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Original Research Article

# Assessment of Common Microorganisms Obtained From Various Environmental Sources Using Cultural, Biochemical, and Microscopic Analysis

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**Abstract**

**Background**: The utility of the biochemical, or metabolic, test has been on display for nearly 150 years for the isolation and identification of microorganisms causing infectious disease due, in large part, to the very nature of microbial versatility. This study aimed to systematically investigate the influence of experimental conditions on various colony morphological features, such as form, margin, texture, size, and color.

**Methods**: The isolation process was conducted between January 2026 and April 2026 at Department of Microbiology with collaboration Department of Biochemistry in SKMCH, Muzaffarpur, Bihar. This study involved the isolation of 11 different types of microorganisms from environmental samples, including soil and water. The microorganisms identified were Fungi, E. coli, Klebsiella spp., Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas putida, Pseudomonas fluorescence, Pseudomonas aeruginosa, Vibrio spp., Salmonella spp., Shigella spp., and Bacillus spp.

**Results**: Several microorganisms have been identified on the habitat, multiplication rate, environmental benefits, virulence factors, growth strategy, physiological properties, genetic variation, metabolic activity and clinical significance of different microorganisms.

**Conclusion**: The biochemical and growth-based approaches in the diagnostic laboratory were initially born out of the discovery of microorganisms and the need to confirm their causative link to infectious disease.

**Keywords:** Microorganisms, Microscopic Characteristics, Cultural Characteristics, Biochemical Characteristics.
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## Introduction

It is common practice, especially in clinical laboratories, to isolate samples using bacteriological techniques such as culture in specific or different media. Scientists and medical professionals can use the colonies that bacteria develop on agar surfaces to differentiate between genera or even species.

A wide range of techniques for identifying, detecting, and distinguishing bacteria are currently available as a result of substantial technological advancements in the bacterial identification process. In order to expedite analysis and minimize handling, molecular techniques including MALDI-TOF MS, PCR, and ELISA have significantly improved bacterial identification.[1] To evaluate the microbiological impact of antibiotic courses and whether phenotypic selection is taking place, culture-based methods are still required to count the number of bacteria both before and after antibiotic delivery. Antibiotic treatments can alter bacterial activity in ways that molecular methods might not be able to detect. Because unusual colony morphologies frequently exhibit distinct biochemical and metabolic traits, clinical diagnosis can be difficult. Small colony variations (SCV) of Staphylococcus aureus, for example, might provide false negative results in coagulase tests due to their changed metabolic activity.[2] Therefore, describing colony morphology often enhances traditional microbiological identification by recognizing intra-strain diversity.[3-6].

Bacterial phenotypic and genetic adaptation leads to intra-population variety, which offers benefits and raises the probability of bacterial survival in changing and evolving environments. [4,5]. A macroscopic depiction of the many biological mechanisms used by microorganisms to deal with a variety of stressful situations, such as starvation, oxygen deprivation, antibiotics, and host defenses, is provided by changes in colony shape.[3-6]. Furthermore, differences in virulence [7], antibiotic resistance [8,9], and persistence can all be reflected in variations in colony morphology.[10] Colony morphology characterisation is still useful for comprehending the diversity of individual microorganisms resulting from genetic modifications and reversible changes, even though some writers believe it is out of date. [11, 12].

Multiple colony morphology variants are frequently seen in chronic infections like cystic fibrosis (CF). Pseudomonas aeruginosa's transformation from a non-mucoid to a mucoid form, which is more challenging to eliminate, is a prominent clinical characteristic in cystic fibrosis.[13, 14].Mucoid variants may exhibit increased resistance to medications such imipenem, gentamicin, aminoglycosides, and ciprofloxacin as well as multidrug resistance [15,16]. Bacteria linked to both acute and chronic illnesses have been found to exhibit a variety of additional morphotypes. Small colony variations (SCV) are among the most prevalent and thoroughly researched examples. [17,18,19,20,21], hyperpili ated colonies, and rough (small) colonies [19,22]

Different morphological patterns emerge in the colonies when bacteria from biofilms are grown on agar media, demonstrating that bacteria can change their phenotype to facilitate the formation of biofilms.[23]

