To Isolate and Screen River Bacteria to Check its Antagonistic Activity Against Antibiotic Resistant Pathogens.

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Abstract

River is one of the main biological resources and has potential to solve threat caused by antimicrobial resistance. From different river water sample and soil sample of Uttarakhand, India the microbes were isolated and screened to check it's antimicrobial activities. The objective was to identified potential antibacterial from river sources which may help in treatment of antimicrobial resistance. The antimicrobial activity of isolates was checked against two pathogens: *Salmonella typhi* and *Acinetobacter baumannii.* Preliminary screening of river microorganism in opposition to antibiotic resistant pathogen is performed by cross streak technique. Secondary screening of positive isolates is done using Agar well diffusion technique, in addition a comparison was also made between centrifuged and non-centrifuged isolates to check whether the antimicrobial substance is produced externally or internally. A zone-of-inhibition was found in non-centrifuged plates around two isolates. Thus, further research on river bodies may lead to find better treatment of antimicrobial resistance.

Keywords: *Antimicrobial resistance, River microbes, Agar well diffusion technique, cross-streak technique*

1. Introduction

The microorganism is capable of developing resistance towards drug making infections harder to treat and increasing the risk of disease spread, severe illness and death. The main causes of antibiotic resistance are over prescribing of antibiotics, patients not finishing their treatment, over-use of antibiotics in livestock and farming, poor infection control in hospitals and clinics, lack of hygiene and poor sanitization, lack of antibiotics being developed. The main mechanism of resistance is: limiting uptake of a drug, modification of a drug target, inactivation of a drug, and active efflux of a drug. The World Health Organization (WHO), has announced antimicrobial resistance as a threat to global health. WHO and center for Disease Control and

Prevention (CDC) have taken initiatives to coordinate various actions to analyze the risk, identify the causative factors and to manage this global concern effectively. (*Https://Www.Who.Int/News-Room/Fact-Sheets/Detail/Antimicrobial-Resistance*, n.d.) It also requires urgent multisectoral action in order to achieve the Sustainable Development Goals (SDGs).(*Https://Sdg.Iisd.Org/News/Antimicrobial-Resistance-Threatens-Development-Sdgs-Tripartite-Report/*, n.d.)

AMR's broad effects on: SDG 3, including that as AMR increases treatment costs, universal health coverage will be unattainable for many countries. In addition, reducing child and infant mortality relies on effective antibiotics. SDG 1 (no poverty), AMR could cause an additional 28.3 million people to be pushed into extreme poverty by 2050 due to high costs of treatment and chronic infections. SDG 2 (zero hunger), animals harmed by AMR affects farmers' livelihood and broader food security. SDG 8 (decent work and economic growth), as AMR increases mortality and morbidity, labor supply will decline and could cause a decrease of 1-3% in global economic output by 2030,

The untapped biological resources present in water bodies may be fundamental in solving several of the world's public health crises, which span from the rise of antibiotic resistance in bacteria, pathogenic fungi and parasites, to the rise of cancer incidence and viral infection outbreaks. River organisms are under a persistent threat of infection by resident pathogenic microbes including bacteria, and in response they have engineered complex organic compounds with antibacterial activity from a diverse set of biological precursors. Therefore, a concerted effort to discover new antibacterial from river sources has the potential to contribute significantly to the treatment of the ever-increasing drug-resistant infectious diseases.

A study commissioned by the Union Water Resources Ministry to probe the "unique properties" of the Ganga found that the river water contains a significantly higher proportion of organisms with antibacterial properties. Other Indian rivers also contain these organisms but the Ganga — particularly in its upper Himalayan stretches — has more of them, the study suggests.(*Https://Www.Thehindu.Com/News/National/Ganga-Has-Higher-Proportion-of-Antibacterial-Agents-Study/Article61560349.Ece*, n.d.)

Understanding more about these mechanisms should hopefully lead to better treatment options for resistive infective diseases, and development of respective antimicrobial drugs.

