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A Study on the Antimicrobial Activities of Hot Spring Bakreshwar Water on Multiple Drug Resistant Bacteria

Disha Roy¹, Bhaskar Narayan Chaudhuri², Partha Guchhait², Satadal Das^{2*}

1.Department of Biotechnology, Kalinga Institute of Industrial Technology, Bhubaneswar, Odisha, India

2.Department of Microbiology, Peerless Hospitex Hospital and Research Centre Limited, Kolkata, India

ABSTRACT

Bakreshwar hot springs have geological significance, and are known to have curative properties for any diseases. In this study, antimicrobial activities of the hot spring Bakreshwar water were assessed against MDR and ATCC microorganisms. A range of bacterial strains, including *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Acinetobacter* sp. (MDR) and *Pseudomonas* sp. (MDR) were used to evaluate the antimicrobial activity of the water. The results showed that the hot spring water expressed significant antimicrobial activity against all tested MDR and ATCC microorganisms. High temperature and strong alkaline pH (9.2) of the hot spring water, the presence of minerals and the physical and chemical agents in the water are probably responsible for the antimicrobial activities that were observed. The findings lead us to believe that the hot spring water from Bakreshwar might be a source of natural antimicrobial properties, which could be utilized for the development of new drugs for treating infections caused by resistant bacteria. Further studies are required to confirm the specific mineral, chemical or physical agent that are responsible for the observed activity.

Keywords: Bakreshwar's hot spring water; antimicrobial activity; *Staphylococcus aureus*; *Escherichia coli*; *Escherichia coli* (MDR), *Klebsiella* sp.; *Acinetobacter* sp.; *Pseudomonas* sp.

*Corresponding Author Email: drsatdas@hotmail.com

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INTRODUCTION

A hot spring is a natural spring of water which is heated by geothermal activity with water temperature exceeding the temperature of a human body (which is normally about 37 degrees Celsius). Hot springs feed communities of microorganisms like thermophiles, a type of extremophile which can adapt to hot and high temperatures of about 80 degrees Celsius [1]. Humans have been enjoying hot springs for thousands of years [2]. Being a source of therapeutic and medicinal values, hot springs with their increasingly healing properties have shown significant improvements in patients with disabilities and diseases like

rheumatoid arthritis and ankylosing spondylitis. However, the therapeutic efficacy of the hot spring water therapy remains undetermined [3]. As a result, it is now time to understand the effectiveness of the antimicrobial activity in hot spring waters and develop new drugs for treatments against MDR bacteria. In this study, the antimicrobial activity of Bakreshwar hot spring water has been analyzed. Bakreshwar hot springs are believed to have curative properties against any ailment. This is due to the high temperatures of these thermal springs, ranging between 31 degrees Celsius and 89 degrees Celsius, which host thermophilic organisms and their strong alkaline pH, ranging between 7.4 to 9.2. Alongside, the physico-chemical contents are characteristic feature of these springs [4]. The bacterial species were grown in Mueller Hinton Broth and the bacterial suspension was prepared by suspending three bacterial colonies from each plate in sterile 0.9% sodium chloride in order to acquire 0.5 McFarland scale (10^8 CFU per ml) standard as inoculums. To adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a specific range to standardize microbial testing, McFarland standards are used as a reference. The hot spring water's minimal inhibitory concentration is evaluated against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Acinetobacter* sp. (MDR) and *Pseudomonas* sp. (MDR). MIC is defined as the lowest concentration of antimicrobial or drug that will inhibit the visible growth of bacteria after overnight incubation [5]. It is used for determining the susceptibility of these bacteria against drugs and helps to estimate the activity of new antimicrobial agents [6].

