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In vitro evaluation of homoeopathic medicine Acidum Muriaticum in selected potencies for its antimicrobial activity against *Salmonella* Typhi

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ABSTRACT

Aims: The main aim of this study was to screen homoeopathic medicine Acidum Muriaticum in selected potencies for its preventive and curative antimicrobial action to use for the general management of typhoid by providing individualized homoeopathic treatment for symptomatic improvement with minimal complications leading to early recovery.
Methodology and results: Homoeopathic medicine Acidum Muriaticum in different Hahnemannian centesimal scale potencies (CH) - (6CH, 12CH, 30CH, 200CH, 1M, 50M) was evaluated for antimicrobial activity against *Salmonella* Typhi (*S.* Typhi*)* (MTCC-1264) using the agar well diffusion method. The diameter of the zone of inhibition was measured (in mm) and the potency of Acidum Muriaticum having high minimum inhibitory concentration (MIC) was determined by using various controls namely, positive control (azithromycin), negative control (nutrient broth + tetracycline), vehicle control (dispensing alcohol, which contains 90% ethanol as per Homoeopathic Pharmacopoeia of India) and blank control (distilled water) in the study. Homoeopathic medicine Acidum Muriaticum in different liquid potencies (6CH, 12CH, 30CH, 200CH, 1M, 50M) was screened. Results suggest that 6CH, 12CH and 30CH potencies of Acidum Muriaticum could inhibit the growth of *S.* Typhi. Acidum Muriaticum showed maximum growth inhibitory zone (GIZ) against *S.* Typhi in agar plates with 6CH, 12CH and 30CH potencies. Acidum Muriaticum in different potencies with different concentrations significantly showed MIC against *S.* Typhi*,* namely 6CH (1:2), 12CH (1:2) in 25 µL/mL and 30CH (1:1) in 50 µL/mL concentration, respectively.
Conclusion, significance and impact of study: The present study concludes that the homoeopathic medicine Acidum Muriaticum in 6CH, 12CH and 30CH potencies could inhibit *S.* Typhi as compared with azithromycin. Hence, it could be an alternative medicine to azithromycin, the antimicrobial drug against *S.* Typhi. However, further research is required to confirm the precise mechanism of action.

Keywords: Acidum Muriaticum, antimicrobial, *Salmonella* Typhi

INTRODUCTION

Salmonella Typhi (*S.* Typhi) bacteria are found in contaminated food or water. It affects 27 million people worldwide and causes 200,000-600,000 deaths annually due to chronic carriers. Nontyphoidal salmonellosis (NTS) causes 12 million illnesses annually (Kasper *et al.*, 2016). *Salmonella* is a genus of rod-shaped Gram-negative bacteria, about 1-3 microns × 0.5 microns in size. They are motile with peritrichous flagella, Gram-negative facultative anaerobic belonging to the Enterobacteriaceae. *Salmonellae* comprise more than 2000 serotypes and all pathogenic clinical ailments are (1) enteric fever, (2) gastrointestinal disorder, producing gastroenteritis and septicemia localized infections (Ananthanarayan and Paniker, 2006).

The first-line drugs are common antimicrobial agents like chloramphenicol, amoxicillin, and ciprofloxacin. However, because of the multidrug resistance developed against these medicines, these drugs' action has decreased, leading to increased dependence on third-era cephalosporins and Azithromycin (Karkey *et al.*, 2018).

According to the World Health Organization, homoeopathy is one of the second most widely accepted systems of medicine. Homoeopathy is both preventive and curative in epidemic diseases by administering genus epidemics. William Boericke, in his 'Pocket Manual' recognizes the prophylactic power of baptisia in low

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dilutions. Production of antibodies against *Baci. typhosus* raises the natural body's resistance to the invasion of bacillary intoxication, which produces typhoid syndrome (Babu, 2009; Boericke, 2022).

This study aimed to screen the antimicrobial activity of Acidum Muriaticum against *S.* Typhi *in vitro.*

MATERIALS AND METHODS

Medium

Xylose lysine deoxycholate agar (XLD agar) plates [Product number MP031, Hi-Media Laboratories Private Limited, C-40, Road No.21Y, MIDC, Wagle Industrial Area, Thane (W), - 400 604, India] were used in the study to observe zone of inhibition by agar well-diffusion method and for determination of MIC value (5 mL nutrient broth + 300 mL of tetracycline hydrochloride) was used as a medium.

Homoeopathic medicines preparation

All the Hahnemannian centesimal scale (CH) liquid potencies (6CH, 12CH, 30CH, 200CH and 1M, 50M) of homoeopathic medicine Acidum Muriaticum were obtained from good manufacturing practices (GMP) - approved Standard Homoeopathic Medicine Manufacturer.

Azithromycin

Azithromycin 500 mg antimicrobial drug for intravenous use (I.V.) was obtained from a local pharmacy as a positive control. It was further dissolved thoroughly in 5 mL of distilled water for preparation of 100 mg/mL concentration.