2. Methodology

2.1 Collection of River water and soil sample

The 6 samples are collected from soil and river water of Uttarakhand. The river samples are from Saraswati river (Mana village, Uttarakhand, India), Ganga river (Haridwar), Chopta hills waterfall (Uttarakhand, India), Yamnotri hot water spring (Uttarkashi, Uttarakhand, India), Gangotri river (Gangotri town, Uttarkashi, Uttarakhand, India) and soil sample is from river bank of Gangotri (Gangotri town, Uttarkashi, Uttarakhand, India).

2.2 Pathogen collection:

The pathogens were collected from nearby microbiological lab (Rajkot, Gujarat). The pathogens are *Salmonella Typhi* (M1) and *Acinetobacter baumannii* (M5).

2.3 Isolation of bacteria from river and soil samples:

For isolation of bacteria from river sample, we used a spread plate technique. 1 ml of each river sample is spread evenly on nutrient agar media and incubated for 24 hours. Soil sample is serially diluted up to 10^5 ratios and 1 ml from 10^2 ,10^3, 10^4, 10^5 dilution was spread on nutrient agar media and incubated for 24 hours. Mixed colony of different microbes are further streaked to obtain pure colonies on same Nutrient agar media. Total 32 isolates were selected for primary screening (as shown in table no.1).

2.4 *Primary screening of isolates*:

Primary screening of isolates to check its antagonistic activity against resistant pathogens is done by a cross-streak method. Muller-Hinton Agar is prepared using distilled water or deionized water and sterilized by autoclaving. The sterilized Muller-Hinton Agar is poured into sterilized petri plates and allowed to solidify. All the isolates (from purified culture) were streaked and incubated for 24 hours and on the next day pathogen *Salmonella Typhi* (M1) and *Acinetobacter baumannii* (M5) were streaked on the same plate. The pathogens were streaked perpendicular to the isolates in such a way that they doesn't overlap each other. Result was observed after 24 hours of incubation at 37˚ C. The six positive isolates were observed.

2.5 Secondary screening of isolates:

For secondary screening using Agar well diffusion method of all six positive isolates obtained from primary screening, nutrient Agar media is prepared using distilled water or

deionized water and sterilized by autoclaving. The sterilized Nutrient Agar is poured into sterilized petri plates and allowed to solidify. Positive isolates are inoculated with the help of wire loop in to test tubes containing sterilized muller Hinton broth. And pathogens *Salmonella Typhi* (M1) and *Acinetobacter baumannii* (M5) were inoculated into nutrient broth. After 24 hours, pathogens were streak on the petri plates containing nutrient agar using cotton swab. The plate was rotated 60˚ /70˚ and streaking procedure is repeated to ensure an even distribution of the pathogens. Now, 3/4 wells are made on the petri plates. In one well we are adding a control which is prepared by adding 0.25mg streptomycin diluted in 10ml distilled water. Streptomycin is an effective antibiotic against *Salmonella Typhi* (M1) and *Acinetobacter baumannii* (M5) pathogens. The positive isolates were inoculated in the remaining well using micropipette. After incubation of 24 hours, 2 Isolates from sample 'A' and 'E' shows clear zone of inhibition against M1-salmonella typhi.

2.6 Morphological characterization

The morphological characterization of positive isolates obtained from secondary screening is done indicating the colony characterization and gram's nature. (As shown in table no. 2)