MATERIALS AND METHOD

The Hot Spring Water

The hot spring water was collected from Bakreshwar's (23.88°N 87.37°E) Agni kunda. Bakreshwar is a small town in West Bengal, India which is manifested with ten hot springs- Paphara ganga, Baitarini ganga, Khar kunda, Bhairav kunda, Agni kunda, Dudh kunda, Surya kunda, Shwet ganga, Bhrahma kunda, Amrita kunda. The water in the Agni kunda is at a high

temperature of 80 degrees Celsius. Being rich in minerals like sodium, potassium, calcium, silicates, chlorides, bicarbonates and sulfates, it is said to hold medicinal properties. Traces of radioactive elements are also present. The water was collected in the month of May, 2023 and was brought to the laboratory in sterile air tight containers at room temperature and stored in dark place.

Test Microorganisms and Culture Media

Six bacterial strains such as *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Acinetobacter* sp. (MDR) and *Pseudomonas* sp. (MDR) were chosen for this study. Among these bacteria, *Staphylococcus aureus* (ATCC 25923) is gram- positive in nature and *Escherichia coli* (ATCC 25922), *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Acinetobacter* sp. (MDR) and *Pseudomonas* sp. (MDR) are gram- negative in nature. These bacterial species were grown in Mueller Hinton Broth (Himedia).

Inoculum preparation

Each bacterial strain taken from the stock cultures, was streaked on the agar plate. The plate was set for incubation at 37 degrees Celsius for 24 hours. The bacterial suspension was prepared by suspending three bacterial colonies from each plate in sterile 0.9% sodium chloride in order to acquire 0.5 McFarland scale (10^8 CFU per ml) as inoculums to determine the MIC value.

Minimum inhibitory concentration (MIC) assays

The antibacterial activity was determined followed by minimum inhibitory concentration. 100 µl of Mueller Hinton Broth (double concentration) was poured in each of the first microtitre well of the first column. 100 µl of Mueller Hinton Broth (single concentration) was added in the other wells. Then, 100 µl of hot spring water was poured in the first microtitre well of the first column and then mixed with the Mueller Hinton Broth. Then 100µl of this was added to the next well with 100µl of Mueller Hinton Broth and this procedure was continued up to the eighth well. Thus, concentration of the hot spring water became half in the first well and serially double diluted till the eighth well. Then 10µl of different bacterial suspension was added to each well in separate rows of the ELISA plate. For each bacterial species, two rows were assigned. Then the absorbance or optical density was measured at 620nm to be utilized as baseline absorbance values. The plate was left for incubation at 37°C for 24 hours after the completion of the optical density measuring. The absorbance was recorded again at 620 nm after 24 hours. Then the optical density values of the wells taken initially were subtracted from the final values recorded after 24 hours. By finding out the lowest concentration of the hot spring water which had a lower optical density value than

the optical density of the control, the MIC value was determined.

RESULTS AND DISCUSSION

The results showed that the hot spring water of Bakreshwar was effective against all the bacterial strains (both ATCC and MDR) that were analyzed in this study. The growth curve for all the microbial strains, individually and in combinations were plotted and the following growth curves (optical density Vs increasing dilutions) were achieved. It showed a recurring fluctuation pattern where most of the MDR strains showed a decrease in growth at a specific dilution of 1:16 (fourth well) and the ATCC strains showed a fall at a dilution of 1:32 (fifth well).

