

Determination of *In vitro* antimicrobial activity of homeopathy medicines

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Homeopathy is a popular alternative medicine. The mechanism of action of homeopathic drug has not been clarified but it has been found to be effective against various diseases including diarrhea, dysentery, otitis media, eczema and even cancer. After qualifying thorough investigation, it can be used against antibiotic tolerance and multi drug resistant bacteria. In a recent study, bactericidal effect of homeopathic remedies has been investigated. But such an effect is not in accordance with the general principles of homeopathy. To test this paradigm, *in vitro* antimicrobial effects of four homeopathic drugs Aconite 30, Arsenicum album 30, Mercuric corrosivus 30 and Mercury solution 200 against five common intestinal pathogens, including- *Escherichia coli*, *Klebsiella* sp., *Salmonella* sp. *Pseudomonas* sp., and *Bacillus* sp have been observed. Standard agar well diffusion technique and macrodilution technique were followed. But, tested homeopathic drugs did not show any significant effect on any of the five pathogens. Rigorous study against other organism is required to fully understand antimicrobial activity and other mechanism of action.

Key words: Homeopathy; Antibiotic; Multidrug resistant bacteria; MIC

Homeopathy is a therapeutic method, first developed by Samuel Hahnemann (1755–1843) and is now practiced throughout the world. This method use preparations of substances whose effects when administered to healthy subjects correspond to the manifestations of the disease (symptoms, clinical signs, pathological states). Homeopathy has two main principals (1, 2). Firstly, ‘like cures like’ principle, patients with particular signs and symptoms can be helped by a homeopathic remedy that produces these signs and symptoms in healthy individuals. Secondly, homeopathic remedies can retain its biological activity after repeated dilution and succession even when diluted beyond Avogadro's number (3).

Though lacking much scientific evidence, it is considered as alternative medicine due to its low cost, fewer side effects and occasional failure of other treatment methodology (4). Shortage of new antibiotics and development of drug resistant organism is a matter of concern for public health worldwide and often lead to the quest of alternative medicine to microbial diseases. Many homeopathy drugs are now in use against common bacterial diseases. Although, mechanism of action of these medicines is not yet identified, they are claimed to be potent antibiotic or similibiotic (5).

In present study, four homeopathy drugs Aconite 30, Arsenicum album 30, Mercuric corrosivus 30 and Mercury solution 200 (6), which are commonly used

against diarrhea and dysentery, were tested for their antimicrobial activity against five common intestinal pathogens, including- *Escherichia coli*, *Klebsiella* sp., *Salmonella* sp. *Pseudomonas* sp., and *Bacillus* sp. Result of this study can provide an evidence to either antimicrobial property or placebo effect of these drugs.

MATERIALS AND METHODS

Sample collections & sample processing. Sample drugs were randomly collected from homeopathic drug stores at different areas of Dhaka city during March, 2018. Standard technique, suggested by American Public Health Association (APHA, 1998) was followed. During collection drug satisfied the manufacturing & expiry dates & storage criteria.

Determination of antimicrobial activity

Agar well diffusion technique. To observe *in vitro* antimicrobial activity, a standard procedure of agar well diffusion method was performed (7, 8). Agar well diffusion assay was observed against five common gastrointestinal pathogens including, *Escherichia coli*, *Pseudomonas* sp., *Klebsiella* sp., *Bacillus* sp., and *Salmonella* sp. Broth cultures of these organisms in Muller-Hinton Broth (MHB) were adjusted to the turbidity of Macfarland standard of 0.5 at first. Then with sterilized cotton swab, bacterial suspension was evenly spread on Muller-Hinton agar for confluent growth of each organism. With sterile cork-borer, wells were made on agar plates and 100 µl of each sample was added. For positive and negative control, disk of gentamicin 10 µg and Sterile MHB were used respectively. After overnight incubation at 37 °C, presence of clear zone was observed and measured for antimicrobial activity (9).

Determination of minimum inhibitory concentration by macrodilution technique. For determination of minimum inhibitory concentration, macrodilution or tube dilution method was followed. Again broth cultures of five tested organisms were adjusted to the turbidity of 0.5 McFarland solutions. For MIC of each drug, 7 vials containing 3 ml Muller Hilton broth were taken. 7 different volumes- 16 µl, 32 µl, 64 µl, 128 µl, 256 µl, 512 µl and 1024 µl of drug was added after withdrawing same amount of media. And finally 100 µl of bacterial suspension was added. After overnight incubation at 37 °C, vials were observed against light to identify the MIC.

