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An *in-vitro* study of antimicrobial activity of homoeopathic medicine pyrogenium against *Escherichia coli*

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Abstract
The discovery of new drugs with antimicrobial activity against *E. coli* is crucial for public health and the fight against other antibiotic-resistant bacteria with the MCR-1 gene detected in China and a polymyxin B-resistant strain in Brazil. Novel approaches to drug discovery and alternative medicine like Homoeopathy possess immense potential in this regard. Studies have proved significant antimicrobial action in mineral and plant based Homoeopathic medicines. Homoeopathic nosode Pyrogenium known as 'dynamic antiseptic' with homoeopathic literature to support might have antimicrobial potential in higher potencies.
Antimicrobial activity of Pyrogenium 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.
Pyrogen 200 C showed a negligible zone of inhibition with negligible relative growth inhibition while Pyrogenium 30 C showed mild to moderate zone of inhibition with relative growth inhibition of 7.14%.

Keywords: Antimicrobial, *E. coli*, homeopathy, nosode, pyrogenium

Introduction
Escherichia coli (*E. coli*) is a gram-negative bacillus known to be a part of normal intestinal flora but can also be the cause of intestinal and extraintestinal illness in humans. It can be transmitted via contaminated food and water, making it the most common cause of food borne illnesses. There are hundreds of identified *E. coli* strains, resulting in a spectrum of disease from anaemia, mild urinary tract infections to severe bloodstream infections, self-limited gastroenteritis to renal failure and septic shock [1, 2].

E. coli: A subtle but dangerous microorganism
E. coli's virulence lends to its ability to evade host defences and develop resistance to common antibiotics and thus outbreaks of *E. coli* infection can have serious consequences for public health and food safety [3].
E. coli isolates in China show emergence of the MCR-1 gene, which confers resistance to the antibiotic colistin [4]. While in Brazil, there is emergence of a polymyxin B-resistant strain of *E. coli* with potential impact of antibiotic resistance on patient outcomes and possesses potential impact on global health [5]. Novel approaches to drug discovery and finding new drugs to combat antibiotic resistance can help reduce the risk [6]. Homoeopathy can offer alternative strategies for minor infections especially in Upper Respiratory Tract Infections [7]. Besides, new antimicrobials developed against *E coli* can be used to control other microbes as some pathogens share similar mechanisms of antimicrobial resistance. Furthermore, new drugs that have antimicrobial activity against *E. coli* could help reduce the use of broad-spectrum antibiotics, which can have negative side effects and contribute to the development of antibiotic resistance [8].
In short, the discovery of new drugs with antimicrobial activity against *E. coli* is crucial for public health and the fight against antibiotic-resistant bacteria and Homoeopathy can help in this domain.

Homoeopathy and its antimicrobial potential

Dr. Christian Friedrich Samuel Hahnemann, German physician discovered Homoeopathy in the 18th century [10]. Homoeopathy is ranked as the second-most popular system of medicine in the world by the WHO [11] and is one of the most widely used types of traditional and complementary medicine [12]. It is based on similia principle '*Similia similibus curentur*' that means diseases are cured by symptom similarity. Homoeopathy uses preparations of substances whose effects when administered to healthy subjects correspond to the manifestations of the disease (symptoms, clinical signs, pathological states) [10].

Homoeopathic medicines have demonstrated antifungal activity by an *in vitro* study inhibiting growth of *Aspergillus niger* [13]. Mineral extract tincture has a greater antibacterial activity than the plant extract tincture and Homoeopathic preparation of *Lycopodium clavatum* is an effective inhibitor of periodontal pathogens bacteria such as *P. gingivalis* [14]. Homoeopathic remedies have also demonstrated both *in vitro* and *in vivo* antiviral activity against BHV-1 and BVDV-1 viruses [15]. Several studies conducted on homoeopathic medicines (Aconite 30c, *Arsenicum album* 30c, *Mercurius corrosivus* 30c, *Aspergillus niger*) especially from plant and mineral sources have demonstrated antimicrobial activity [16].