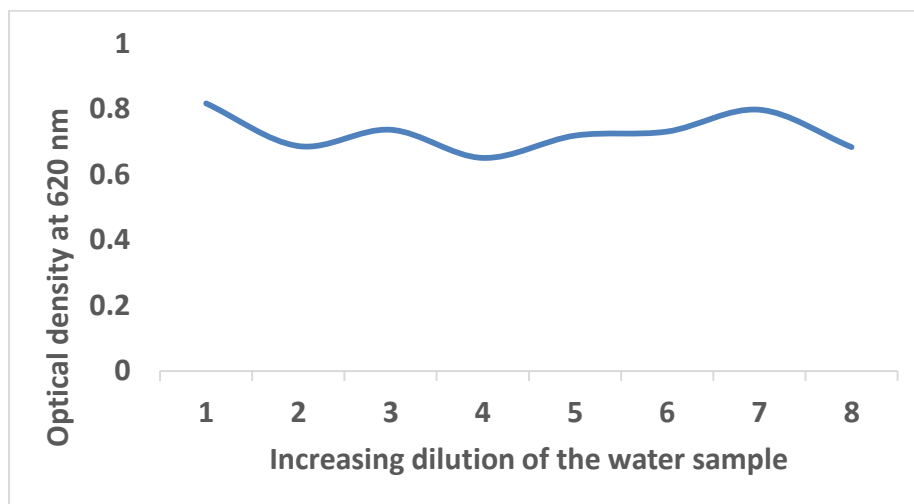


Figure 1: Antimicrobial activity of Bakreshwar's hot water sample against *Staphylococcus aureus* (ATCC 25923). Concentration of the sample: 1- 1: 2 dilution; 2- 1:4 dilution; 3-1:8 dilution; 4- 1:16 dilution; 5- 1:32 dilution; 6- 1:64 dilution; 7- 1:128 dilution; 8: - 1: 256 dilution.

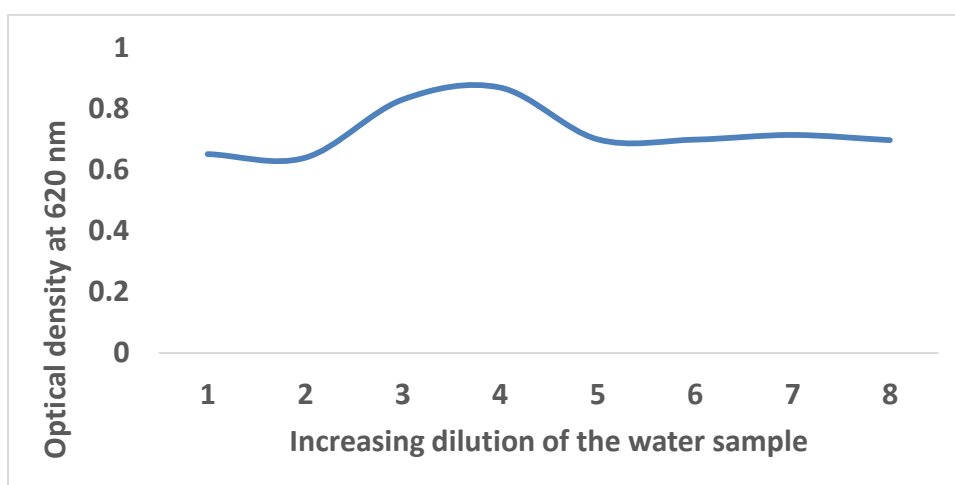


Figure 2: Antimicrobial activity of Bakreshwar's hot water sample against *Escherichia coli* (ATCC 25922). Concentration of the sample: 1- 1: 2 dilution; 2- 1:4 dilution; 3-1:8 dilution; 4- 1:16 dilution; 5- 1:32 dilution; 6- 1:64 dilution; 7- 1:128 dilution; 8: - 1: 256 dilution.

