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Unveiling the Antimicrobial Potential of Homoeopathic Sarcode and Bowel Nosode: A Study on *Histaminum* and *Dysenteriae Co* against Escherichia Coli And Klebsiella Bacteria

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ABSTRACT

Background - There has been a rapid emergence of antibacterial resistance in the past two decades showing the rapid shift in the epidemiology of bacterial infection. **Objective** - The current study aimed at screening various Homoeopathic dilutions of *Histaminum* and *Dysenteriae co* for their antimicrobial activity against Escherichia coli and Klebsiella. **Methods** - The antibacterial activity of Homoeopathic dilutions in 12C, 30C, and 200C potency of Sarcode *Histaminum* and Bowel Nosode *Dysenteriae co* was assessed on clinical isolates of Escherichia coli and Klebsiella. **Results** - Limited reduction in the growth of Bacteria was observed by *Histaminum* and *Dysenteriae co* in all of the selected potencies as compared to 90% alcohol. **Conclusion** – Both sarcode *Histaminum* and Bowel nosode *Dysenteriae co* had limited antibacterial activity against E. coli and Klebsiella.

Keywords: Antimicrobial activity, Escherichia coli, Klebsiella, *Histaminum, Dysenteriae co*.

INTRODUCTION

Escherichia coli is a non-pathogenic, anaerobic flora of the human intestine. Few E. coli strains have developed the ability to cause disease of the gastrointestinal, urinary, or central nervous system in even the most robust human hosts[1]. Infants are more susceptible to E. coli diarrhoea as they have not yet developed immunity. In adults, it causes traveller's diarrhoea due to the release of exotoxins. Reabsorption of Na+ and $Cl^-$is inhibited by these exotoxins, and secretion of Cl^- and $HCO3^-$ into the intestinal lumen is stimulated resulting in watery diarrhoea of 20 litres per day and loss of electrolytes. Enterohemorrhagic E. coli (EHEC) secretes a powerful Shiga-like toxin (verotoxin), that inhibits protein synthesis by inhibiting the 60S ribosome, which results in haemorrhagic colitis with bloody diarrhoea. Infection by the strain of EHEC, O157:H7, results in Haemolytic uremic syndrome, with anaemia, thrombocytopenia, and renal failure. E. coli is the most common cause of Urinary Tract Infection, causing cystitis or pyelonephritis. Gram-negative sepsis, septic shock, and pneumonia in debilitated hospitalized patients are commonly caused by E. coli [2]

Klebsiella - Klebsiella are encapsulated (O antigen), Gram-negative bacilli; fimbriated, non-sporing, non-motile (no H antigen) that causes Klebsiella pneumonia, rhinoscleroma with foul-smelling nasal discharge, sepsis, and UTI. In Klebsiella pneumoniae, sputum looks like red currant jelly, due to the presence of an O antigen capsule. During the culture, string test is positive. In Nutrient agar and MacConkey, convex, viscid, large mucoid colonies are seen [2]

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Klebsiella are opportunistic human pathogens that can be isolated from various animal and human clinical specimens. They are responsible for seven to ten percent of all hospital-associated bloodstream infections. Antimicrobial resistance represents a serious problem in this bacterial group, especially due to the increasing prevalence of extended-spectrum β-lactamase (ESBL)-producing isolates[3]

Laboratory diagnosis of E. coli is by culture of urine, stool, pus, CSF, blood, sputum, peritoneal exudate, etc. Antimicrobial susceptibility is determined by Mueller-Hinton agar. Whereas Klebsiella on MacConkey agar produces large, dome-shaped, sticky, pink colour lactose-fermenting colonies [4]. In conventional treatment, chloramphenicol, gentamicin, ampicillin, nalidixic acid, furadantin etc are used as antibiotics. E. coli is abundant in the human gastrointestinal tract and also serves as a reservoir or carrier of antibiotic-resistant genes, especially to third-generation cephalosporins. The resistance of Klebsiella strains to different generations of cephalosporins, especially the third generation, was first reported in 1981; since then, these bacteria have become more resistant to antibiotics[5].

