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# Dissimilar Types of Isolation Microbiota

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**Abstract:** *The human body contains many different and many varities of microorganisms, including a large number of bacteria, viruses, fungi, and protozoa etc, which are also referred to as the microbiota respectively. Compared with the basic number of cells majorly comprising the human body, that of the microbiota has been found or got to be much larger. The microbiome is defined or can be stated as microorganisms and their basic genomes have been shown to contain about 100 times more genes than the basic human genome. The microbiota also affects many vital functions in the human body too.*

**Keywords:** Human Body, Microbiota*, S. pyogenes, C. diphteria*

## I. INTRODUCTION

All plants and animals too, even humans contain many microorganisms, which invoke many relationships with mainly the host, such as symbiosis, commensalism, and parasitism etc[1, 2]. The basic cells of the human body, around 90% of which are alien and unknown to it [3], are referred or called to as the microbiota or microbiome. The microbiota is basically an assemblage of microorganisms that form an simple ecological community in a specific specified area. The first ever scientists to mention this term or terminology were Lederberg and McCray when they referred to the role of microorganisms in human health as well as diseases [1]. The terminology microbiome, on the other way or hand, focuses on the basic genomes of all microorganisms in a proper specific environment nook [4]. Prebiotics are basically selectively fermented components that majorly lead to specific changes in the composition and/or activity of the intestinal microbiota respectively, thereby providing major benefits to host health [3]. The word probiotic originally came from the Latin word pro (meaning *for*) and also from Greek word *biōtikós* (meaning *of life*) Probiotics are alive, also beneficial, nonpathogenic bacteria such as *Lactobacillus* and *Bifidobacterium* and yeasts like *Saccharomyces* that provide major and important health benefits to the host when administered in sufficient amounts respectively[5]. On the very other hand, prebiotics are selectively and originally fermented components that basically lead to specific changes in the basic composition and/or activity of the intestinal microbiota respectively, thereby providing essential benefits to host health respectively[6].

There are around more than even 100 trillion microorganisms in the human gut alone, and they have also 150-times more genes than the entire human genome combined[7]. The basic development of molecular methods that relyals on 16S rRNA, 18S rRNA, and other marker genes respectively has also helped in determining and knowing of microbes found in a specific area. These methods have opened the doors for us to studying and clarifying and seeing the roles of microorganisms in the basic human body [8].

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Humans have a variety of microbiota, including bacteria, archaea, fungus, protists, and viruses. A considerable portion of ocular inflammations are caused by infections of the external eye, some of which invade the cornea and cause vision loss. [9]

The conjunctiva, lid, and cornea are the most frequently afflicted areas of the eye when there is an ocular disease caused by pathogenic microorganisms. The main culprit responsible for many eye infections and potential vision loss is bacteria. Clinical symptoms such conjunctivitis, scleritis, keratitis, blepharitis, canaliculitis, and dacryocystitis are frequently recorded. The most frequent cause of "red eye" is conjunctivitis, and corneal ulceration is a significant factor in mono-ocular blindness in impoverished nations. Infection (infective conjunctivitis), allergic responses, and irritation are the three most typical causes of conjunctivitis (loose eyelash).

Bacteria and viruses are the most frequent causes of infectious conjunctivitis. The discharge from bacterial conjunctivitis is pus-filled, whereas the discharge from viral conjunctivitis is aqueous. Second only to cataracts, infectious keratitis is a leading cause of vision loss and blindness. Endophthalmitis can result in vision-threatening ocular complications after intraocular surgeries and during open-globe accidents, while blepharitis is an inflammation of the eyelid margins that can cause patient pain and a reduction in visual function. Lacrimal sac and duct inflammation is known as dacryocystitis.[10]

One of the few points where germs can enter the body directly is the nose. The nasal passage is crucial for filtering the air we breathe in and for preventing the body from absorbing microscopic foreign particles or bacteria. The nasal passages and nose are, or can be, the ideal environment for some bacteria, both healthy and dangerous, which many people are unaware of. The nose, which is situated on the face, contains the nostrils, which serve as the primary entrance to the nasal tube. The passageway continues to the throat from there. The nose, which is situated in the centre of the face, is the organ of smell. The internal part of the nose is located above the roof of the mouth. It is supported by cartilage and bone. The frontal process of the maxillary bone and the nasal bones on each side make up the majority of the bony portion. The airway starting at the nostril (where the nose opens) and terminating at the back of the throat is called the nasal cavity. A partial or total obstruction of one or both of these air channels is referred to as nasal obstruction. One of the few bodily openings via which germs and microorganisms may enter the body is the nose.

