by a corresponding increase in the cost and danger to society. The adverse effects of resistant diseases are being experienced globally. Drug-related illnesses claim the lives of a minimum of individuals in Europe and the United States annually, with a much higher number of fatalities occurring in other regions worldwide. Recent projections suggest that drug-resistant infectious illnesses might potentially impact an extra 10 million people worldwide. The World Health Organisation (WHO) is calling for collaboration between governments and the clinical sciences to address the issue of drug-resistant infections. They are pushing for significant investments in antibacterial drug research to meet the global public health need (Hussain et al., 2023).

### Biologically Active Compounds with Reaction of Medicinal plants

Regarding the discovery of novel bioactive compounds, such as antibacterial pharmaceuticals. Small organic materials have long fascinated pharmaceutical and biology researchers due to their profound influence on the function of biomolecules in biological systems (Bilal & Iqbal, 2020). Hemmerling and Piel (2022) found that the combination of these compounds has the capacity to affect a wide range of potential locations. Organic compounds for biological screening may be acquired via many means. The bulk of antimicrobial agents used in clinical practice consist of natural chemicals or semi-synthetic derivatives (Mondal et al., 2018). In addition, natural medicines have the capacity to interact with particular proteins, and their success rates in high-throughput screening are frequently much higher than those of growth factors obtained from conventional plant sources (Ghosh et al., 2022). Plants provide an inexhaustible reservoir of novel biological substances that find use in medicine and several other domains (Wang et al., 2023). Flavonoids, alkaloids, phenols, resins, fixed oils, volatile oils, glycosides, tannins, and steroids are naturally occurring bioactive chemicals found in certain parts of plants such as fruits, seeds, bark, flowers, and leaves (Wani et al., 2023). The therapeutic influence of medicinal plants is often good due to the buildup of bioactive substances (Dhiman et al., 2023). Saeed et al. (2023) discovered that these substances particularly hinder the functioning of physiologically active molecules. Herbal compounds, together with their modifications and analogues, make up almost half of all clinically used medications, with higher plant-derived natural products accounting for 25% of the total (Moyo et al., 2023). The utilisation of Galegine, derived from Galega officinalis L., has been employed in the synthesis of metformin and other antihyperglycemic medications (Khezri et al., 2023). Similarly, papaverine, obtained from Papaver somniferum L., has been utilised as a model compound for the production of verapamil, a medication used for the treatment of hypertension (Agrawal et al., 2023; Ashrafi et al., 2023).

### Plant Extraction

Several challenges need to be addressed when treating viral illnesses with natural solutions. One of these challenges is the identification and categorization of initial screening assay methods, which play a crucial role in ensuring that researchers have a recuperation of isolates or elements with relevant therapeutic implications. The test should be user-friendly, efficient, uncomplicated, and provide prompt results, often at a low cost (Arya et al., 2023; Yadav et al., 2023). Kamil Hussain et al. (2019) identified the plant components with biological activity, it is necessary to subject the plants to a suitable extraction process. Extraction, as it is often referred to as in the pharmaceutical industry, is a technique used to isolate the bioactive components with therapeutic properties. The powdered

sample undergoes testing to determine its potential biological potential (Shen et al., 2023). The methanolic extraction is then divided into fractions, with each fraction being subjected to further bio-assay testing (Samra et al., 2021).

## Streptococcous Pneumoniae in Pakistan

The global prevalence of antimicrobial resistance is a significant challenge, as it raises worries about the potential loss of efficiency in antibiotic therapy. This might result in therapeutic futility and reduced efficacy of older medications (Shahid et al., 2023). These bacteria are often present in the nasal cavity and have been associated with community-acquired respiratory infections (CA-RTIs). These illnesses, such as pneumonia and meningitis caused by S. pneumoniae, result in significant illness and death. Due to inadequate lab diagnostic and antimicrobial susceptibility diagnostic examination in Pakistan, as well as in some other Asian countries, the antibiotic management choices for Respiratory Tract Infections are mostly based on anecdotal evidence. Furthermore, the availability of laboratory testing in this scenario may be financially prohibitive (Shah et al., 2023). The increasing prevalence of drug-resistant bacteria poses a significant threat to the current and future management of community-acquired respiratory tract infections (CA-RTIs) (Keita, 2021). Epidemiological research is valuable for determining the specific risk factors in a particular area or locality and guiding appropriate antibiotic therapy (Matran et al., 2023).