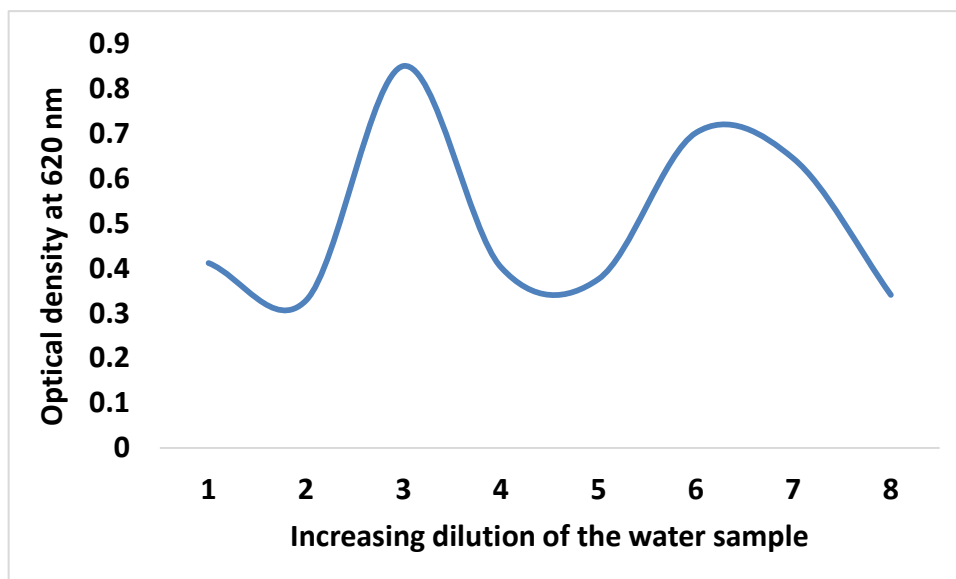


Figure 3: Antimicrobial activity of Bakreshwar's hot water sample against *Escherichia coli* (MDR). Concentration of the sample: 1- 1: 2 dilution; 2- 1:4 dilution; 3-1:8 dilution; 4- 1:16 dilution; 5- 1:32 dilution; 6- 1:64 dilution; 7- 1:128 dilution; 8- - 1: 256 dilution.

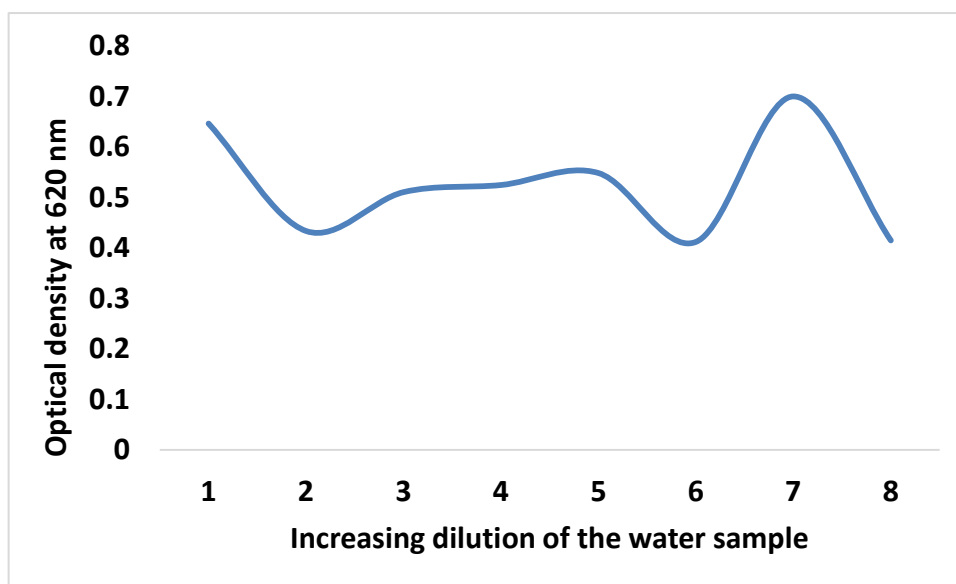


Figure 4: Antimicrobial activity of Bakreshwar's hot water sample against *Klebsiella* sp. (MDR). Concentration of the sample: 1- 1: 2 dilution; 2- 1:4 dilution; 3-1:8 dilution; 4- 1:16 dilution; 5- 1:32 dilution; 6- 1:64 dilution; 7- 1:128 dilution; 8- - 1: 256 dilution.