3. Result And Discussion:

3.1 Following table no. 1 shows total no. of isolates isolated from collected samples.

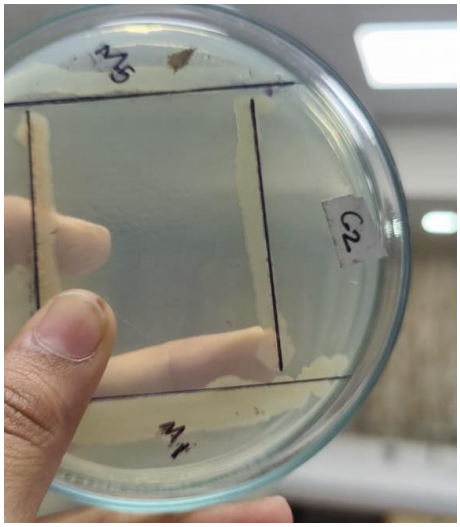
Sample No.	Sample Name.	Location	Dilution Factor	Total No. Of Isolates	Primary Screening	Secondary Screening
A	Saraswati river water	Mana village, Uttarakhand	-	6	1	1
B	Ganga river water	Haridwar	-	7	1	-
C	Chopta waterfall hill	Uttarakhand	-	4	2	-
D	Yamnotri hot water spring	Uttarkashi, Uttarakhand	-	5	1	-
E	Gangotri river water	Gangotri town, Uttarakhand	-	5	1	1
S	Gangotri river bank soil sample	Uttarkashi, Uttarakhand	10^{-2} 10^{-3} 10^{-4} 10^{-5}	2 1 0 2 Total- 5	-	-

3.2 Primary screening

In primary screening Following 6 positive isolates were observed: Sample A- Isolate no. A3 [Saraswati River, Mana village, Uttarakhand] showing antimicrobial resistance against M1 pathogen, Sample B- Isolate no. B2 [Ganga River, Haridwar] showing antimicrobial resistance against M5 pathogen, Sample C- Isolate no. C2 [Chopta waterfalls, Uttarakhand hills] showing antimicrobial resistance against M5 pathogen and Isolate no. C3 [Chopta waterfalls, Uttarakhand hills] showing antimicrobial resistance against M1 pathogen. Sample D- Isolate no. D2 [Yamnotri hot water spring, Uttarkashi, Uttarakhand] showing antimicrobial resistance against M5, Sample E - Isolate no. E31 [Gangotri water, Uttarkashi, Uttarakhand] showing antimicrobial resistance against M1 and M5 pathogen.

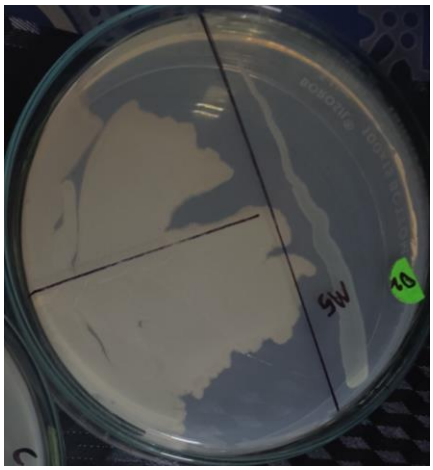
Sample -A (A3)

Sample -B (B2)

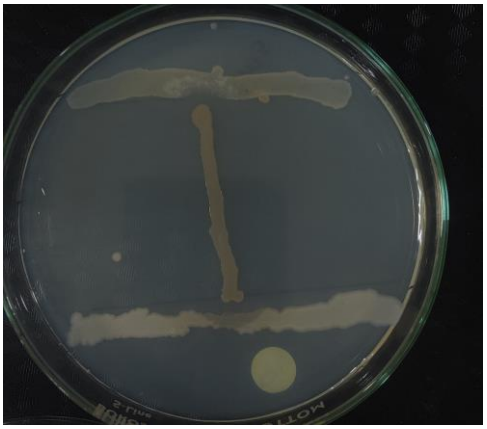


Sample -C (C2)

Sample -C (C3)



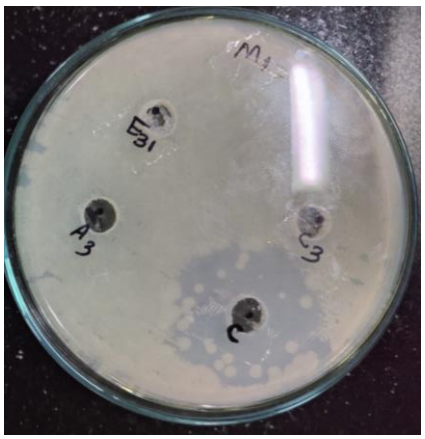
Sample -D (D2)