Nosodes homeopathic remedies that are prepared using diseased tissue and are used to treat illnesses that share similar symptoms based on drug proving or pathogenesis. While the term "isopathy" is used for treating a specific infection, different nosodes are used to treat corresponding diseases without necessarily being classified as isopathic. The nosode category is currently experiencing significant changes, with new remedies such as the HIV and Hepatitis C nosodes being introduced and evaluated for their effectiveness through laboratory and *in vitro* models, as well as human trials. An animal study demonstrated that piglets that received homeopathy treatment experienced less *E. coli* diarrhoea than those given a placebo. In contrast, non-nosode drugs, such as *Apis mellifica*, Cantharis, and *Causticum hahnemanni*, were found to have no significant inhibitory effects in one study [16].

A study titled 'Antibacterial activity of homeopathic drugs *in vitro*' concluded that 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 and more research is needed in this unexplored field of study in order to offer new hope in situations where germs are resistant to all known antibiotics [17].

Pyrogenium: A potential antimicrobial nosode

Pyrogenium, also called pyrexin or pyrozen, is a nosode prepared from the product of decomposition of chopped lean beef in water, allowed to stand in the sun for two or three weeks. Its dilutions are made directly according to Burnett and without glycerine. It was proved by Dr. Drysdale, Dr. Burnett, Dr. Swan, Dr. Yingying, Dr. Sherbino, Dr. Heath, Dr. H.C. Allen [18] the drug Pyrogenium is indicated for all septic conditions in Homoeopathic literature. HC Allen says Pyrogenium has greatest action in septic fever especially in puerperal fever, he called pyrogen as "homoeopathic dynamic antiseptic" [19].

The Repertory of the Homoeopathic Materia Medica by J.T Kent has mentioned Homoeopathic medicine Pyrogenium as a 3 mark remedy in 'Fever' chapter under the rubrics 'Puerpural', 'Septic', and as a 2mark remedy in the rubric 'Zymotic' [20]. The term "zymotic" is an archaic medical term that was used in the 19th century to describe diseases caused by fermentation or the action of microbes, particularly those that were contagious or infectious. This term was used prior to the discovery of specific disease-causing microorganisms, such as bacteria and viruses, and was based on the observation that certain diseases seemed to spread rapidly and infectiously, similar to the way that yeast or other microorganisms cause fermentation. Now, the term "zymotic" is rarely used in medical contexts, as we now have a much better understanding of the specific microorganisms that cause infectious diseases and how they are transmitted. Instead, we use more specific terms such as "bacterial infection," "viral infection," "contagious disease," etc [21].

Pyrogen in lower potency (6C) have shown little to no zones of inhibition [22]. Meanwhile the *in vivo* antipyretic activity of Pyrogen in infective fevers has been established in ultrahigh homoeopathic dilutions [23]. Therefore higher potencies of Pyrogen might have antimicrobial potential. Also, homoeopathic literature and experimental studies hint at the possibility of positive antimicrobial activity.

Homoeopathic indications of pyrogen in microbial affections

- Pyæmia. Sepsis. Typhilitis. Labour - puerperal fever. Ovary, abscess of. Peritonitis. Ptomaine poisoning. Puerperal fever [24].
- All discharges are horribly offensive. Great pain and violent burning in abscesses. Chronic complaints that date back to septic conditions. Threatening heart failure in zymotic and septic fevers. Influenza, typhoid symptoms [25].
- For sapraemia or septicemia; puerperal or surgical from ptomaine or sewer gas infection; during course of diphtheria, typhoid or tyhpus; when the best selected remedy fails to > or permanently improve. 'homoeopathic dynamic antiseptic' [26].
- Septic puerperal infection. Pelvic cellulitis [27].

Materials and Methods

Materials

- **Homoeopathic medicine:** Homoeopathic medicine Pyrogenium 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:** 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 were heat sterilised