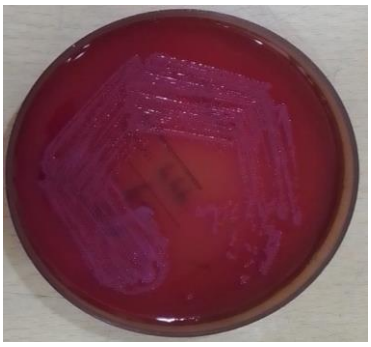

Figure (1) Heavy growth of E.coli

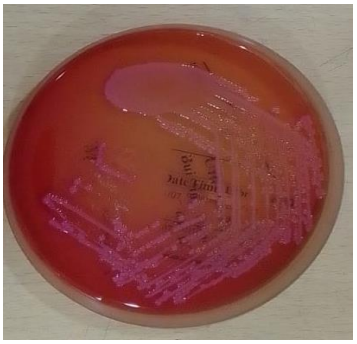

Figure (2) Heavy growth of Klebsiella

Relevance of antimicrobial study in current times.

The first chemotherapeutically effective antibiotic penicillin was discovered by Alexander Fleming in 1929. There are several ways of classifying antibiotics, but the most common classification schemes are based on their molecular structures, mode of action, spectrum of activity, route of administration, and chemical or molecular structures [6]

The major antibiotics are the following[7]

Table – (1) Antibiotics

Antibiotic	Activity
Penicillin	Anti-bacterial
Cephalosporin	Anti-bacterial

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Streptomycin	Anti-bacterial
Erythromycin	Anti-bacterial
Tetracycline	Anti-bacterial
Gentamycin	Anti-bacterial
Terramycin	Anti-bacterial
Chloramphenicol	Anti-bacterial
Rifampin	Anti-bacterial
Neomycin	Anti-bacterial

One of the main causes of death and morbidity worldwide is the rising burden of infectious illnesses that have a substantial effect on community health all over the world. Bacterial infections are a global issue due to the careless use of antibiotics, which causes resistance to emerge quickly. Antibiotic-resistant illnesses place a heavy strain on nations whose healthcare systems are already overworked. The resistant organism has increased the risk and expense of current treatment approaches[8]**.** Antimicrobial resistance (AMR) had a global incidence and mortality rate of 1.27 million and 5 million in 2019, respectively. AMR in India is caused by six pathogenic microorganisms, including Escherichia coli and Staphylococcus [9].

Antibiotics as chemotherapeutic agents, in the host, sometimes produce undesirable side effects like allergic reaction or sensitivity, nerve damage, and irritation of the gastrointestinal tract or kidneys. The normal microbial flora of the host should not be eliminated by these antibiotics [10]. The usage of antibiotics inevitably leads to the evolution of antibiotic resistance. The replacement of vulnerable pathogens with more resistant species or variations has persisted, and clinical isolates are becoming more and more multidrug-resistant (MDR). Methicillin-and multiply-resistant S. aureus (MRSA), vancomycin-resistant enterococci (VRE), penicillin-resistant pneumococci, and multidrug-resistant Gram-negative bacilli like Escherichia coli, Klebsiella, Enterobacter, and Pseudomonas aeruginosa are examples of multidrug-resistant (MDR) organisms that cause serious hospital or community infections and are currently difficult to treat[11]. When antibiotic therapy is not administered immediately via an appropriate route or is ineffective in vitro against the infecting organism, it is considered "inappropriate." [12].

Data from other developed nations suggest that 80% or more of antibiotic use occurs among outpatients. Reducing inappropriate use is essential to reduce both antibiotic resistance and adverse events[13]. Addressing misuse of restricted antibiotics is problematic and requires improved compliance with the pre-approval process and more effective measures to identify and limit their ongoing, unnecessary use[14]. The World Health Organisation (WHO) has long advocated for the introduction of essential medicines policies to encourage the rational use of medicines. These include specific measures to promote the prudent use of antibiotic drugs to minimize the development of resistance[15].

Significance of Homoeopathic Sarcode *Histaminum* and Bowel Nosode *Dysenteriae Co.*

Histaminum

The symptomatology of *Histaminum* was established in 1950 by J Gringuaz of Beunos Aires on thirty-nine provers using the potencies 3C, 6C, 12C, 30C, 200C, and 1M. It is an arteriole vessel constrictor, a capillary vessel dilator, and a hypertensive. It constricts the bronchi and stimulates the tissue of the uterus, intestine, gastric, and pancreatic salivary secretions. It is normally used in the form of histamine bi-chlorhydrate, which serves as the stock for the Homoeopathic preparations. Symptoms are pruritus and burning of the nose, throat, and ears. Taste is lost. All the symptoms of skin and mucous membrane are allergic type. The mucus

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membrane becomes dry, and there is burning and redness of the skin. The remedy relationship of *Histamine* is *Apis mel* in acute inflammations, *Ars alb* in anxiety and burning pains, and *Secale* in haemorrhages. [16].