Organism

Salmonella Typhi (MTCC-1264) was obtained from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh. The culture was grown in nutrient broth + tetracycline and added in 1 mL of prepared nutrient broth. The culture of *S.* Typhi was then transferred into two test tubes containing nutrient broth + tetracycline and incubated at room temperature for 24 h.

Antimicrobial assay (Agar well diffusion)

The agar well diffusion method determined the antimicrobial activity of homoeopathic potencies of medicine Acidum Muriaticum 6CH, 12CH, 30CH, 200CH, 1M and 50M.

Suspension of *S.* Typhi culture with 0.143 O.D. at 600 nm was swabbed on XLD agar plates and kept for incubation for 30 min at room temperature.

After incubation, XLD (xylose lysine deoxycholate) agar plates were punched with a sterilized borer of 0.5 cm. Various potencies of the selected homoeopathic medicine Acidum Muriaticum 6CH, 12CH, 30CH, 200CH, 1M, 50M (each potency of 20 µL), azithromycin 20 µL (100 mg/mL) were added in wells (0.5 cm diameter) made on the agar. It was followed by incubation at room temperature for 24 h. Azithromycin 20 µL (100 mg/mL) as a positive control, distilled water as blank control and dispensing alcohol (ethanol 90%) as vehicle control was added. After 24 h of incubation at room temperature, the antimicrobial activity was scored by measuring the diameter of the zone of inhibition around the wells. Out of six potencies, namely 6CH, 12CH, 30CH, 200CH, 1M and 50M, only the first three (i.e., 6CH, 12CH and 30CH) potencies of homoeopathic medicine Acidum Muriaticum showed a zone of inhibition around the well which ranged from 0.2-0.8 cm for different potencies. Each antimicrobial assay was performed and the mean diameters of the zone of inhibition (cm) and standard deviation using computer Microsoft Excel were reported (Benson, 2002; Munshi *et al*., 2022).

MIC

MIC was determined by checking the growth of *S.* Typhi, after adding 500 µL of different drug dilutions in Eppendorf tubes containing 500 µL of broth and 500 µL of the pathogen. There were different tube preparations for positive control (azithromycin), negative control (nutrient broth + tetracycline), vehicle control (dispensing alcohol) and blank control (distilled water). Readings were taken to calculate minimum inhibitory concentration values (Sharmin *et al.*, 2014; Mukherjee *et al.*, 2019).

RESULTS AND DISCUSSION

From the results shown in Tables 1 and 2, it is evident that the homoeopathic medicine Acidum Muriaticum in different liquid potencies like 6CH, 12CH and 30CH in agar-well diffusion assay (Figures 1, 2 and 3) as well as in MIC assays has better efficacy to inhibit the growth of *S.* Typhi*.* Acidum Muriaticum in different potencies with different concentrations significantly showed MIC against *S.* Typhi namely 6CH (1:2), 12CH (1:2) in 25 µL/mL and 30CH (1:1) in 50 µL/mL concentration, respectively, after comparison with positive control (azithromycin), negative control (nutrient broth + tetracycline), vehicle control (dispensing alcohol which is ethanol 90% as per Homoeopathic Pharmacopoeia of India) and blank control (distilled water).

Antimicrobial assay of homoeopathic medicine and controls by agar well diffusion method

Out of various literature review studies, five *in vitro* (Sharmin *et al.*, 2014; Mokarroma and Shammi, 2018; Shinde *et al.*, 2018; Mukherjee *et al.,* 2019; Munshi *et al.*, 2022) and one *in-vivo* (Ahmad *et al*., 2017) studies assayed the effects of homoeopathic drugs and were estimated for antimicrobial exertion against *S.* Typhi*.* The majority of the studies show the consequences of the base minimum inhibitory concentration (MIC) of the

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Table 1: Antimicrobial activity by agar well diffusion method.

Sr. No.	Homoeopathic potency and controls	Zone of inhibition (cm)			SD
		First reading	Second reading	Mean	
1	Acidum Muriaticum 6CH	0.8	0.7	0.75	0.07
2	Acidum Muriaticum 12CH	0.6	0.5	0.55	0.07
3	Acidum Muriaticum 30CH	0.3	0.2	0.40	0.07
4	Acidum Muriaticum 200C	0.0	0.0	0.00	0.00
5	Acidum Muriaticum 1M	0.0	0.0	0.00	0.00
6	Acidum Muriaticum 50M	0.0	0.0	0.00	0.00
7	Dispensing alcohol (vehicle control)	0.0	0.0	0.00	0.00
8	Azithromycin (chemical control)	0.15	0.17	0.16	0.01
9	Distilled water (blank control)	0.0	0.0	0.00	0.00

Table 2: Summary of highest MIC values at various concentrations.

