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Assessing the efficacy of homoeopathic medicine psorinum against *Escherichia coli*: An *in-vitro* analysis of antimicrobial activity

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Abstract

Antibiotic resistance in *E. coli* is a global health concern, with the MCR-1 gene detected in China and a polymyxin B-resistant strain in Brazil. Novel drug discovery approaches and alternative therapies like homoeopathy can help especially using nosodes. Even though Psorinum is a powerful Homoeopathic nosode, it has little to no studies on its antimicrobial activity.

Antimicrobial activity of Psorinum 30C and 200C against *Escherichia coli* O157:H7 is tested using the Kirby-Bauer method with Müeller-Hinton Agar (MHA) as the medium. The zone of inhibition and relative growth inhibition percentage is analysed.

Psorinum 200 C showed significant zone of inhibition with relative growth inhibition of 28.57% while Psorinum 30 C showed mild to moderate zone of inhibition with relative growth inhibition of 7.14%.

Keywords: Antimicrobial, E. coli, homeopathy, nosode, psorinum

Introduction

Escherichia coli (*E. coli*) is a bacterium that is commonly found in the gut of humans and warm-blooded animals. Most strains of *E. coli* are harmless. Some strains however, such as Shiga toxin-producing *E. coli* (STEC), can cause severe foodborne disease. It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and sprouts [1].

The potency of *E. coli* enables it to resist the natural defences of the host and become immune to commonly used antibiotics. As a result, outbreaks of *E. coli* infection can pose significant risks to public health and food safety ^[2].

The emergence of antibiotic resistance in *E. coli* isolates is a global health concern. In China, the MCR-1 gene has been detected, conferring resistance to colistin ^[3]. In Brazil, there is emergence of a polymyxin B-resistant strain of *E. coli*, which could have potential impact on patient outcomes and global health ^[4]. Novel approaches to drug discovery and finding new drugs can help reduce the risk of antibiotic resistance ^[5] and alternative strategies like homoeopathy can offer options for minor infections like Upper Respiratory Tract Infections ^[6]. Additionally, new antimicrobials developed against *E. coli* can be used to control other microbes, as some pathogens share similar mechanisms of antimicrobial resistance and new drugs with antimicrobial activity against *E. coli* can help reduce the use of broad-spectrum antibiotics and minimise their negative side effects ^[7].

In general, the identification of novel medications that exhibit antimicrobial effects against *E. coli* is vital for safeguarding public health and combating antibiotic-resistant bacteria.

Antimicrobial action of homoeopathic medicines

In the 18th century, Dr. Christian Friedrich Samuel Hahnemann, a German physician, developed homoeopathy^[8]. Homoeopathy is one of the most extensively practised forms of traditional and complementary medicine^[9] and is recognised by the WHO as the second most popular medical system in the world^[10]. One of its basic principles is 'Similia similibus curentur' or 'diseases are cured by similarity of symptoms'. In homoeopathy, chemicals are prepared whose effects, when given to healthy persons, correlate to the symptoms of the disease. (Symptoms, clinical signs, pathological states)^[11].

By reducing the growth of *Aspergillus niger* in an *in vitro* investigation, homoeopathic medications have proven their antifungal effectiveness ^[12]. Tinctures made from mineral extracts have stronger antibacterial properties than those made from plant extracts, and a homoeopathic preparation of *Lycopodium clavatum* can effectively control periodontal infections like P. gingivalis ^[13]. Additionally, homoeopathic medicines have shown *in vitro* and *in vivo* antiviral effectiveness against the BVDV-1 and BHV-1 viruses ^[14]. Homoeopathic medicines used to treat infections are not antibiotics but rather similibiotics (similar to bacteria), meaning that we give the patient medicine that can cause symptoms in them that are similar to those produced by bacteria in order to activate the host's defence system, which in turn kills the bacteria ^[15].

Nosodes in homoeopathy are prepared from diseased tissue and used to treat diseases with similar symptoms as per drug proving or pathogenesis. Though treating a specific infection is called isopathy, various nosodes are used for their corresponding diseases without being labelled as isopathic. The category of nosodes is undergoing a revolutionary overhaul of the old drugs (Mycobacterium tuberculosis nosode, Cancer nosode), new remedies (HIV nosode, Hepatitis C nosode, *Escherichia coli* nosode) with efficacy in laboratory and *in vitro* models, and human studies. One animal trial showed that piglets in the homoeopathy-treated group had significantly less *E. coli* diarrhoea than those in the placebo group. In a study, nonnosode drugs were observed to have no significant inhibitory effect ^[16].