Dysenteriae co

In the early 1900s, British pathologist and bacteriologist Edward Bach D.O. (1886-1936) began to associate the presence of certain bacteria found in the large intestine with chronic disease. Within several years, these organisms were classified and prepared as homoeopathic potencies collectively called the 'bowel nosodes'. From the beginning, practitioners using these nosodes found great success in the treatment of all types of chronic disease[17]. The bacterial content of stool included coliforms, streptococci, and spore-bearing organisms, but also non-lactose-fermenters. If the person was placed on an uncooked vegetarian diet for several weeks, non-lactose fermenting bacilli were absent in the stool. The non-lactose fermenting bacilli were not strictly pathogenic, but a source of toxins. They stressed that the corrective diet to contain nuts, cereals, and bananas. These stimulated the growth of Lactobacilli. The vaccines were prepared from a colony of the non-lactose-fermenting bacillus[18].

Dr Bach started an investigation about the role of intestinal bacteria in the pathogenesis of chronic diseases. He found that certain intestinal germs which belong to the non-lactose fermenting, gram-negative coli typhoid group have a close association with the pathogenesis of chronic disease. He successfully treated many chronic cases by potentising various non-lactose fermenting bowel bacteria and administering them clinically[19].

Research on bowel nosodes was first conducted by Dr. Edward Bach (1886–1936) and his coworker, Dr. John Paterson (1890–1955). The first full preparation of clinical proving was conducted in 1929 by Thomas Dishington[20].

John Paterson (1890- 1955), a co-worker of Bach, began his research on bowel nosodes and studied more deeply the characteristics of the bowel flora, especially their behaviour in health, disease, and drug proving. He conducted research for about 20 years and examined around 20,000 stool specimens. He concluded that the non-lactose fermenting non-pathogenic bowel flora undergoes definite changes in the disease condition, and the balance of the bowel flora is disturbed in the disease. Paterson advocated specific recommendations on potency, dose, and repetition of bowel nosodes. He was the one who related each of the bowel nosodes to a group of homoeopathic remedies[21]..

Dysenteriae co is a bowel nosode prepared from Bacillus dysenteriae. Symptoms include nervous tension, very sensitivity to criticism, anxiety, and shyness; pylorus-specific activity resulting in spasms and retention of digested matter. Gastric symptoms worsen from 12 midnight to 1 a.m. Inflammation of the pharynx, eyes, and nose. Associated remedies are Arg. nit, Ars alb, and Kalmia.[22]

Homoeopathic medicines particularly bowel nosodes and microorganisms

During the era when germ theory was gaining prominence, Dr. Bach, a pathologist and bacteriologist, recognized a link between gut flora and overall health. Inspired by The Organon of Medicine by Dr. Samuel Hahnemann, Bach saw similarities between the homoeopathic law of similars and conventional vaccination principles. He began to potentize bacterial cultures taken from the human intestine, transforming them into nosodes rather than traditional vaccines. After treating over 500 patients with these nosodes, Dr. Bach observed a 95% improvement rate over ten years, publishing his findings in 1920[23].

Dr. Edward Bach, Dr. John Patterson and his wife Elizabeth Patterson, Dr. Wheeler, and Dishton all worked on this. Dr. Bach and Dr. Patterson identified various types of bacteria in stool samples from hospitalized patients, which they isolated and studied to produce these nosodes. Bowel nosodes are believed to holistically support health by regulating organ function and promoting balance within the body.

The competition between pathogenic bacteria and commensal microbes—a crucial aspect of human health—plays a central role in gut health. When harmful bacteria find favourable conditions in the gut, they may

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proliferate, disrupting the balance of beneficial bacteria and occasionally causing neutral microbes to behave pathologically. This disruption can increase the body's susceptibility to disease[23].

Need for study

Gaining a deeper comprehension of the existing techniques for evaluating and measuring the antimicrobial effi cacy of an extract or a pure compound for use in human health, agriculture, and the environment is crucial, as t he attributes of novel antimicrobial products, such as their ability to combat bacteria resistant to multiple drugs , are increasingly appealing.[24].