RESULTS AND DISCUSSIONS

All four drugs showed small zone or no zone of inhibition against five tested organisms. Mercury

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solution (Q=200) produced zone of inhibition against three of the pathogens. A 5 mm in diameter against *E. coli*, 4 mm against *Salmonella* sp. and *Bacillus* sp. and no inhibition against *Klebsiella* sp. and *Pseudomonas* sp. *Mercuris corrosivus* showed inhibition zone of 2 mm in diameter against three of the pathogens whereas *Aconite* and *Arsenicum album* against two and one pathogens respectively. Zone of inhibition exerted by *Aconite* against *Klebsiella* sp. was 6 mm in diameter, which was maximum in this study. No zone of inhibition was found against *Pseudomonas* sp. by any of the medicine. Result of agar well diffusion is shown in Table 1.

All four drugs were found to have minimum inhibitory concentrations (MIC) between 256 µl/3ml to 1024 µl /3ml for five of the pathogens. Result of (MIC) is shown in Table 2.

DISCUSSION

Diarrhoea and dysentery are two forms of gastroenteritis often caused by consumption of food contaminated with various pathogens (10). Along with oral rehydration therapy, various antibiotics are prescribed to shorten the duration of these diseases. But repetitive antibiotic treatment can increase the risk of antibiotic tolerance and selecting multi drug resistant bacteria. Again antibiotic can cause various side effects

in patients. In the present study, four different homeopathic drugs were tested against five different pathogens. The four tested drugs showed to have insignificant bacterial inhibition in agar well diffusion method but showed variable efficiency in inhibiting bacteria by macrodilution process.

The principle of homeopathic drug is, it can retain its medicinal property even in extremely high dilutions (1, 3). But in the present study, the result was not directly relatable with this principle. Cause most of the drugs were found to be inhibitory at high concentrations, between 256 µl/3ml to 1024 µl/3ml.

Though, in some studies treatment with homeopathic drugs found to be effective against bacterial diseases (3, 11-14) and there are few report that homeopathic drugs found to be effective against bacterial diseases (3, 11-14) and there are few report that homeopathic drugs can work as similibiotics, that is they can induce immune system of the host as like a bacteria and therefore can cure patients (6). But, by so far, most of these studies do not have strong scientific background (15, 16) and extensive scientific research should be done to understand mechanism of action as well as antimicrobial activity of homeopathy medicines.

CONCLUSION

Homeopathy drugs have long been used as alternative

TABLE 1. Zone of inhibition by agar well diffusion (diameter in mm) against different homeopathic drugs

Selected Homeopathic Drugs (n=4)	Potency (Q/ MT ⁵)	<i>Salmonella</i> sp. (mm)	<i>Klebsiella</i> sp. (mm)	<i>E. coli</i> (mm)	<i>Pseudomonas</i> sp. (mm)	<i>Bacillus</i> sp. (mm)
<i>Aconite</i> ¹	30	0	6	3	0	0
<i>Arsenicum album</i> ²	30	0	2	0	0	0
<i>Merc. cor.</i> ³	30	2	0	2	0	2
<i>Merc. sol.</i> ⁴	200	4	0	5	0	4

¹ Symptoms: Stools small, frequent, bloody or slimy; fever, restlessness, anxiety, fear of death.

² Symptoms: Stools dark, putrid, mixed with blood ; during stool tenesmus and burning in rectum, great *anguish, restlessness, fear of death*; extreme thirst, aggravated at night, or after eating or drinking.

³ *Mercuris corrosives*. Symptoms: Stools pure blood or bloody mucus. During stool straining and tenesmus. *Severe pains in rectum after the discharge*. Almost constant cutting pain in the abdomen.

⁴ Mercury solution. Symptoms: Similar to *Merc. cor.* Symptoms are worse at night, and where there are profuse night sweats, especially on the head.

⁵ Mother Tincture.

TABLE 2. Minimum Inhibitory Concentration (MIC) (µl /3ml) of different homeopathic drugs

Selected Homeopathic Drugs (n=4)	Potency (Q/ MT)	<i>Salmonella</i> sp. (µl)	<i>Klebsiella</i> sp. (µl)	<i>E. coli</i> (µl)	<i>Pseudomonas</i> sp. (µl)	<i>Bacillus</i> sp. (µl)
<i>Aconite</i>	30	512	1024	1024	Nil	256
<i>Arsenicum album</i>	30	256	512	1024	256	512
<i>Merc. cor.</i>	30	256	512	256	256	512
<i>Merc. sol.</i>	200	1024	512	1024	256	256

medicine. But their antimicrobial activity has not been clearly identified yet. In present study, no significant antimicrobial activity has been observed against five common intestinal pathogens. Antimicrobial activity against other pathogens can be observed in future endeavor.

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