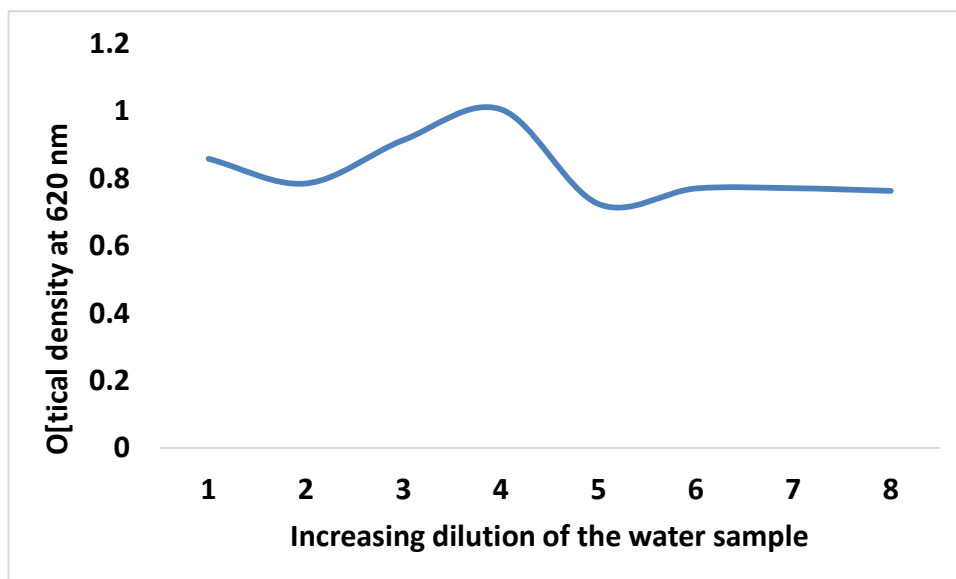


Figure 5: Antimicrobial activity of Bakreshwar's hot water sample against *Acinetobacter* sp. (MDR). Concentration of the sample: 1- 1: 2 dilution; 2- 1:4 dilution; 3-1:8 dilution; 4- 1:16 dilution; 5- 1:32 dilution; 6- 1:64 dilution; 7- 1:128 dilution; 8: - 1: 256 dilution.

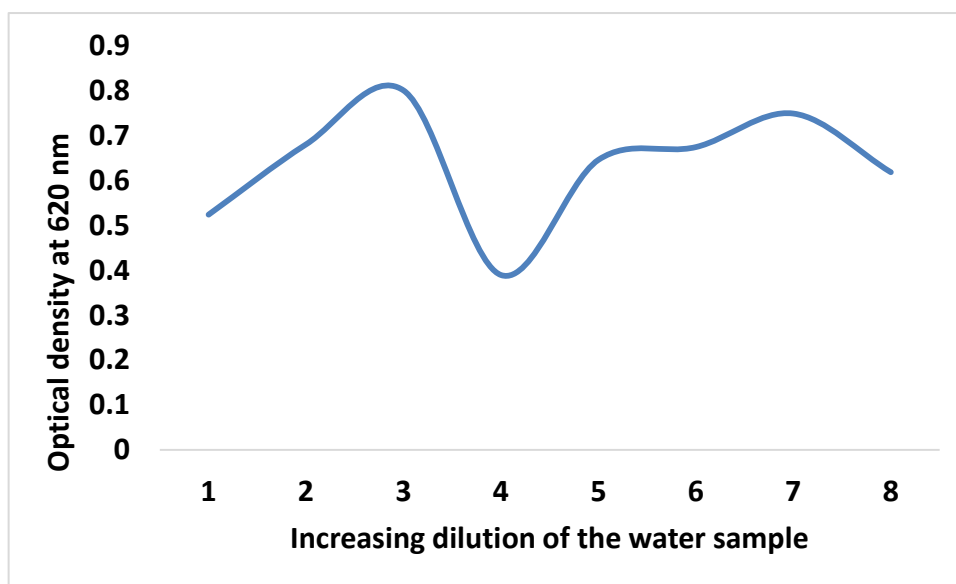


Figure 6: Antimicrobial activity of Bakreshwar's hot water sample against *Pseudomonas* sp. (MDR). Concentration of the sample: 1- 1: 2 dilution; 2- 1:4 dilution; 3-1:8 dilution; 4- 1:16 dilution; 5- 1:32 dilution; 6- 1:64 dilution; 7- 1:128 dilution; 8: - 1: 256 dilution.