The Study of Antimicrobial Resistance (SCAR) conducted investigations in Pakistan during the years 2002-03, 2004-06, 2007-09, and 2014-15. Similar tests were also carried out in various regions including Asia, the Middle East, Africa, Latin America, the Asia-Pacific, and the Confederation of Independent Countries. The purpose of these tests was to gather specific and regional data regarding the trend of strains among isolates from S. pneumoniae CA-RTIs. Specimens were collected from two sites in Pakistan: Shaukat Khanam Memorial Cancer Hospital and Aga Khan University Hospital in Karachi (during the time of study from 2002 to 2009) and Research Institute in Lahore (during the period of study from 2002 to 2009 and for the course of the research duration of 2014-15) (Jabeen et al., 2022).

## Methods and Procedures

The research included the collection of sputum samples from individuals diagnosed with pneumonia at District Headquarters Hospitals (DHH) Jahangira, Nowshera and Peshawar. During the morning, sterile tubes/swabs were used to collect sputum samples. This was done since the bacteria populations in the thoracic cavity were most active overnight. A total of 47 sputum samples were meticulously collected, and patients were instructed to get samples in a manner that minimizes contamination. The samples were promptly labelled and relocated in the Research Laboratory at Peshawar, Khyber Pakhtunkhwa (KP), Pakistan. The study used sheep blood agar, chocolate agar, and nutrient agar as well as selected sensitive and specific medium for cultivating S. pneumoniae. Construct the foundation in accordance with the prescribed regulations. After autoclaving at an ambient temperature of 121°C for about 30 minutes, introduce the Nutrient agar medium into a water bath set at a temperature of 50°C. After the base has reached a lower temperature, incorporate the Nutrient agar into it, ensuring an entire mixture. Furthermore, during the culture isolation process the test growth and plates incubated at 37°C for 24 hours.

## Cultural Cleanness and Refreshment

After streaking the old culture over the sterile Nutrient Agar plates, pure isolates from the plate were subcultured on these sterile plates and treated for 24 to 48 hours. S. pneumoniae cultures were obtained and identified after a 24-hour development period on Nutrient agar by the examination of morphological characteristics, Gram staining, microscopic inspection, and biochemical tests as well as noted morphological colony with help of nutrients agar plates. The study used

bacteria, biochemical test for the *S. pneumoniae.* Similarly, caused the bubble form with the catalase enzyme consumes hydrogen peroxide (H2O2). The role of oxidase test was important and 3 to 4 drops tetra methyl-p-phenylenediamine reagents were performed for the negative and positive color changes. The test of the sensitivity was performed for the bacteria at 370C for 24 hours. As conclusion, broth cultures (100µl) were liquified in 250l sterile phosphate buffer saline (PBS) and mixed with McFarland standard solution ("0.6ml 1 percent Bacl2.H2O and 99.4ml 1 percent conc. H2SO4 using a spectrophotometer set to 540nm"). The research study prepared Muller-Hinton agar in 38g and 1liter of distilled water. The process of plant extraction was methanol and distilled water for aerial parts (see Table 1).

Table 1

Cultural Cleanness and Refreshment (n=47)

| Scientific name | Family | Local name | Part used | Medicinal use | Extract |
|---|---|---|---|---|---|
| Azadirachta indica | Meliaceae | Neem | Leaves | Leprosy | Distill water, Methanol |
| Vachellia nilotica | Fabaceae | Kikar | Leaves | Strengthen gums | Distill water, Methanol |
| Syzygium aromaticum | Myrtaceae | Clove | Leaves | Nausea, treat cold | Distill water, Methanol |
| Allium sativum | Amaryllidaceae | Garlic | Leaves | Lower blood pressure | Distill water, Methanol |
| Piper nigrum | Piperaceae | Black pepper | Leaves | Asthma | Distill water, Methanol |
| Coriandrum sativum | Apiaceae | Coriander | Leaves | Stomach pain | Distill water, Methanol |

The method of minimum inhibitory concentration (MIC) was used for the lowest concentration of sensitive plants. Similarly, the Bacteria culture mixed with N.B media and inoculum. This process makes diluted into 96 wells of micro titer plate and 50µl concentration as well as with the time of incubate 24 hours.