Sr. No.	Name of the medicine	Concentrations (Drug: Distilled water)	MIC value in a ratio (Growth inhibition: Growth)
1	Acidum Muriaticum 6CH	25 µL/mL	1:2
2	Acidum Muriaticum 12CH	25 µL/mL	1:2
3	Acidum Muriaticum 30CH	50 µL/mL	1:1
4	Azithromycin	100 mg/mL	0:016

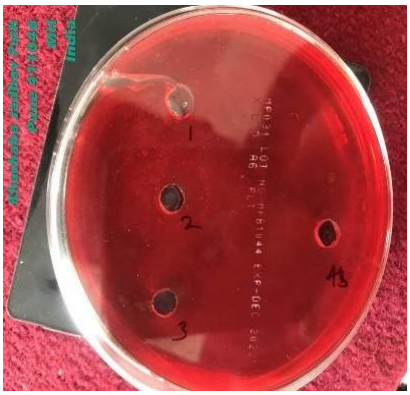

Figure 1: Zone of inhibition against S. Typhi with Acidum Muriaticum. No.1, 6CH; No.2, 12CH; No.3, 30CH and Positive control AB, Antibiotic (azithromycin).

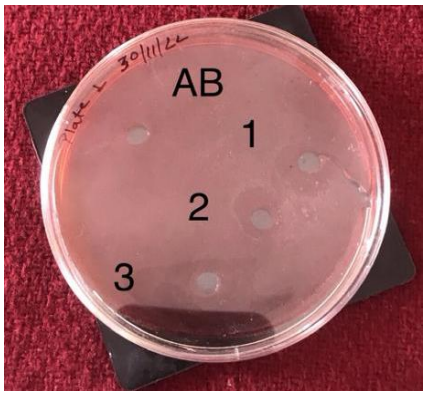

Figure 2: Zone of inhibition against S. Typhi with Acidum Muriaticum. No.1, 6CH; No.2, 12CH; No.3, 30CH; AB: Antibiotic (azithromycin).

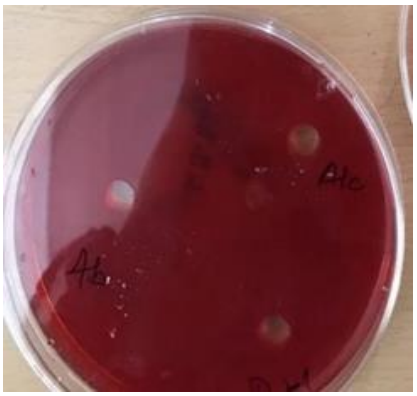

Figure 3: Zone of inhibition against *S.* Typhi with positive control, AB*:* Antibiotic (azithromycin), vehicle control (dispensing alcohol) and blank control (distilled water).

homoeopathic drug displayed antimicrobial capability against the related atomic organisms.

Four studies explain that multidrug resistance (MDR) is characterized by protection from chloramphenicol, amoxicillin and trimoxazole, followed rapidly by the lowered inability to fluoroquinolones. Expanding dependence is presently being put on the move on third-period cephalosporins and azithromycin. Adding antimicrobial opposition to *S.* Typhi is a serious general well-being concern, particularly in industrializing nations (Su *et al.*, 2004; Harish and Menezes, 2011; Karkey *et al.*, 2018; Herekar *et al.*, 2022).

One study assayed the generally reported side effects of azithromycin, including diarrhoea and nausea. Other side effects include abdominal pain and vomiting. Hepatotoxicity is comprised principally of hepatocellular injury within 1 to three weeks of drug use (Sandman and Iqbal, 2021).

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In seven epidemiological studies from 1999 to 2010, locally picked-up cases represented 18 of 3373 detailed typhoid fever cases. It showed the possibility of recognizing occurrences of typhoid fever that might be down from identification by routine examination of observation information (Lynch *et al.*, 2009; Buckle *et al.*, 2012; Crump *et al*., 2015; John *et al*., 2016; Vos *et al.*, 2016; Mukhopadhyay *et al*., 2019).

From 1990 to 2017, it showed how a significant part of the total crowd encounters-lethal well-being mischance with emotional diversity among colourful causes, areas, periods and genders. Worldwide systematic survey with an investigation of typhoid and paratyphoid assessed 13.5 million typhoid fever scenes in 2010 (Buckle *et al.*, 2012; Vos *et al.*, 2017).

Homeopathy cures gently and permanently without adverse side effects by potentized Homoeopathic drugs at a low cost. Homeopathy has a broader and better range of giving gentle and endless results in similar conditions. Coming experimental studies are needed in homeopathy for further scientific witness. During the review, it was set up that Homoeopathic drugs can inhibit the growth of bacteria and homoeopathic medicine has good results in delivering a permanent cure.

CONCLUSION

The present study concludes that 6CH, 12CH and 30CH potencies of the homoeopathic drug Acidum Muriaticum had the highest MIC value and can successfully inhibit *S.* Typhi compared with azithromycin. Therefore, it could be an alternative medicine to azithromycin, an antimicrobial treatment currently available. However, additional *in vitro* testing and further research are required to confirm the antimicrobial properties with the precise mechanism of action.

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CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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