Homoeopathy can be highly beneficial in preventing the development of bacterial resistance and mutations, thereby averting the emergence of dangerous new types of infections within society. More research is required in this uncharted area of study in order to offer new hope in situations where bacteria are resistant to all known antibiotics.

Psorinum - a potential antimicrobial nosode

Psorinum is a nosode, a product of an exudate in patients suffering from itch eruptions. It's prepared according to the Hahnemannian method of preparation of drugs class IX in the ratio of 2:9. The exudate is collected from the itch pustule of an infected person, and kept in a phial adding some alcohol. After shaking it well, it is allowed to stand. It is proved by Constantine J Herring ^[17].

The Repertory of the Homoeopathic Materia Medica by J.T Kent has mentioned Homoeopathic medicine Psorinum as a 3-mark remedy in 'Fever' chapter under the rubrics 'Intermittent' and 'Remittent - prone to become typhoid' ^[18].

Psorinum in higher potencies has relatively low potential in controlling fungal growth in cellulose materials compared to other nosodes ^[19]. At the same time, Psorinum has prominent action against lung cancer cells in lower potency ^[20]. The antimicrobial potential of Psorinum in various potencies needs to be further explored in light of its homoeopathic indications and its nature as a nosode.

Homoeopathic indications of psorinum in microbial affections

 Herpetic eruptions, especially on scalp and bends of joints with itching; worse, from warmth of bed. Indolent ulcers, slow to heal. Crusty eruptions all over. Urticaria after every exertion. Pustules near fingernails. Tongue and gum ulcerated. Blepharitis ^[21].

- Hay fever, red small pimples on, pyorrhea in mouth, chronic gonorrhoea; painless discharge from urethra staining yellow^[22].
- Otorrhoea with headache^[23].

Materials and Methods

Materials

- **Homoeopathic medicine:** Homoeopathic medicine Psorinum in 30th and 200th potencies are purchased from Schwabe pharmaceuticals India Pvt Ltd.
- **Test organism:** Human enteric pathogen *Escherichia coli* O157:H7 is purchased from HiMedia Laboratories Pvt. Ltd.
- Media used for antibacterial sensitivity test: Müeller-Hinton Agar (MHA) Medium is purchased from HiMedia Laboratories Pvt. Ltd.

Methods

- Handling of laboratory apparatus and glassware: All glassware was cleaned with mild detergents four or five times in tap water, then rinsed twice in distilled water prior to use. Then it was permitted to air dry. Glassware, such as Petri plates, were, when required, heat sterilised for one hour at 180°C in a hot air oven (Binder ED23, Germany). Eppendorf containers, glass pipettes, and micropipette tips were autoclaved at 121°C for 15 minutes at 15 pressure to sterilise them. (Hirayama, Model HA-300M, Japan).
- **Preservation and Maintenance of** *E coli*: For the broad consideration of a wide range of microorganisms, nutritional broth is used. *E. coli* was sub cultured on plates for pure colonies in nutrient agar plates and stored in nutrient agar slants using the streak plate method.
- **Preparation of inoculum**: A small part of the colony is suspended for 24 hours at 32°C in nutrient broth medium under aseptic conditions.
- Preparation of Mueller Hinton Agar plates: 38 grams of the medium are dissolved in one litre of distilled water to make the agar. The medium must be heated while being constantly stirred and cooked for one minute before going through a 15-minute autoclave at 121°C and cooling to room temperature in order to fully dissolve it. Mueller Hinton Agar needs to be applied to sterile petri dishes at a constant depth and on a level, horizontal surface. It is guaranteed that the pH is 7.3 ± 0.1 at 25°C. The temperature range for the plates is 2 °C to 8 °C ^[25]. The McFarland standards (108 CFU/ml) were used to evaluate the MHA culture ^[26].
- **Inoculation of previously prepared MHA plates:** A sterile cotton swab was dipped into the inoculum suspension after the turbidity had been adjusted. Multiple rotations of the swab were performed while it was firmly pressed against the interior tube wall above the fluid level. The swab was used to inoculate the dry surface of an MHA plate by streaking the sterile agar's surface. Streaking was carried out twice more, with the plate being rotated by roughly 60 degrees each time.
- Impregnation of disc with Psorinum: In a hot air oven (Barnstead Labline, USA), discs (6mm) were impregnated with 200 µl of each homoeopathic

medication individually, dried at 40 °C for 30 minutes, and then stored at 4 °C until use. Only 95% ethanol was used to make the negative control.