## Data Analysis

The data was analyzed in the real laboratory collected pictures and colony of *s. pneumoniae*, which were creamy, smooth shiny and opaque color (see Figure 1).

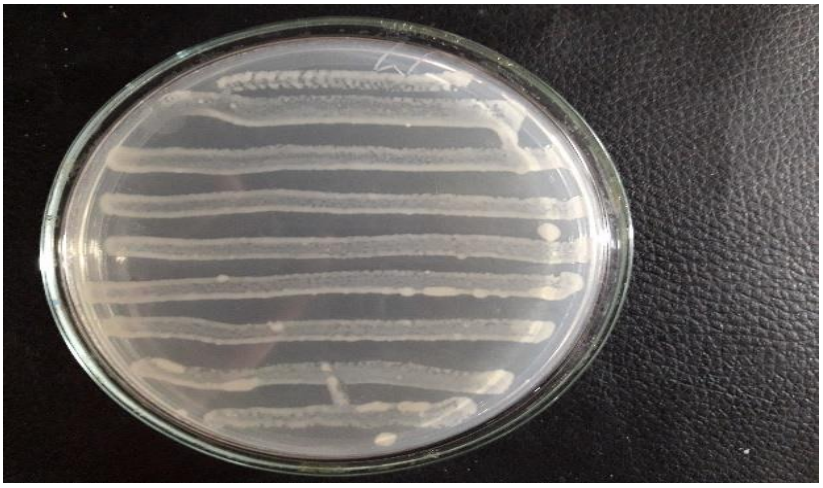

Figure 1

The result showed that *S. pneumoniae* gram was positive and diplococci with microscopic green color (see Figure 2).

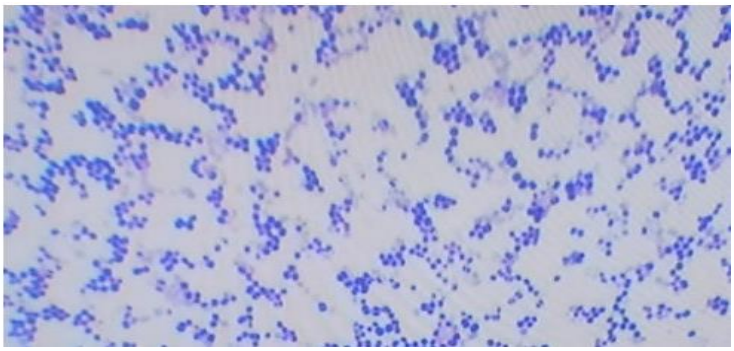

Figure 2

## Biochemical Testing

The process of Biochemical analysis ("catalase test, oxidase test") and identified colonies to know the pathogen. The microscopic and microbiological cultural were examined (see Table 2).

Table 2

Test Isolates and Biochemical Classification

| No. | Isolate | Shape | Gram | Biochemical tests | Result |
|---|---|---|---|---|---|
| 1. | *Streptococcous Pneumoniae* | Cocci | +ve | Oxidase | _ |
| 2. | *Streptococcous Pneumoniae* | Cocci | +ve | Catalase | _ |

The colonial morphology of isolates 1 and 2 seemed smooth, shiny and had a wet-like mucoid appearance. The object had a transparent appearance and may be similar to a water droplet. It could either have a concave shape or be flat with a smooth, rounded edge. The colony (Table 1) included Gram-positive cocci bacteria. The biochemical tests conducted, namely catalase and oxidase assays (as shown in Table 1), confirmed that the microorganism is Streptococcus spp. On the other hand,

the catalase test and positive results of S. pneumouniae were shown in the Figure 3. The sample and its Oxidase test results were draw in the Figure 4.