Different manuals of phytotherapy report the use of cranberry juice (Vaccinium macrocarpon) and bearberry (Arctostaphylos uva-ursi) to treat urinary tract infections, while species like garlic (Allium sativum), lemon balm (Melissa officinalis), and tea tree (Melaleuca alternifolia) are described as broad-spectrum antimicrobial agents. Homoeopathic medicines have antibacterial activity against "Klebsiella pneumoniae". Klebsiella infections can be successfully treated with varying doses of Senega, sulphur, and Lobelia inflata[25]. Similarly, against Staphylococcus species, with an average zone of inhibition peaking at 8.7±1.15 mm. Additionally, Graphites exhibited antibacterial activity with a larger mean zone of inhibition of 7.7±0.58 against Salmonella spp. (30C and 200C), Vibrio spp. (30C), and Pseudomonas spp. (30C and 200C). Along with Staphylococcus, Pulsatilla 30C inhibited the growth of Salmonella and Pseudomonas; Apis mellifica 30C had an inhibitory effect against Pseudomonas.[26]

Numerous in vitro and in vivo studies, as well as case reports, have looked at the homoeopathic remedy Silicea terra's antibacterial qualities; these studies typically involve the remedy in combination with other therapies. These studies assessed the homoeopathic medicine's efficacy against a variety of pathogens, focusing on the drug's antibacterial activity and minimum inhibitory concentration, or MIC [27]. Water, positive and negative controls, and alcohol (10%, 30%, and 90%) as the vehicle control were employed in the comparison. Before this experiment, a pilot study was conducted to evaluate the inhibitory effect of 90% alcohol-prepared nosodes, to determine if the alcohol (vehicle) itself possesses microbial inhibitory activity. Nosodes produced in 10% and 30% alcohol were employed as test samples since it was observed that 90% vehicle alcohol hindered the development of E. coli and S. typhi, respectively.

It's important to note that even in cases where the antibiotics (ceftriaxone, ofloxacin, amoxicillin) did not cause the inhibitory impact, K. pneumoniae and E. coli polyvalent nosode have demonstrated inhibitory activity against K. pneumoniae species growth. In many trials, nosodes exhibited inhibitory activity akin to that of antibiotics such as ciprofloxacin, ofloxacin, amoxicillin, meropenem, and ceftriaxone. This observation could potentially stimulate further exploratory studies in this domain[28]

These days, the invasion of an organism's body tissue by the pathogen, its growth, and the host tissue's response to the pathogen and the toxins it produces are all considered infectious diseases. Infectious diseases account for 43% of the worldwide disease burden, according to the WHO. Various microorganisms are responsible for causing it, with "Klebsiella pneumoniae" being one of the main culprits.

MATERIAL AND METHODS

Materials

Homoeopathic medicine: Homoeopathic medicine *Histaminum* and *Dysenteriae co* in 12C, 30C, and 200C potencies were purchased from SBL Pharmaceuticals India Pvt Ltd.

Test organisms: Klebsiella and the Human enteric pathogen Escherichia coli O157:H7 were purchased from HiMedia Laboratories Pvt. Ltd.

Media used for antibacterial sensitivity test: Müeller-Hinton Agar (MHA) Medium and SDA medium were purchased from HiMedia Laboratories Pvt. Ltd.

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Methods

Handling of laboratory apparatus and glassware: Before use, all glassware was rinsed twice in distilled water after being washed with mild detergents four or five times in tap water. It was then allowed to air dry. Glassware such as Petri plates was heat sterilised where necessary in a hot air oven (Binder ED23, Germany) at 180°C for 1 hour before use. Sterilization was accomplished by autoclaving Eppendorf tubes, glass pipettes, and micropipette tips at 121°C for 15 pounds per square inch (approx. 10500 kilograms-force per square meter). (Hirayama, Model HA-300M, Japan).

Preservation and Maintenance of E. coli and Klebsiella: Nutrient broth was used for the general consideration of a wide variety of microorganisms. Using the streak plate approach, E. coli and Klebsiella were sub cultured on plates for pure colonies in nutrient agar plates and preserved in nutrient agar slants.

Preparation of inoculum: A small portion of the colony is suspended in a nutrient broth medium in aseptic conditions for 24 hours at 32°C.

Preparation of Mueller Hinton Agar plates: 38 grams of the medium are dissolved in one litre of distilled water to create the agar. To completely dissolve the medium, it is heated while being stirred frequently and cooked for one minute, followed by a 15-minute autoclave at 121°C and cooling to room temperature. Mueller Hinton Agar should be poured onto sterilized petri plates on a level, horizontal surface at a constant depth. At 25°C, it is ensured that the pH is 7.3 ± 0.1. The plates are stored between two and eight degrees Celsius. The MHA culture was compared with McFarland standards (108 CFU/ml).