## Material and Methods

Between January and April of 2026, the isolation procedure was carried out at the Department of Microbiology at Sri Krishna Medical College, Muzaffarpur, Bihar, in cooperation with the Department of Biochemistry. Eleven distinct kinds of microorganisms were isolated from environmental materials, such as soil and water, for this investigation. The microorganisms identified were Fungi, E. coli, Klebsiella spp., Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas putida, Pseudomonas fluorescence, Pseudomonas aeruginosa, Vibrio spp., Salmonella spp., Shigella spp., and Bacillus spp. The spread plate, streak plate, and membrane filter methods were among the methods used to count the bacteria in this investigation. For soil samples, 90 milliliters of distilled water were used to homogenize 10 grams of dirt, which was then diluted to a factor of 10-7. To separate the total live bacteria and fungus from the 10-7 dilution, 0.1 ml of the material was plated onto Nutrient agar and Sabouraud Dextrose agar, respectively. Additionally, certain microbes were detected using selective media as Salmonella-Shigella agar, MacConkey agar, Mannitol Salt agar, Cetrimide agar, TCBS agar, and Starch agar.

Similarly, one loop full of the inoculum was streaked over the same media using the four-quadrant streaking procedure with the same dilution. A membrane filter with a hole size of 0.45 µm was used to filter 100 milliliters of water samples. After that, the filter paper with the microorganisms on it was put on several selective media. With the exception of Sabouraud Dextrose agar plates, which were incubated at $25^0$C for 48 hours, all inoculation plates were incubated at $37^0$C for 24 hours.

Following incubation, the isolates' size, pigment, form, margin, elevation, and other cultural or colony traits were assessed on the agar plates. The isolates' inocula were then moved from the agar plates onto liquid media in order to measure the turbidity of their growth. Bacterial cultures from the agar plates were prepared for gram staining in order to identify the shape, organization, and various groupings of the isolates. Additionally, simple staining was done using cultures from the Sabouraud Dextrose agar (SDA) plate.

Lastly, all of the pathogenic isolates discovered in the samples were identified using standard biochemical techniques. Triple Sugar Iron, citrate, IMVIC (Indole, Methyl Red, Voges-Proskauer, and Citrate), MIU (Motility, Indole, and Urease), catalase, nitrate, gelatin, starch, and oxidase were among these tests.

## Results

**Table 1: Isolation of different microorganisms from environmental samples**

| Media | Isolates | Positive/Nagative |
|---|---|---|
| Nutrient agar | Total Viable Bacteria | + |
| Saborout Detroseagar | Fungi | + |
| MacConkey agar | E.coli/Klebsealia | + |
| ManitolSalt agar | Staphylococcus spp. | + |
| Pseudomonas agar | Pseudomonas spp. | + |
| TCBS agar | Vibrio spp. | + |
| Salmonella-Shigella agar | Salmonella/Shigella spp. | + |
| Starch agar | Bacillus spp. | + |
| Clostridium agar | Clostridium spp. | - |

For this reason present study tried to archive the different characteristics of some common microorganisms such as Fungi, E. coli, Klebsealia spp., Staphylococcus aureus, Staphylococcus epidermidies, Pseudomonas putita, Pseudomonas fluorescence, Pseudomonas aeruginosa,Vibrio spp., Salmonella spp., Shigella spp., Bacillus spp. (Table 1)

**Table 2A: Biochemical identification of the microorganisms**

| Microorganisms | TSI | | | $H_2S$ reaction | Indole | MR | VP | Citrate | Motility |
|---|---|---|---|---|---|---|---|---|---|
| | Slant | Butt | Gas | | | | | | |
| E.coli | Y | Y | + | - | - | + | - | - | + |
| Klebsiella spp. | Y | Y | + | - | - | - | - | + | - |
| Shigella spp. | R | Y | - | - | - | + | - | - | + |
| Salmonella spp. | R | Y | - | + | - | + | - | + | + |
| Vibrio spp. | Y | Y | - | - | + | + | - | + | + |
| Staphylococcus spp. | Y | R | + | + | - | + | - | - | + |
| Bacillus spp. | Y | R | - | - | + | + | - | - | +/- |
| Pseudomonas spp. | R | R | - | - | - | - | - | + | + |