Evaluation of antibacterial activity of homoeopathy . medicines: With a standard ruler held near to the agar surface, the zones of inhibition were measured in millimetres (including the 6mm disc). The findings are

Results



Fig 1: Antibacterial assay of Homoeopathic potencies of Psorinum and control by Kirby Bauer method in Escherichia coli.

 Table 1:
 Zone of inhibition of psorinum and control by kirby bauer method

Sl.no	Homoeopathic medicine	Zone of inhibition (mm)
1	Psorinum 30	$7.5 \pm 0.1 \text{ mm}$
2	Psorinum 200	$9.0 \pm 0.1 \text{ mm}$
3	95% Ethanol (control)	$7.0 \pm 0.1 \text{ mm}$

Homoeopathic medicine Psorinum 30 showed 7.5 ± 0.1 mm of zone of inhibition, Psorinum 200 showed 9.0 ± 0.1 mm of zone of inhibition, and 95% Ethanol (control) showed 7.0 \pm 0.1 mm of zone of inhibition.

Table 2: Relative growth inhibition percentage of different
 potencies of psorinum

Sl.no	Homoeopathic medicine	Relative growth inhibition percentage (%)
1	Psorinum 30C	7.14285714 %
2	Psorinum 200C	28.5714285714 %

Homoeopathic medicine Psorinum 30C showed a relative growth inhibition of 7.14285714% while Psorinum 200C showed a relative growth inhibition of 28.5714285714%.

Discussion

Escherichia coli, one of the most prevalent bacteria, was used in this investigation to assess the antimicrobial characteristics of the homoeopathic medicines Psorinum 30C and 200C. A higher potency, Psorinum 200, showed 9.0 ± 0.1 mm of zone of inhibition with relative growth inhibition of 7.14285714% while Psorinum 30 showed 7.5 \pm 0.1 mm with relative growth inhibition of 28.5714285714%.

noted, and it is determined whether the homoeopathic medications have any antibacterial action against the tested organisms. The end point was regarded as the growth inhibition visible to the unaided eye.

Determination of percentage of zone of inhibition The equation below is used to calculate relative growth inhibition:

(%)Relative Growth Inhition = $\left(\frac{\text{Diameter of Sample - Diameter of Control}}{\text{Diameter of Control}}\right)$ 100 Diameter of Control

> Antimicrobial activity of 95% Ethanol can be attributed to the 7.0 ± 0.1 mm zone of inhibition shown by the control. Both potencies of Psorinum were able to produce mild to moderate zone of inhibition against Escherichia coli when compared to other trials using different homoeopathic drugs [16] Homoeopathic medicine Psorinum 200C has significantly higher antimicrobial activity than Psorinum 30C with mild to moderate zone of inhibition.

> To further examine the extent of Psorinum's antibacterial capabilities, the Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) must be performed.

Conclusion

In conclusion, the in vitro testing of Psorinum, a homoeopathic drug, against E. coli revealed modest to moderate zones of inhibition using the Kirby Bauer method. These findings suggest that Psorinum might be able to combat E. coli as an antibacterial agent. However, more analysis is required to determine Psorinum's efficiency in clinical situations. Future research should also focus on developing new medications that can fight off other pathogenic microbes that have similar antimicrobial resistance mechanisms. This would aid in reducing the usage of broad-spectrum antibiotics, which have negative effects and contribute to the emergence of antibiotic resistance. In the fight against bacteria that are resistant to antibiotics, discovering powerful as well as safe antimicrobial medicines is essential and Homoeopathy has a lot of potential in this area.

Conflict of Interest

Not available

Financial Support

Not available

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