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# Isolation And Identification Of Microorganisms From Homing Pigeon Droppings (*Columba Livia Domestica*)

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**ABSTRACT**
The homing pigeon is a variety of domestic pigeon (*Columba livia domestica*), selectively bred for its ability to find its way home over extremely long distance. The homing pigeon droppings are vehicle of diseases both for humans and other animal species. The present study aims to isolate and identify microorganisms in pigeon droppings collected from the households of pigeon breeders in different places. The collected pigeon droppings were powdered. Bacteria were isolated from pigeon dropping powder by using nutrient agar. The isolated bacteria were identified on the basis of their colony characteristics, morphology, Gram's staining, microscopy and biochemical test. Microbial load of pigeon droppings was calculating by cfu/gm of samples. The maximum number of bacterial population was exhibited in dilution $10^{-4}$ which ranged from $76\times10^5$ cfu/ml different bacteria isolate of *Campylobacter sp., Salmonella sp*., and *Micrococcus sp.,* was observed. Sabouraud Dextrose Agar (SDA) is used for fungal isolation. The maximum number of fungal population was exhibited in dilution $10^{-3}$ which ranged from $155\times10^4$ cfu/ml different fungus colonies of *Fusarium sp., Candida sp.,* was observed. It was determined that homing pigeon droppings pose a potential human health risk. The accumulation of pigeon droppings constitutes a public health danger and causes diseases.

**Keywords:** Pigeon dropping powder, Microbial load, Biochemical tests, Serial dilution, Bacteria, Fungi.

## 1.Introduction

Humans have been growing pigeons for approximately 10,000 years in most region of the planet (Levi,1977; Bhowmik *et al*., 2014). Pigeons can be found in every city and village in the world (Marques *et al*., 2007; Pavez *et al*., 2016). Pigeons are one of the oldest poultry species that have been domesticated by humans (Johnston & Janiga, 1995). Domestic pigeons have a grey body with an iridescent feather around the neck, a black band on the tail, and salmon- colored feet. Breeders have generated colour variations such as white, black or a combination of different colours (Pavez *et al*., 2016). Pigeons coexist with humans as a source of food, as carriers of letters, as a hobby, and for scientific purposes (Seri *et al*., 2008; Pavez *et al*., 2016). Along with other minerals, pigeon droppings contain nitrogen, phosphorus, and potassium. These ingredients offer a good environment that encourages the growth of different microbes (Nyakundi & Mwangi, 2011). Pigeon droppings are caustic and have been demonstrated to be the most acidic of all bird droppings (Vasiliu & Bruiana, 2010). Pigeon droppings promote the growth of the fungus and germs (Haag-Wackernagel & Moch, 2004). Pigeon droppings can spread sickness to people and other animals (Catroxo *et al*., 2011). Some microbes obtained from pigeon droppings have been shown to be harmful in immunodeficient individuals, although immunocompetent individuals have also been infected with these germs (Hamasha *et al*., 2004; Kwang & Soo, 2005; Millar *et al*., 2007). Epidemiological investigations on pigeon populations found at least 100 harmful species to humans, including eight of them being viruses, 41bacteria, 55 fungi and 6 protozoa (Haag-Wackernagel & Moch, 2004). Bird droppings do pose a public health risk and causes illness. Humans even become infected by inhaling dust containing dried droppings (Chang *et al*., 2004). The intimate contact that pigeons have with both humans and animals may lead to the spread of infectious diseases. Pigeons are frequently infected with *Streptococci*, *Salmonella*, and *E. Coli*, among other bacterial agents (Herdt *et al*.,1994). Pigeons are the source of various diseases that can be transmitted to humans, primarily by contact with dried droppings, feather dust and mites (Kozdrun *et al*., 2015; Coudert *et al*., 2015).