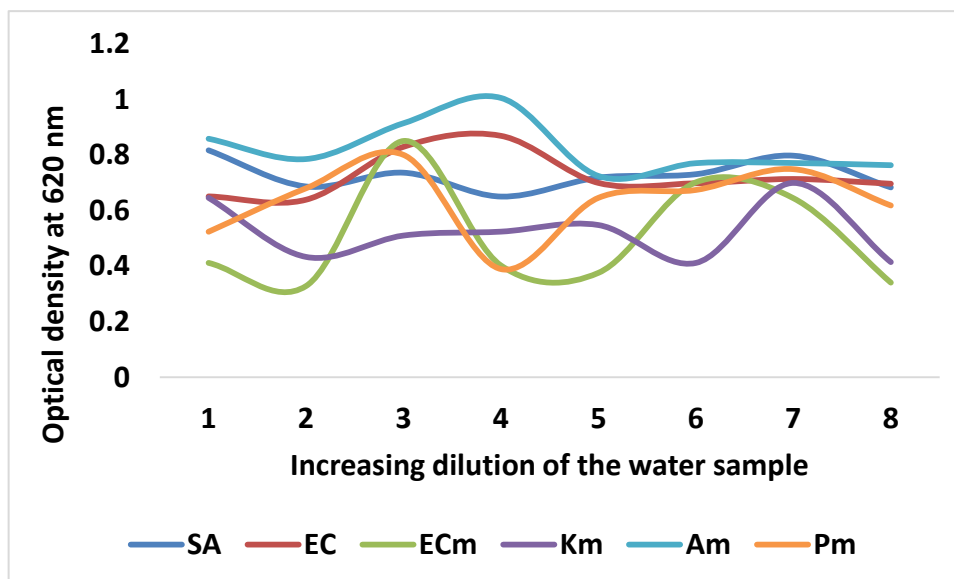


Figure 7: Antimicrobial activity of Bakreshwar's hot water sample against all the six microbial strains. SA- *Staphylococcus aureus*, EC-*Escherichia coli*, ECm –*E. coli* MDR, Km –*Klebsiella* MDR, Am- *Acinetobacter* MDR, Pm- *Pseudomonas* MDR. Concentration of the sample: 1- 1: 2 dilution; 2- 1:4 dilution; 3-1:8 dilution; 4- 1:16 dilution; 5- 1:32 dilution; 6- 1:64 dilution; 7- 1:128 dilution; 8- - 1: 256 dilution.

