

## Isolation, Identification and Antimicrobial Susptability of Bacteria from Arpa River, Bilaspur, Chhattisgarh

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Article History	Abstract
<p>Received: 06 June 2023 Revised: 03 Sept 2023 Accepted: 01 Oct 2023</p> <p>CC License CC-BY-NC-SA 4.0</p>	<p><i>The isolation and characterization of bacteria from the Arpa River water (Bilaspur, India) could give a better knowledge of their variety, distribution, and possible influence on antibiotic usage. Several species of bacteria were found and their potentiality against antibiotics was determined as a result of this research. Isolation entails collecting sample water and cultivating the microbial culture in a suitable medium. Bacteria were separated from microbial cultures and subjected to biochemical assays, antibiotic susceptibility tests, and microbial evaluation studies. The goal of the research is to determine the types of microorganisms found in the Arpa River as well as to expand our understanding of the river. The potential of microorganisms is assessed in this study. The antibacterial activity of isolated microorganisms was tested using five drugs. The bacterial colony exhibits a zone of inhibition against various antibiotics, including amoxicillin, ofloxacin, streptomycin, and ciprofloxacin, but not against Cefixime &amp; L.A. The antibacterial activity of five different oils and extracts from plants were determined using the well diffusion method.</i></p> <p><b>Keywords:</b> Water, Microorganisms, Isolation, Antibiotics, Biochemical tests, gram staining, Enzymatic activity, Antibiotic susceptibility</p>

### 1. Introduction

Water microbiology is the scientific study of all biological features of microorganisms that reside in water (bacteria, archaea, viruses, fungus, parasites, and protozoa). This is sometimes referred to as marine microbiology, which is a subfield of environmental microbiology. These organisms perform several functions, including agricultural fertility, pollution removal from the environment, carbon storage stock regulation and the consumption of numerous major greenhouse gases, such as methane and nitrous oxide. and represent a risk to consumers.

On the one hand, untreated water bacteria can help to clean it by biodegrading possible human infections.

### 2. Materials and Methods

All the chemicals used in the study were obtained from HiMedia, Mumbai, Maharashtra.

#### Chemicals

Gram's crystal violet, Safranin, Gram's iodine, Phenol red, Hydrogen peroxide, Mannitol 1%, Malachite green, Alcohol, Cresol red, Bromocresol purple, Basic fuchsin, Bromocresol green.

Others: Different types of Antibiotics (Amoxicillin, Ciprofloxacin, Cefixime & L.A., Ofloxacin, Streptomycin). Different types of oils (Almond oil, Soybean oil, Coconut oil, Cashew oil, Mustard oil). Different types of plant extracts (Azadirachta indica (Neem), Mentha spicata (Mint), Murraya koenigii (Curry leaves), Tinospora cordifolia (Giloy), Calotropis gigantea (Crown flower) were used.

Source of sample: water samples were collected from Arpa River, near Seepat Chowk, Bilaspur, Chhattisgarh state, India.

### Collection of samples

Water samples were collected in sterile 200 mL plastic bottles and immediately taken to the laboratory for further analysis and stored for evaluation of microbes. Cultured the microorganisms on nutrient agar media by maintaining appropriate culture conditions for master Petridis plate preparation.

### Isolation of microorganisms

Serial dilutions and repeated cultivation of diluted microorganisms were used to isolate pure cultures.

### Preservation of pure culture

Pure bacterial cultures recovered from nutrient agar were maintained as well in nutrient broth, and bacterial slants were prepared on nutrient agar and used for future study.

### Biochemical tests

**a) Catalase test:** Catalase is a protein that aids in the conversion of hydrogen peroxide ( $H_2O_2$ ) into oxygen and water. A tiny quantity of isolated bacteria is combined with a 3% hydrogen peroxide solution to measure the synthesis of the bacterial enzyme catalase. A recent study revealed that oxygen bubbles were produced shortly after  $H_2O_2$  was added to the bacterial culture. The presence of gas bubbles were interpreted positively, signifying catalase activity. Bacteria that produce catalase are either stringent aerobes or facultative anaerobes.

**b) Amylase test:** Microbial isolates produced amylase enzymes are distinguished by the formation of clear zones surrounding the colony following the addition of a 1% iodine solution to microbe overgrowth medium. Amylase enzyme activity was tested using a dinitrosalicylic acid reagent assay.