where necessary in a hot air oven (Binder ED23, Germany) at 180 °C for 1 hour prior to use. Sterilisation 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*:** Nutrient broth is used for the general consideration of a wide variety of microorganisms. Using the streak plate approach, *E coli* was sub cultured on plates for pure colonies in nutrient agar plates and preserved in nutrient agar slants.
- **Preparation of inoculum:** Small portion of the colony is suspended in 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 sterilised petri plates on a level, horizontal surface at a constant depth. At 25 C, it is ensured the pH is 7.3 ± 0.1. The plates are stored between 2 and 8 C [28]. The MHA culture was compared with McFarland standards (108 CFU/ml) [29].
- **Inoculation of previously prepared MHA plates:** After adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. The dried surface of an MHA plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60 degrees each time.
- **Impregnation of disc with Pyrogenium**: Following the standardized single disc diffusion method [30], the discs (6 mm) were impregnated with 200 µl separately of each homoeopathy medicine and were dried at 40 °C for half an hour in a hot air oven (Barnstead Labline, USA) and were stored at 4 °C until use. Negative control was prepared only with 95% ethanol.
- **Evaluation of antibacterial activity of homoeopathy medicines:** The zones of inhibition were measured in millimetres (including the 6mm disc) with 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 end point.
- **Determination of percentage of zone of inhibition:** Relative growth inhibition is determined using following the equation:

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

Results

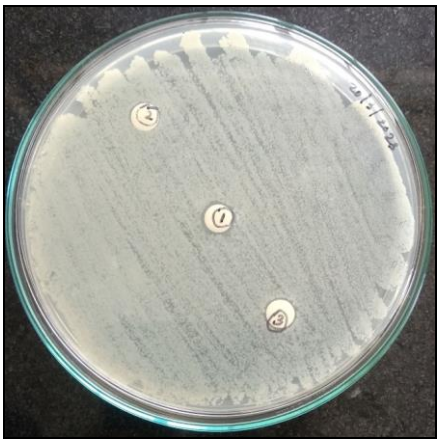

Fig 1: Antibacterial assay of Homoeopathic potencies of Pyrogenium and control by Kirby Bauer method in *Escherichia coli*

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

Sl.no	Homoeopathic medicine	Zone of inhibition (mm)
1	Pyrogenium 30C	7.5 ± 0.1 mm
2	Pyrogenium 200C	7.0 ± 0.1 mm
3	95% Ethanol (control)	7.0 ± 0.1 mm

Homoeopathic medicine Pyrogenium 30C showed 7.5 ± 0.1 mm of zone of inhibition, Pyrogenium 200C showed 7.0 ± 0.1 mm of zone of inhibition, and 95% Ethanol (control) showed 7.0 ± 0.1 mm of zone of inhibition.

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

Sl.no	Homoeopathic medicine	Relative growth inhibition percentage (%)
1	Pyrogenium 30C	7.14285714%
2	Pyrogenium 200C	0%

Homoeopathic medicine Pyrogenium 30C showed a relative growth inhibition of 7.14285714% while Pyrogenium 200C showed a relative growth inhibition of 0%.

Discussion

In this study, Homoeopathic medicine Pyrogenium 30C and 200 C were evaluated for their antimicrobial properties against one of the most relevant microorganisms, *Escherichia coli*. Pyrogenium 30C had the most antimicrobial activity against E.coli by showing 7.5 ± 0.1 mm zone of inhibition with relative growth inhibition of 7.14%, while the higher potency Pyrogenium 200C showed 7.0 ± 0.1 mm zone of inhibition with negligible relative growth inhibition. The zone of inhibition in control can be attributed to the antimicrobial activity of 95% ethanol.

A study done with Pyrogenium 6C showed no zone of inhibition [21] similar to the results showcased by Pyrogenium 200C in this study. This shows that Homoeopathic medicine Pyrogenium in potencies 6C and 30C has negligible antimicrobial activity while 200C has observable antimicrobial activity with mild to moderate zone of inhibition.

The Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) need to be done

to further analyse the extent of antimicrobial properties of Pyrogenium.

Conclusion

In conclusion, the *in vitro* study on the antimicrobial activity of Homoeopathic medicine Pyrogenium, against *E. coli*, demonstrated mild zones of inhibition in Kirby Bauer method. These findings suggest that certain potencies of Pyrogenium have potential antimicrobial properties against *E. coli*. However, further studies are necessary to determine the efficacy of Pyrogenium as an antimicrobial agent in clinical settings. Additionally, future research should focus on finding new drugs that can combat other pathogenic microorganisms that share similar mechanisms of antimicrobial resistance. The development of such drugs could reduce the use of broad-spectrum antibiotics, which contribute to the development of antibiotic resistance and have negative side effects. Overall, finding effective and safe antimicrobial agents is crucial for public health and the fight against antibiotic-resistant bacteria and homoeopathy has great scope in this regard.

Conflict of Interest

Not available

Financial Support

Not available

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