Inoculation of previously prepared corresponding plates: After adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swabbing was done uniformly to produce a uniform lawn of bacteria on the MH plate. This procedure was repeated by streaking two more times, rotating the plate approximately 60 degrees each time.

Impregnation of the sterile disc into media: The Sterile discs are impregnated into the corresponding media, MH media for bacteria, using sterile forceps which is alcohol sterilized, followed by heat sterilization after every use.

Loading of sterile discs with Medicine: According to the standardized single disc diffusion method, the discs (6mm) were loaded with 10µl of the corresponding test medicine or control.

Four plates were set up for each organism. In the first plate, after inoculation and impregnation of sterile discs, the discs were loaded with 10µl of one of the following: *Histaminum* 12C, *Histaminum* 30C, *Histaminum* 200C, positive control, and negative control. In the second plate, after inoculation and impregnation of sterile discs, the discs were loaded with 10µl of one of the following: *Dysenteriae co* 12C, *Dysenteriae co* 30C, *Dysenteriae co* 200C, positive control, and negative control, respectively.

This protocol was followed for E. coli and Klebsiella.

The positive controls used against E. coli, and Klebsiella were Cefixime and Azithromycin, respectively. Each tablet was separately powdered and dissolved in distilled water. 10µl of the well-mixed solution was loaded into separate sterile discs and used as a positive control in the corresponding plates.

The negative control used was 10µl of Ethanol.

Incubation of loaded plates: The bacterial plates were incubated at 37°C for 24 hours.

Evaluation of the antibacterial activity of homoeopathy medicines: The zones of inhibition were measured in millimetres with a standard ruler close to the agar surface. The results are recorded, and the antibacterial activity of the homoeopathic medicines against the test organisms used was determined. The growth inhibition as seen by the naked eye was considered the endpoint.

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Figure (3) positive control – Azithromycin solution

RESULTS

Antimicrobial activity of different potencies of *Histaminum* and *Dysenteriae co* against Escherichia coli and Klebsiella bacilli are as follows -

Table (2) – Zone of Inhibition by various potencies

Sl no	Bacteria	Medicine	Zone of inhibition (cm)
1.	Klebsiella	*Dysenteriae co* 12 C	0.4
2.	Klebsiella	*Dysenteriae co* 30C	0.6
3.	Klebsiella	*Dysenteriae co* 200C	0.5
4.	Klebsiella	Ethanol (Negative Control)	1.0
5.	Klebsiella	Azithromycin (Positive control)	1.4
6.	Escherichia coli	*Dysenteriae co* 12 C	0.4
7.	Escherichia coli	*Dysenteriae co* 30C	0.4
8.	Escherichia coli	*Dysenteriae co* 200C	0.6
9.	Escherichia coli	Ethanol (Negative Control)	0.8
10.	Escherichia coli	Cefixime (Positive control)	0.8
11.	Escherichia coli	*Histaminum* 12 C	0.6
12.	Escherichia coli	*Histaminum* 30C	0.6
13.	Escherichia coli	*Histaminum* 200C	0.2
14.	Escherichia coli	Ethanol (Negative Control)	0.4
15.	Escherichia coli	Cefixime (Positive control)	0
16.	Klebsiella	*Histaminum* 12 C	0.4
17.	Klebsiella	*Histaminum* 30C	0.3

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18.	Klebsiella	*Histaminum* 200C	0.4
19.	Klebsiella	Ethanol (Negative Control)	0.5
20.	Klebsiella	Azithromycin (Positive control)	1.1