**TSI: Triple Sugar Iron Test; Y: Yellow (Acid); R:Red(Alkaline); MR: Methylred; VP: Voges-Proskauer**

**Table 2B: Biochemical identification of the microorganisms**

| Microorganisms | Oxidase | Catalase | Nitrate | Unease | Starch hydrolysis | Manitol | Gelatin utilization |
|---|---|---|---|---|---|---|---|
| | - | + | + | - | - | + | - |
| E.coli | - | + | + | + | - | + | - |
| Klebsiella spp. | - | + | + | - | - | - | - |
| Shigella spp. | - | + | + | - | - | - | - |
| Salmonella spp. | + | - | - | - | - | - | - |
| Vibrio spp. | - | + | + | - | - | + | + |
| Staphylococcus spp. | - | + | + | - | + | - | + (rapid) |
| Bacillus spp. | + | + | + | - | - | + | + (rapid) |
| Pseudomonas spp. | | | | | | | |

All the isolates were introduced for the identification of their biochemical properties against different parameters like their ability of different carbohydrate utilization, ability of the production of $H_2S$ gas, motility, citrate, oxidase, catalase, starch, nitrate and gelatin utilization ability (table 2A). E. coli utilized lactose, sucrose and dextrose in TSI, showed the positive result MR, motility, nitrate and manitol.

Klebseilla spp. showed positive result for citrate, catalas, nitrate, urease, manitol Klebseilla spp. also utilized lactose, sucrose and dextrose. Shigella spp. showed positive result for MR, motility, catalas and nitrate. Salmonella spp. showed positive result for MR, motility, citrate, catalase, and nitrate. Salmonella only produced H2S.

Vibrio spp. showed positive result for indole, MR, citrate, motility and oxidase. Staphylococcus spp. showed positive result for MR, motility, catalase, nitrate, manitol and gelatin. The special characteristic of Bacillus spp. was to hydrolyze gelatin and starch. Pseudomonas spp. showed positive result for citrate, motility, oxidase catalase and nitrate (table 2B).

**Table 3A: Morphological and microscopic characteristics of isolated microorganisms on nutrient agar plate**

| Isolates | Cultural Characteristics | | | | |
|---|---|---|---|---|---|
| | Size | Pigment | Form | Margin | Elevation |
| Fungi | Large | Blackish | Rhizoid | Filamentous | Convex |
| E.coli | Small | Dry pink | Circular | Entire | Convex |
| Klebsealia spp. | Small | Gummy pink | Circular | Entire | Flat |
| Staphylococcus aureus. | Large | Yellow | Irregular | Endulate | Raised |
| Staphylococcus epidermidies | large | Pink | Irregular | Endulate | Raised |
| Pseudomonas putita. | Pin point | Whitish | Circular | Entire | Raised |
| Pseudomonas fluorescence. | Large | Greenish | Irregular | Lobate | Raised |
| Pseudomonas aeruginosa | Small | Non fluorescent | Irregular | Lobate | Raised |
| Vibrio spp. | Large | Yellow | Irregular | Undulate | Raised |
| Salmonella spp. | Small | Black centre | Circular | Entire | Convex |
| Shigella spp. | Small | Pink | Circular | Entire | Convex |
| Bacillus spp. | Moderate | Violet | Irregular | Lobate | Raised |

**Table 3B: Morphological and microscopic characteristics of isolated microorganisms on nutrient agar plate**

| Isolates | Microscopic Characteristics | | |
|---|---|---|---|
| | **Shape** | **Arrangement** | **Staining** |
| Fungi | Bird's nest & flask fungi | Chain | - |
| E.coli | Rod | Single | Gram negative |
| Klebsealia spp. | Rod | Single | Gram negative |
| Staphylococcus aureus. | Cocci | Cluster | Gram positive |
| Staphylococcus epidermidies | Cocci | Cluster | Gram positive |
| Pseudomonas putita. | Rod | Single | Gram negative |
| Pseudomonas fluorescence. | Rod | Single | Gram negative |
| Pseudomonas aeruginosa | Rod | Single/Pairs/short chain | Gram negative |
| Vibrio spp. | Curved/Comma | Single polar flagellum | Gram negative |
| Salmonella spp. | Rod | Cluster | Gram negative |
| Shigella spp. | Rod | Short chain | Gram negative |
| Bacillus spp. | Rod | Chain | Gram positive |

On agar plates to describe the colony of different bacteria can use different terms like colony morphology, colony types, colony variants and morphotypes.