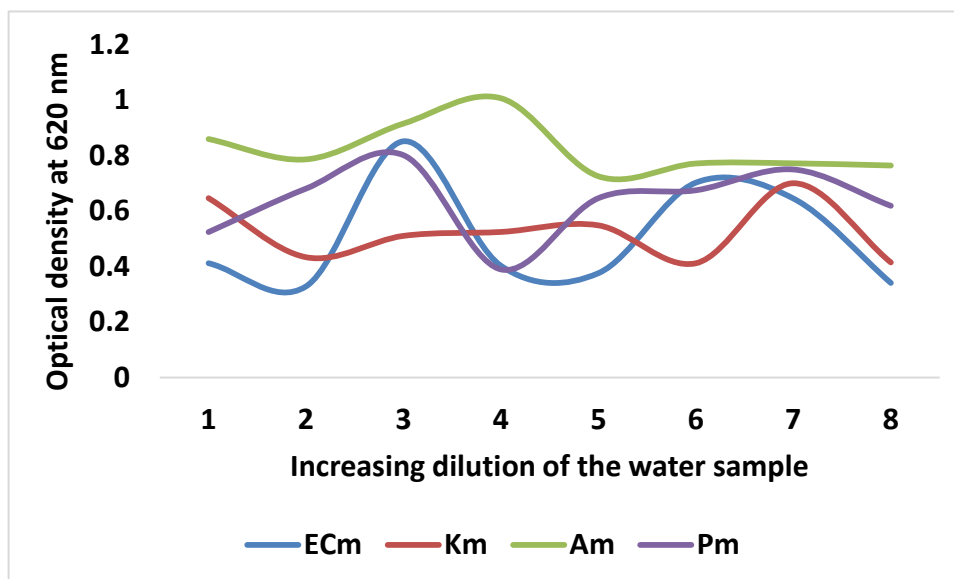


Figure 8: Antimicrobial activity of Bakreshwar's hot water sample against MDR strains- ECm [*Escherichia coli* (MDR)], Km [*Klebsiella* sp. (MDR)], Am [*Acinetobacter* sp. (MDR)] and Pm [*Pseudomonas* sp. (MDR)]. Concentration of the sample: 1- 1: 2 dilution; 2- 1:4 dilution; 3-1:8 dilution; 4- 1:16 dilution; 5- 1:32 dilution; 6- 1:64 dilution; 7- 1:128 dilution; 8- - 1: 256 dilution.

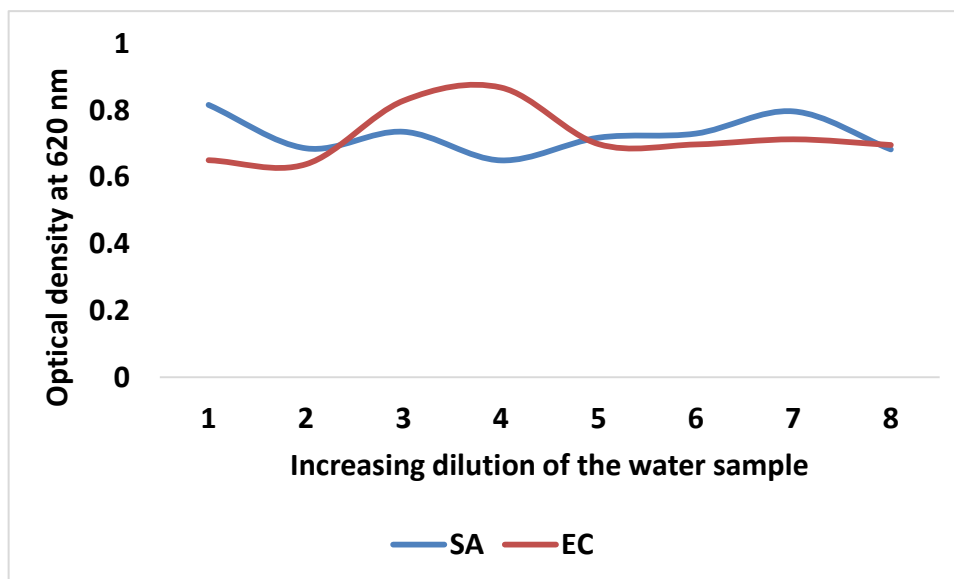


Figure 9: Antimicrobial activity of Bakreshwar's hot water sample against ATCC strains- SA [*Staphylococcus aureus* (ATCC 25923)], EC [*Escherichia coli* (ATCC 25922)]. Concentration of the sample: 1- 1: 2 dilution; 2- 1:4 dilution; 3-1:8 dilution; 4- 1:16 dilution; 5- 1:32 dilution; 6- 1:64 dilution; 7- 1:128 dilution; 8: - 1: 256 dilution.

People have been using hot spring waters throughout ancient times for its therapeutic properties and its abilities to treat ailments. To prove their therapeutic effectiveness, scientific studies are being conducted. Due to the rise of multiple drug resistant bacteria and the emerging mutated bacterial strains which can be quite dangerous [7], there is an immediate need to develop new drugs against MDR strains and the Bakreshwar hot spring water show promising antimicrobial activity against both MDR and ATCC strains thereby, proving itself to be a natural source of antimicrobial agents, which is evident from the findings of this study.