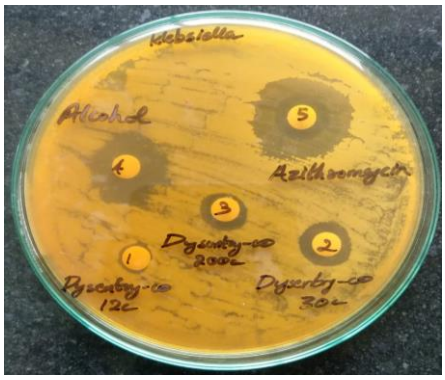

Figure (4) Plate 1- *Dysenteriae co* against Klebsiella

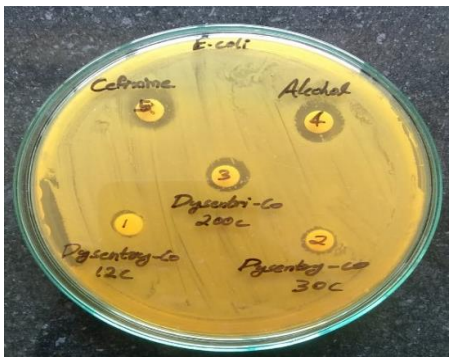

Figure (5) – Plate 2 - *Dysenteriae co* against E.coli

Figure (6) – Plate 3 - *Histaminum* against E. coli

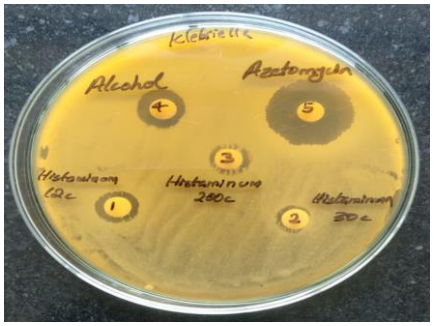

Figure (7) – Plate 4 - *Histaminum* against Klebsiella

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Plate (1) - Considering the zone of inhibition, *Dysenteriae co* 30 has the maximum antibacterial activity against Klebsiella, followed by 200th potency and then 12C potency. Azithromycin was used as the positive control. **Plate (2)** - *Dysenteriae co* 200 showed maximum antimicrobial activity against E. coli, followed by 30C and 12C. Cefixime was taken as a positive control. **Plate (3**) - *Histaminum 12C* and 30C showed greater antibacterial activity against E. coli than its 200th potency. Here, Cefixime, the antibiotic that was used as a positive control, had no zone of Inhibition. So, its antimicrobial resistance nature is further to be tested. **Plate (4)-** *Histaminum 12C* had greater antibacterial activity against Klebsiella than its 30th and 200th potencies. The positive control used was Azithromycin.

DISCUSSION

This study aimed to assess the antimicrobial potential of *Dysenteriae co* and *Histaminum* against Klebsiella and Escherichia coli. The results showed small zones of inhibition, ranging from 0.4 cm to 0.6 cm, which were less than those produced by positive control and negative controls - Azithromycin and Cefixime. These findings suggest that while there is some activity, the antimicrobial potential of these homeopathic remedies is limited. Even though antibiotics are powerful in preventing infectious diseases, certain antibiotics are being threatened because of the multidrug-resistant strains of the microbes. Thus, compared with the expensive antibiotics, the potency of *Histaminum*, possessing limited antimicrobial activity against E. coli, needs further investigation.

Potential sources of bias include variations in the preparation of the remedies, strain-specific bacterial responses, and the possibility that the concentrations tested were not ideal for the strains used.

The lack of significant antimicrobial activity observed in this study aligns with its literature, which has not reported any antimicrobial activity. While some studies using homoeopathic drugs report effects, most fail to show substantial antimicrobial action. This suggests that homeopathic remedies like *Dysenteriae co* and *Histaminum* may not work in the same way as conventional antibiotics, which have well-established mechanisms such as cell wall disruption and protein synthesis inhibition.

Given the in vitro nature of the study and the use of only two bacterial strains, the findings have limited generalizability. The results may not necessarily reflect how these remedies would behave in vivo, and their bioavailability or effectiveness in the human body remains uncertain. Focusing on just two remedies and bacterial strains limits the broader applicability of these findings to the full range of homeopathic medicines and pathogens.

The Kirby-Bauer zone of inhibition method was chosen because it is the gold standard in antimicrobial activity testing, known for its simplicity and reliability. However, this method may not fully capture the nuanced effects observed to be exerted by homeopathy, especially the energetic and subtle mechanisms of action. Future research could explore alternative models that more accurately reflect homeopathic principles and offer a more comprehensive understanding of the potential antimicrobial effects.

CONCLUSION

This study observed that homeopathic medicines exhibit limited antibacterial activity, suggesting that further clinical studies are needed to validate the role of homeopathic treatment in managing a wide range of pathogen-related medical conditions. This work aims to strengthen the evidence base for homeopathy as a holistic science by demonstrating the potential of homeopathic medicines in inhibiting bacterial growth.

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Conflicts of Interest - None declared.

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