The physical state of the nasal passages varies depending on the health of the nose. When suffering from infectious diseases like sinusitis, the nose may flow or get clogged and secrete a thick fluid. In polyps, the airway is obstructed and no longer produces mucus. It is one of the factors that contributes to sinusitis and causes the nasal membrane to enlarge. Other bacteria secrete themselves as a result of this environment. Additionally, environmental changes like a dry or cold temperature, swimming, and air pollution cause the bacteria to thrive. Given that they produce rhinitis, which makes breathing difficult and causes congestion, colds and the flu provide the bacteria with an ideal environment in which to proliferate. The immune system deteriorates, which allows the germs to spread more quickly. [10]

One of the most distinctive and diverse ecosystems of bacteria in the human body may be found in the mouth cavity. The mouth is a biome that has a variety of distinct habitats, each with its own set of microorganisms. Numerous studies have been conducted to characterise the microbiomes of the

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saliva, tongue, buccal mucosa, teeth surfaces, gums, palate, subgingival and supragingival plaque, as well as the throat and tonsils. These studies revealed general similarities but also small-scale differences, such as higher levels of the genus Corynebacterium in both types of plaque or higher levels of the phylum Firmicutes in both saliva and buccal mucosa as compared to plaque . It is usual to employ an oral rinse as a sample collection technique in metagenomics investigations to get a representative sample of the whole oral microbiome, even though some of these habitats are examined independently in other studies. [11]

## II. MATERIAL AND METHOD

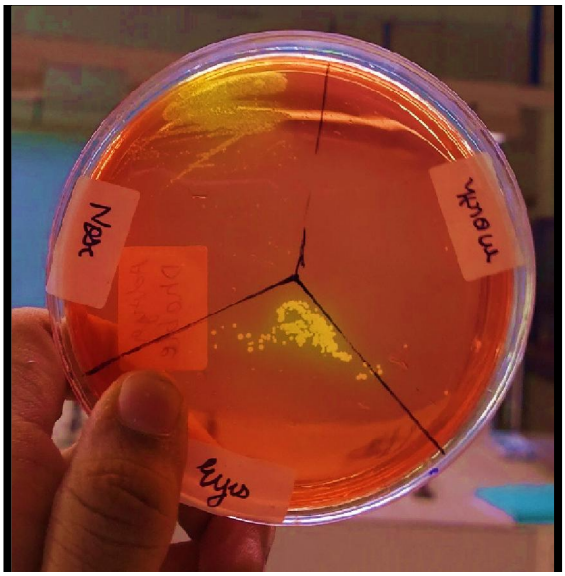
### 2.1 Collection of Samples

3 samples were isolated from the nose, mouth, and eyesfrom 5 different people. These people worked at different places and were not employed in a common occupation.

### 2.2 Isolation and enrichment of samples

0.1 ml of the sample has added in different 10 ml broths name as Nutrient broth, Nutrient agar, CLED Agar, Mannitol Salt Agar and BHI agar. We carried the dilution technique for getting isolated colonies

After 24 hours several colonies were spotted and streaked on different medias such as Nutrient agar, CLED Agar, Mannitol Salt Agar and BHI agar.


**Identification of Microorganisms by Gram nature and Biochemical technique**

**Identification of microorganism which highest colonies found in water samples**

The bacterial strain was identified on the basis of Cultural, "Morphological and Biochemical Characteristics as given in the Bergey's Manual of Synematic Bacteriology vol. 2 (Holt et al., 1984).

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**Morphological and Cultural Identification:**

Strain showing highest antibacterial activity was Gram stained and stained microscopically for cellular morphology and Gram stain phenotype. Colonies developed on Certrimide agar were characterise by observing various parameters viz, shape, size, colour, elevation, margin, surface etc. and recorded in the table.