Figure 3

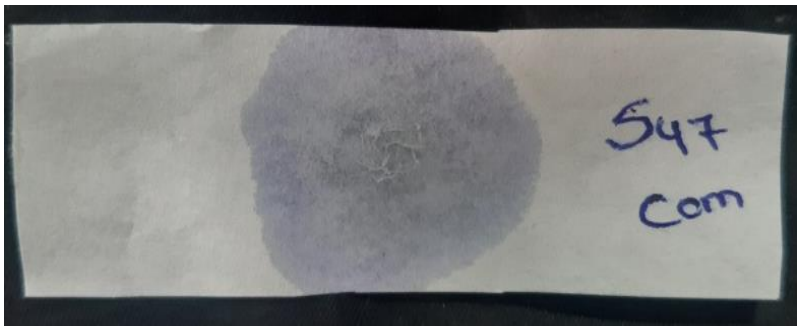


Figure 4

Table 3
Zone of inhibition (mm)of different test isolate

| Antibiotic Tested | Antibiotic resistant profile of *S. pneumoniae* isolated from sputum sample (n=47) | | |
|---|---|---|---|
| | Resistant n (%) | Intermediate n (%) | Sensitivity n (%) |
| Levofloxacin | 0(≤13) | 0(14-16) | 29 (≥21) |
| Erythromycin | 0 (≤15) | 0 (16-20) | 24(≥21) |
| Clindamycin | 0(≤15) | 0 (16-18) | 22(≥19) |
| Clarithromycin | 14 (≥15) | 7 (15-20) | 17(≤16) |
| Ampicilin | 0 (0) | 0(0) | (0) |
| Meropenem | 0(0) | 0 (0) | 0 (0) |