The presence of a large amount of minerals and the physical and chemical agents in the water might have led to the curing of resistant plasmids of MDR bacteria. It is probably because of an extremely alkaline pH of about 9.2, lysis of the resistant plasmids are occurring thus[8], leading to the denaturation of the plasmid DNA of the MDR bacteria. This phenomenon is most likely causing the MDR strains to show some change in behavior which is visible through the fluctuations in the growth curve. To successfully characterize and specify the mineral or the physical and chemical agent responsible for the frequent fluctuation in the optical density values and the curve plot, further scientific studies are required.

CONCLUSION

It can be concluded that the Bakreshwar hot spring water has demonstrated considerable antimicrobial activity against a range of pathogenic bacteria, which also includes multiple drug

resistant strains. The high alkaline conditions and the presence of large quantities of minerals and physical and chemical agents are probably responsible for its antimicrobial activity. The results and observations of this study suggest that the hot spring water might be a source of natural antimicrobial agents which can be used for the development of novel drugs and therapeutic agents. In spite of that, further studies are needed in order to confirm the specific chemical and physical agents or the minerals that are responsible for the respective antimicrobial activity shown by the hot spring water and its safety and effectiveness has to be determined.

Conflict of interest

The author expresses no conflict of interest.

AUTHOR'S CONTRIBUTION

The collection of the hot spring water sample and the study was designed and conceived by SD. DR carried out the experiment. The data analysis and the writing of the manuscript was done by DR. The manuscript was edited and reviewed by all Authors.

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REFERENCES

1. Mehta R, Singhal P, Singh H, Damle D, Sharma AK. Insight into thermophiles and their wide-spectrum applications. 3 Biotech. 2016 ;6(1):81. doi: 10.1007/s13205-016-0368-z.
2. Mario-César Suárez Arriaga, A Short Story of the Long Relationship Between The Human Race and Geothermal Phenomena , Ed. B, Michoacan University – UMSNH, 58090 Morelia, Mexico, Proceedings World Geothermal Congress 2005 Antalya, Turkey, 24-29 April 2005
3. Wangchuk, P.; Yeshi, K.; Ugyen, K.; Dorji, J.; Wangdi, K.; Samten; Tshering, P.; Nugraha, A.S. Water-Based Therapies of Bhutan: Current Practices and the Recorded Clinical Evidence of Balneotherapy. *Water* 2021, 13, 9. <https://doi.org/10.3390/w13010009>.
4. Shukla A, Maniar K, Pillai A, Siddiqui S, Bambhrolia M, Shah M, Panchal J, Geothermal water in Bakreshwar-Tantoli region in West Bengal, India: Implications on water quality for irrigation and drinking purposes, *Groundwater for Sustainable Development*, 2022, 18, 100773, <https://doi.org/10.1016/j.gsd.2022.100773>.

5. Kowalska-Krochmal B, Dudek-Wicher R. The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance. *Pathogens*. 2021, 10(2):165. doi: 10.3390/pathogens10020165.
6. Balouiri M, Sadiki M, Ibsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. *J Pharm Anal*. 2016,6(2):71-79. doi: 10.1016/j.jpha.2015.11.005.
7. van Duin D, Paterson DL. Multidrug-Resistant Bacteria in the Community: Trends and Lessons Learned. *Infect Dis Clin North Am*. 2016,30(2):377-390. doi: 10.1016/j.idc.2016.02.004.
8. Delaney S, Murphy R, Walsh F. A Comparison of Methods for the Extraction of Plasmids Capable of Conferring Antibiotic Resistance in a Human Pathogen From Complex Broiler Cecal Samples. *Front Microbiol*. 2018, 9:1731. doi: 10.3389/fmicb.2018.01731.

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