**Biochemical Characterization:**

Catalase test was performed by inserting loop full colony into tube containing 3% hydrogen peroxide. A citrate utilization test was carried out by Koser citrate Agar (Schillinger, 1996) and assay for nitrate reduction were performed (Harrigan, 1998).

IMViC tests was also performed and ability to ferment various carbohydrates were evaluated using Sugar fermentation medium broth supplemented with filter sterilized sugar solutions to a final concentration of w/v and 0.004% Phenol red (Schillinger, 1996).

## III. RESULT AND DISCUSSION

### 3.1 Collection of Sample

Table 1:

| **Sr. no** | **Person no.** | **Occupation** |
|---|---|---|
| 1. | Person 1 | Industry, Mumbai |
| 2. | Person 2 | Printing office |
| 3. | Person 3 | Security guard |
| 4. | Person 4 | Student |
| 5. | Person 5 | Peon |

**Isolation and Enrichment of water Sample:**

**Microbial colonies isolated from Mouth:**

Table 2:

| **Person Sample** | **Nutrient agar** | **Mannitol Salt agar** | **BHI agar** | **CLED agar** | **Total** |
|---|---|---|---|---|---|
| Person 1 | 21 | 0 | 19 | 16 | 56 |
| Person 2 | 11 | 0 | 10 | 10 | 31 |
| Person 3 | 19 | 1 | 20 | 19 | 58 |
| Person 4 | 16 | 0 | 19 | 18 | 53 |
| Person 5 | 9 | 0 | 13 | `21 | 43 |
| **Total** | 76 | 1 | 81 | 84 | 241 |

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**Microbial colonies isolated from Nose:**

Table 3:

| Person Sample | Nutrient agar | Mannitol Salt agar | BHI agar | CLED agar | Total |
|---|---|---|---|---|---|
| Person 1 | 24 | 28 | 20 | 22 | 94 |
| Person 2 | 21 | 10 | 18 | 12 | 61 |
| Person 3 | 26 | 30 | 20 | 29 | 105 |
| Person 4 | 22 | 29 | 16 | 29 | 96 |
| Person 5 | 20 | 24 | 19 | 29 | 92 |
| **Total** | 113 | 121 | 93 | 121 | 448 |

**Microbial colonies isolated from Eyes:**

Table no. 4:

| Person Sample | Nutrient agar | Mannitol Salt agar | BHI agar | CLED agar | Total |
|---|---|---|---|---|---|
| Person 1 | 29 | 1 | 24 | 26 | 80 |
| Person 2 | 28 | 0 | 18 | 19 | 65 |
| Person 3 | 22 | 0 | 19 | 29 | 70 |
| Person 4 | 18 | 0 | 29 | 23 | 70 |
| Person 5 | 27 | 0 | 12 | 21 | 60 |
| **Total** | 124 | 1 | 102 | 118 | 345 |

**Identification of microbe:**

**Gram nature of the Microbe isolated from the Mouth has been shown in table no. 5**

| Colony Characteristics | Colony 1 |
|---|---|
| Size | 2mm |
| Colour | White |
| Shape | Circular |
| Margin | Smooth |
| Opacity | Flat |
| Consistency | Sticky |
| Elevation | Flat |
| Gram nature | Gram Positive |
| Morphology | Cocci shaped |

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**Gram nature of the Microbe isolated from the Nose has been shown in table no. 6**

| **Colony Characteristics** | **Colony 1** |
|---|---|
| Size | 1mm |
| Colour | Golden yellowish |
| Shape | Circular |
| Margin | Smooth |
| Opacity | Opaque |
| Consistency | Sticky |
| Elevation | Slightly raised |
| Gram nature | Gram positive |
| Morphology | Cocci in cluster |

**Gram nature of the Microbe isolated from Eyes been shown in table no. 7:**

| **Colony Characteristics** | **Colony 1** |
|---|---|
| Size | 2 mm |
| Colour | Off white |
| Shape | Irregular |
| Margin | Irregular |
| Opacity | Opaque |
| Consistency | Soft |
| Elevation | Flat |
| Gram nature | Gram Positive |
| Morphology | Rod shaped |