Presence of colonies on the agar media indicated that a group of bacteria grown from the single colony on agar surface those were different by their character on different media.

All the strain on different agar media showed distinct size, shape, arrangement, margin, elevcation, pigmentation and also gram reaction through microscope (table 3A and 3B).

**Table 4: Morphological characteristics of isolated microorganisms on liquid medium**

| Isolates | Cultural Characteristics | | |
|---|---|---|---|
| | **Degree of growth** | **Nature of turbidity** | **Nature of surface growth** |
| Fungi | High | Cloudy | Wrinkled |
| E. coli | High | Cloudy | Smooth |
| Klebsiella spp. | High | Cloudy | Smooth |
| Staphylococcus aureus. | High | Cloudy | Smooth |
| Staphylococcus epidermidies | High | Murky | Smooth |
| Pseudomonas putita. | High | Murky | Rough |
| Pseudomonas flourescence. | Moderate | Murky | Rough |
| Pseudomonas aeruginosa | Moderate | Murky | Rough |
| Vibrio spp. | Low | Murky | Rough |
| Salmonella spp. | Moderate | Murky | Rough |
| Shigella spp. | Moderate | Cloudy | Rough |
| Bacillus spp. | Moderate | Murky | Rough |

Suspension on liquid broth is essential for conducting different experiment such as Antibiogram, anti-microbiual activity, MIC, MBC, response of bacterial growth against different temperature, pH and salinity.

**Discussion**

Numerous microorganisms have been identified, and scientists are investigating their habitat, rate of multiplication, environmental advantages, virulence factors, growth strategy, physiological characteristics, genetic variety, metabolic activity, and clinical importance. [24, 25]. Some of the results of our research on the antimicrobial qualities of certain medicinal plants against various pathogenic laboratory strains and the antibiogram characteristics of various pathogens were reported from the microbiology lab of SKMC, Muzaffarpur.

Understanding the cultural, microscopic, and biochemical traits of the isolates that are frequently used in our basic research is crucial for performing the fundamental study of microbiology. The growth parameters of various bacteria on liquid media were determined in this study using three key categories: degree of growth, turbidity type, and surface growth type. The fungus E. coli Klebsiella spp., Staphylococcus spp., and Staphylococcus epidermidies all had extremely high growth rates. The proliferation of Staphylococcus species was hazy. For E. Coli Klebsiella spp., the colony's surface area was smooth. Staphylococcus species and Staphylococcus epidermidies, whereas Pseudomonas putita, Pseudomonas fluorescence, Pseudomonas aeruginosa, Vibrio species, Salmonella species, Shigella species, and Bacillus species were found in the rough area.

With the exception of Shigella species, Pseudomonas putita, Pseudomonas fluorescence, Pseudomonas aeruginosa, Vibrio species, Salmonella species, and Bacillus species all had murky cell turbidity appearances. Pseudomonas fluorescence, Pseudomonas aeruginosa, Salmonella spp., Shigella spp., and Bacillus spp. all demonstrated moderate growth, however Vibrio spp. shown lesser growth.

The degree of growth According to a number of earlier research, the identification of the colony morphology of various bacteria on various media is strongly dependent on the environmental conditions, but the variability is not very noticeable. [26, 27, 28, 3-6].

## Conclusion

The key component for clinical diagnosis, among other things, is the identification of characteristic colony morphologies. The colony morphology manual of bacteria on agar media is extensively employed in scientific and clinical laboratories as an extra tool to identify distinct bacterial species, which makes this sort of study quite common. In order to help with clinical diagnosis on bacterial profiling, the ultimate goal is to predict antibiotic resistance, expression of virulence factors, and persistence capacity based on morphological features. The current study will provide guidelines for many authors, clinicians, and technicians to better understand bacterial adaptation and evolution for fundamental science purposes.

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