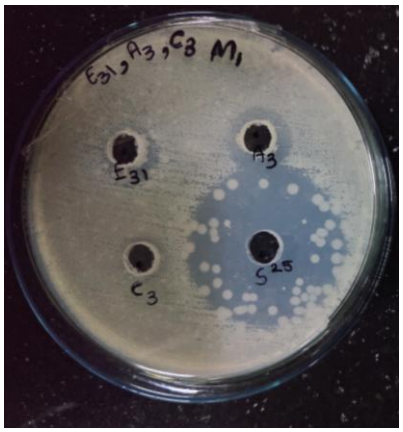
Sample -E (E31)

3.3 Secondary Screening

In secondary screening 2 Isolates from sample 'A' and 'E' shows clear zone of inhibition against- *Salmonella Typhi* (M1) pathogen while no result is observed against *Acinetobacter baumannii* (M5) pathogen. The isolates which are centrifuged and then inoculated doesn't show zone of inhibition suggesting that microbes are not producing antimicrobial substance externally. The non-centrifuged plates of sample A and E shows zone of inhibition against *Salmonella Typhi* (M1) pathogen. The zone of inhibition of Streptomycin is 9mm whereas zone of inhibition of sample A (isolate no. A3) is 6mm, and sample E (isolate no. E31) is 4mm.



Centrifuge plate containing M1 pathogen.



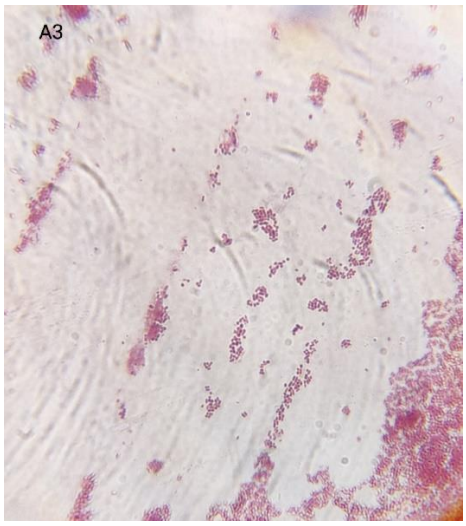
Clear zone of inhibition was observed in non-centrifuge plate around sample 'A' isolate no. A3 of 6mm and sample 'E' isolate no. 'E31' is of 4mm: showing antimicrobial resistance activity against *Salmonella Typhi* (M1).

3.4 Morphological Characterization

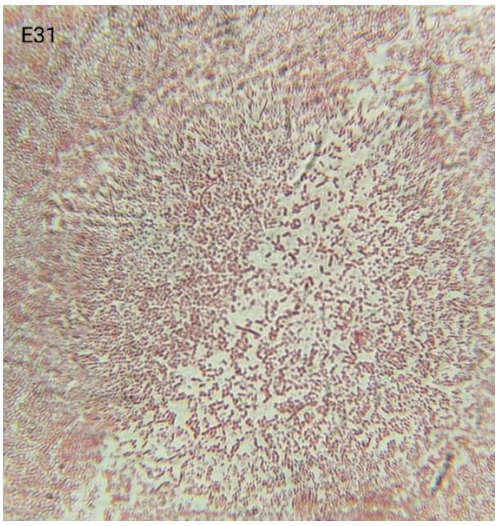
3.4.1 Table no.2 showing morphological characterization of colony

Characters	**Sample A**	**Sample E**
Colony size	Small	Small
Colony Shape	Round	Round
Colony Colour	Off-white	Off-white
Opacity	Translucent	Transparent
Texture	Smooth	Smooth
Surface morphology	Low convex	Low convex
Pigment	Not present	Not present

3.4.2 Gram staining



Sample A- Isolate no. A3 is Gram-negative in nature. This are rod shaped bacteria.



Sample E- Isolate no. E31 is Gram-negative in nature. This are rod shaped bacteria.

4. Conclusion

The 2 isolates were successfully isolated and screened from the river showing antagonistic activity against resistant pathogens which can be further tested and purified for production of antibiotics. Further research on river microbial flora may lead to better way of solving infections caused by pathogens, has potential to treat antimicrobial resistance by producing antimicrobial substances which can be isolated and purified to produce an effective antibiotic.

5. Acknowledgement

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