**Biochemical Testing of bacteria isolated from mouth is shown in table no. 8:**

| **Characteristics** | **Result** | **Characteristics** | **Result** |
|---|---|---|---|
| | **Morphological Characteristics** | | |
| Shape | Long rod-shaped | Gram Staining | Gram Positive |
| Size | 0.5 to 0.8 µm | Motility Test | Non - motile |
| Colour | White color | Spore | Non-sporulating |
| | | | |
| | **Decomposition/Enzymatic studies** | | |
| Oxidase Test | _ | Gelatine Hydrolysis Test | _ |

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| | | | |
|---|---|---|---|
| Catalase Test | Negative | $H_2S$ production test | _ |
| Nitrate Reduction Test | _ | Urease Test | Negative |
| | **IMViC Set** | | |
| Indole test | _ | Methyl- red test | _ |
| Voges-Proskauer Test | Negative | Citrate Utilization test | _ |
| | **Sugar Fermentation Test** | | |
| Glucose | Positive | Lactose | Positive |
| Maltose | Positive | Mannitol | Negative |
| Sucrose | Positive | Xylose | Negative |

**Biochemical Testing of bacteria isolated from the nose is shown in the table no.9:**

| **Characteristics** | **Result** | **Characteristics** | **Result** |
|---|---|---|---|
| | **Morphological Characteristics** | | |
| Shape | Cocci | Gram Staining | Gram Positive |
| Size | 0.5 – 1.0 µm | Motility test | Non motile |
| Colour | Yellow or white colonies | Spore | Non Sporulating |
| | | | |
| | **Decomposition/ Enzymatic studies** | | |
| Oxidase Test | Negative | | |
| Catalase test | Positive | $H_2S$ production | Negative |
| Nitrate reduction test | Positive | | |
| Urease test | Positive | Gelatine Hydrolysis test | Positive |
| | **IMViC set** | | |
| Indole test | Negative | Methyl - red test | Positive |
| Voges -Proskauer test | Positive | Citrate utilization test | Positive |
| | | | |
| | **Sugar Fermentation Test** | | |
| Glucose | Positive | Lactose | Positive |
| Maltose | Positive | Mannitol | Positive |
| Sucrose | Variable | Xylose | Positive |

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**Biochemical Testing of bacteria isolated from the eyes is shown in the table no. 10:**

| **Characteristics** | **Result** | **Characteristics** | **Result** |
|---|---|---|---|
| | **Morphological Characteristics** | | |
| Shape | Irregular | Gram Staining | Gram Positive |
| Size | 0.5 – 1.0 µm | Motility test | Non motile |
| Colour | White colonies | Spore | Non Sporulating |
| | | | |
| | **Decomposition/ Enzymatic studies** | | |
| Oxidase Test | Negative | | |
| Catalase test | Positive | $H_2S$ production | Positive |
| Nitrate reduction test | Positive | | |
| Urease test | Negative | Gelatine Hydrolysis test | Negative |
| | **IMViC set** | | |
| Indole test | Negative | Methyl - red test | Positive |
| Voges -Proskauer test | _ | Citrate utilization test | Negative |
| | | | |
| | **Sugar Fermentation Test** | | |
| Glucose | Positive | Lactose | Negative |
| Maltose | Positive | Mannitol | Negative |
| Sucrose | Negative | Xylose | Negative |

From Table no. 8 we can conclude that isolated microorganism is *Streptococcus Pyogens,* While table no.9 indicates that the microorganism isolated is *Staphylococcus aureus* and table no. 10 indicated that the microorganisms isolated is *Cornybacterium Diphtheriae.*

Similar studies were carried out by Wang and Rogers, in which normal in the oral cavity, various different species of the genus Streptococcus, Lactobacillus, Lactococcus, Enterococcus, Staphylococcus, Corynebacterium, as well as Bacteroids are prominent (10). Similar experiment were too carried out by Marita Chakhtoura, UsamahHadiwrere they isolated *Staphylococcus aureus* from nasal polyps of patients in an survey(5). Miroslav Pátek in an another named "Current Evidence for *Corynebacterium* on the Ocular Surface" showed the presence of Corynebacterium Diphteria in the eyes (11).

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