**c) Gelatinase Test:** The gelatin hydrolysis test was used to measure the ability of microorganisms to produce gelatinase, which liquefies gelatin. This process took place in two consecutive events. In the first reaction, gelatinase reduced gelatin to polypeptides. After that, the polypeptides converted into amino acids. Bacteria took these amino acids and use them for metabolic processes. To carry out this test medium with gelatin was prepared in Petridis plates and inoculum was added in that and seen partial liquefaction that indicates positive result.

### Production of the acid (Carbohydrate fermentation test)

The fermentation of mannitol was tested using phenol red. In this experiment, pure culture inoculums was added to mannitol broth and fermented. Before testing, the inoculated broth culture was kept at 35-37°C for 24 hours. A positive test involves a colour change from red to yellow, suggesting an acidic pH alteration.

### Antimicrobial activity

This was done by employing an antibacterial activity technique i) Disc Diffusion (31) (ii) The Well Diffusion Methods (32) were used to achieve this objective.

a) Bacterial culture potentiality against drugs: Antimicrobial drugs were used at a concentration of 1mg/1mL to evaluate the potentiality of bacterial cultures against drugs. Activity was tested with five antibiotics i) amoxicillin ii) ciprofloxacin, iii) cefixime & L.A., iv) Ofloxacin, and v) streptomycin.

b) Bacterial culture potentiality against oils Almond oil, Coconut oil, Soybean oil, Cashew oil, and Mustard oil were used to demonstrate antimicrobial activity.

Antimicrobial activity of plant extracts: Five types of plant extracts were used for antimicrobial activity using methanol as a solvent (1 g/1mL): Azadirachta indica (Neem), Mentha spicata L. (Mint), Murraya koenigii (Curry leaves), Tinospora cordifolia (Giloy), Calotropis gigantea (Crown flower).

### Dyes degradation studies by bacterial cultures

The five types of synthetic dyes used for degradation are as follows

a) Bromocresol Purple b) Bromocresol Green c) Malachite Green d) Basic Fuchsin e) Cresol Red

5ml culture broth was placed in a test tube, followed by 5ml of aqueous dye solution (0.01 of the above dyes diluted in 100 ml of distilled water) and kept in a orbital shaker for 4 to 5 days to observe the colour change.

### Biofuel production

Biofuels are made from live resources or trash. One of the most common biofuels, ethanol, evolved from plants. In this work, microbial fermentation was used to generate ethanol for medical and industrial purposes.

Steps in biofuel (ethanol) production are:

1. Preparation of nutrient broth
2. Adding organism and yeast to broth

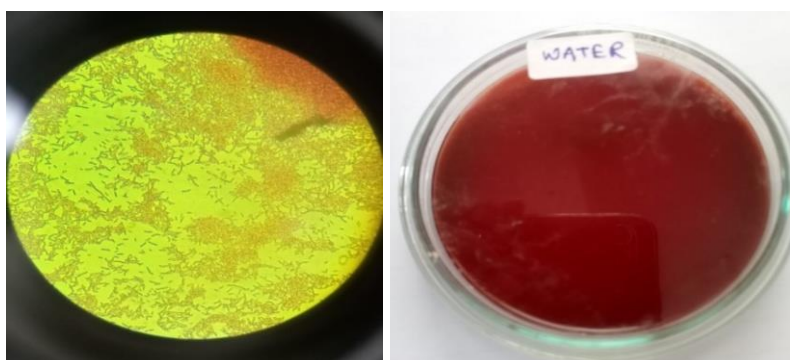
### 3. Fermentation

The fermentation conditions are nearly same (pH5,35°), however the culture and cultivation are not.

## 2. Results and Discussion



**Fig 1:**a)Isolation of microbes from water b) Pure bacterial culture maintenance



**Fig 2:** c) Gram staining d) Macconkey agar

Purple colour observed under the microscope which indicates presence of gram positive bacteria.

Motility test results: Under the microscope, the bacterial colony shown motility, which indicates that the bacterial colonies were motile. Motile organisms contain flagella which helps them to travel beyond the point of inoculation.