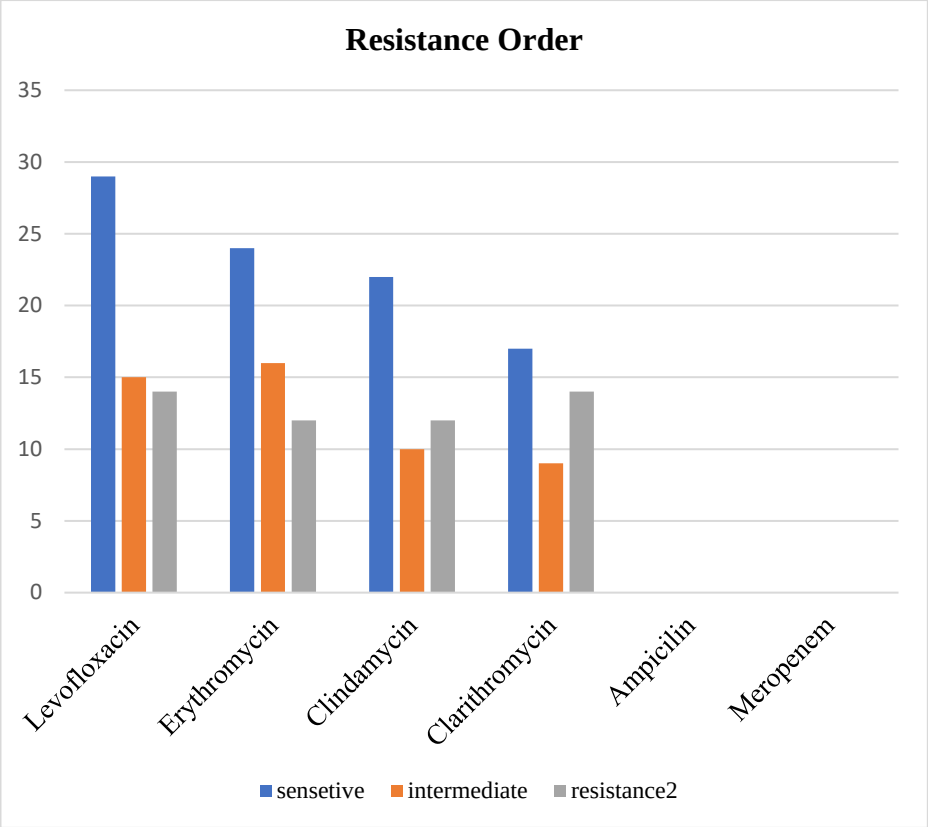


Figure 5

The method of diffusion was used for the sensitivity test. Complete antibiotics tested; inhibition zones were recorded. The antibacterial sensitivity break point and test isolate for the antibiotics are shown in Table 3 and Figure 5.

Table 4

MIC and of Allium Sativum Plant

| **Concentration of plant** | **MIC** |
|---|---|
| 1µgml | 8µg/ml |
| 2µg/ml | 4µg/ml |
| 4µg/ml | 2µg/ml |
| 8µg/ml | 1µg/ml |
| 16µg/ml | |
| 32µg/ml | |
| 64µg/ml | |
| 128µgmll | |
| 256µg/ml | |
| 512µg/ml | |

The Garlic plant was ascertained, and MIC was measure by micro titer 96 wells plate. There is different isolate was inhibited (such as, "512µgram/ML, 256µgram/ML, 128µgram/ML, 64µgram/ML, 32µgram/ML, 16µgram/ML, 8µgram/ML, 4µgram/ML, 2µgram/ML and 1µgram/ML") as well as see the isolated inhibit in Table 4. Similarly, the MIC level of sensitiveness of plant were measured in the Figure 6.

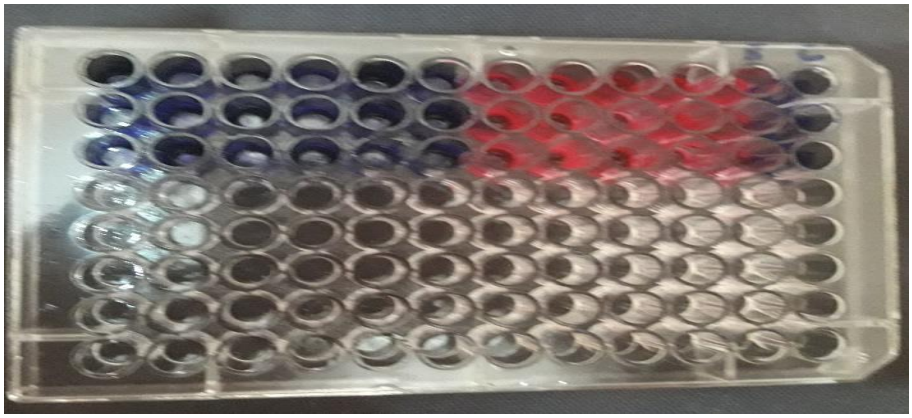

Figure 6

## Conclusion

Based on the statistics results, Levofloxacin, Erythromycin, and Clindamycin are recommended as the most responsive antibiotics. The study advised to save these medications for specific cases and patients were recovered. The antimicrobial susceptibility characteristics are crucial to ensure the prescription of the most efficacious drug for the patient, especially in situations when experimental treatment is necessary. The routine testing of bacteria's susceptibility to antibiotics is very competent, and doctors and medical practitioners may readily use the study's results to treat bacterial illnesses by providing different medications according to the data. The study concludes that all of the plant extracts tested ("Allium sativum, Coriandrum Sativum, Syzygium aromaticum, Vachellia nilotica, and Azadirachta indica") exhibit strong antibacterial properties against S. pneumoniae. Specifically, Allium sativum, Syzygium aromaticum, and Vachellia nilotica demonstrate potential antibacterial effects. The lowest MIC of several extracts is derived from the active medicinal botanical Allium sativum.

**Author Contribution**

The Conception and design: Haleema Bibi. Collection of Sample Size and assembly of data: Analysis and interpretation of the data: Drafting and Critical revision of the article for important intellectual content: Rashid Waqas. Statistical expertise and final approval and guarantor of the article.

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