No bacterial culture colonies were detected in Macconkey agar, indicates the negative results

**Table 1: Biochemical test results**

Biochemical tests	Catalase test	Amylase test	Gelatin hydrolysis test
Results	Positive (Bubble formation) (+)	Positive (Clear zone appears) (+)	Positive (Gelatin degrades) (+)

All the performed biochemical tests shown positive results

**Table 2 : Antimicrobial potatality by bacterial culture**

Antibiotics	Results	Zone of inhibition (mm)
Amoxicillin	+	19 mm
Ciprofloxacin	+	39 mm
Cefixime & L.A.	-	No Zone
Ofloxacin	+	40 mm
Streptomycin	+	35 mm

All antibiotics shown zone of inhibition excluding ceixime, L.A. However, Ofloxacin shown maximum zone of inhibition whereas Amoxicillin shown less zone of inhibition.

**Table 3:** Antimicrobial activity of plant extracts

Extract Plant	Results	Zone of Inhibition
Azadirachta indica (Neem)	+	6 mm
Mentha spicata (Mint)	-	No Zone
Murraya koenigii (Curry leaves)	-	No Zone
Tinospora cordifolia (Giloy)	-	No Zone
Calotropis gigantea (Crown flower)	-	No Zone

In the antimicrobial activity of plants *Azadirachta indica* shown zone of inhibition whereas remaining all plants extracts were not shown (1mg/1mL concentration).

### Carbohydrate fermentation test

In the carbohydrate fermentation test purple colour was seen which indicates the positive results acid, gas production was also observed.

**Table 4:** Antimicrobial study by oils

Oils	Results	Zone of Inhibition
Almond oil	-	No Zone
Soyabean oil	-	No Zone
Coconut oil	+	5 mm
Cashew oil	-	No Zone
Mustard oil	-	No Zone

Coconut oil showed a zone of inhibition, whereas the remaining oils did not, indicating their ineffectiveness against bacterial cell walls.

**Table 5:** Dyes degradation studies by bacterial cultures

Dyes	Dye Reducing property
Malachite Green	+
Cresol Red	-
Bromocresol Purple	-
Basic Fuchsin	-
Bromocresol Green	-

Malachite Green shown dye reduction, all the remaining dyes have not shown any dye reduction by microbial culture.

Bio-fuel production: Bacterial culture did not produce bio-fuel and also there was no gas production. Several factors influence antibiotic efficacy: (a) illness (sensitivity and resistance, tolerance, persistence, biofilm) and inoculum volume; (b) antimicrobial concentration and inhibitory concentration (c) Host factors (impact on the blood and digestive tract).

A greater understanding of the relationship between antibiotic use, bacterial infections, and host responses provides new insights and aids attempts to produce effective antibiotics, which results in better clinical outcomes while lowering drug side effects.

An important characteristic of essential oils and their components is hydrophobicity, which enables them to partition with the lipids present in the cell membrane of bacteria and mitochondria, rendering them more permeable by disturbing the cell structures. In this study bacterial culture were shown sensitivity towards coconut oil.

### 3. Conclusions

Consequently, the isolation and characterization of bacteria from the water source provides a better understanding of their diversity, distribution, and potential impact on antibiotic use. Many different species of bacteria were found, and their potentiality against antibiotics was studied. The bacteria are isolated by

collecting sample water and culturing them in a suitable medium. Pure strains were obtained and characterized utilizing spreading and serial dilution procedures. Characterization procedures such as morphological analyses and biochemical testing allow isolated organisms to be identified and classified. This approach reveals more about the microbial populations in the Arpa River in Bilaspur district, Chhattisgarh state, India. Antibiotic resistance testing gives useful information on antibiotic potentiality against isolated bacteria. This knowledge is crucial for comprehending the emergence and spread of antibiotic resistance in environmental organisms. The result of this study shows that there are many potential types of bacteria in River water, each with its own unique and antibacterial properties. The presence of antibiotic resistant bacteria highlights their potential Impact on Public health and the environment. Identification of bacteria and immune signatures helps monitor and manage anti-bacterial susptability. By doing all the above experiments by suitable methods, we concluded that the property of isolated microorganisms from Arpa river water, Bilaspur district, Chhattisgarh state, India and their evaluation has been done. However further studies are needed for pathological aspect.

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