In recent years, opportunistic fungal infections have expanded significantly, with species from the genera *Aspergillus, Penicillium, Mucor, Rhizopus, Paecilomyces, Fusarium, Alternaria,* and *Cladosporium* emerging as the cause of a wide range of human illnesses (Shah *et al*.,2001; Dannaoui *et al*.,2007; Nucci *et al*.,2007; Courville *et al*.,2002; Aguilar *et al*., 1998). Pigeon droppings, found in public areas, may be a significant carrier for the spread of pathogenic yeasts into the environment and potentially humans (Chee & Lee, 2005). This study aims to isolate and identify microorganisms from pigeon droppings.

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## 2. Materials and Methods

### 2.1 Sample Collection:

A total of 60 pigeon droppings were gathered from residences of pigeon breeders in various locations in Tirunelveli District. The research was conducted between the year 2022 and 2023. Pigeon droppings were collected in clean sterile plastic bags (plastic packets containing disposable hand gloves), which were appropriately sealed and labelled with the date and location of collection. The average sample weight ranged from 40 to 100 g. The pigeon droppings were finely ground using a grinding machine and then they were collected in sterilized and sealed containers and directly transported to the microbiology laboratory for analysis.

### 2.2 Preparation of Pigeon dropping powder suspension:

1 g of the dry dropping powder was thoroughly vortexed in 9.0 ml of normal saline. Aliquot (1.0 ml) was transferred into the subsequent screw capped tubes and diluted serially in one-tenth to$10^{-9}$ dilutions. 0.1 ml aliquot from each dilution was transferred aseptically onto freshly prepared nutrient agar plates and spread with a sterile spreader. The inoculated plates were incubated at 37°C in bacteriological incubator for 24 h. Colonies developed on the plate were counted and expressed as cfu/ml. Pure cultures of bacteria were obtained by streak plate method. Based on the macro-morphological colony characteristics, discrete bacteria colonies that developed were sub cultured on nutrient agar and incubated at 37°C for 24 h.

### 2.3 Isolation and Identification of Microorganisms:

The bacterial isolates were identified using morphological characterization, Gram staining, physiological and biochemical characteristics presented in Bergey's Manual of Determinative Bacteriology and the APi Kit profiles (Holt *et al*., 1994). The identification of fungi was based on classic taxonomy (macro and microscopic characteristics). The surface and the reverse of the colonies were observed, as well as diameter, conidial colour, texture, and presence of soluble pigments (Tell, 2005; Dugan, 2006; Balajee *et al*., 2007). For isolation and identification, of the bacterial and fungal culture on the surface of the agar plate are incubated and inoculated in petri plates and kept at $37^0$C for bacteria and $28^0$C for fungi.

### Morphological and Microscopic Characterization of Microorganisms:

Microorganisms were identified by morphological characters and the microscopic examination. Bacterial morphology characteristics such as colony diameter, growth, colour, form, were observed on culture medium. Morphological features such as shape and arrangement were observed after Gram's staining according to standard protocol. The fungal morphology was studied macroscopically by observing the colony features (colour, shape, size and hyphae) and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue stained slide mounted with a small portion of the mycelium (Gaddeyya *et al*., 2012).

### 2.4 Biochemical Test:

For the identification of bacterial isolates routine standard biochemical tests such as Indole test, Triple sugar test, Methyl red test, Citrate utilization test, Urease test, Voges-Proskauer test, Nitrite reduction test and Mannitol motility were performed. Isolates were inoculated in these medium and incubated at $37^0$C for 24 hours. Next day various biochemical reactions such as indole production, starch hydrolysis, gelatin hydrolysis, urease hydrolysis, citrate utilization, fermentation of sugars and motility were observed.

#### 2.4.1 Indole test:

Indole production of isolates was tested with a medium composed of tryptone, 10g., CaCl2, 0.03M; NaCl, 5g in 1 litre of distilled water and autoclaved at 121° C for 15 minutes. Each isolate was inoculated into the tryptone broth and incubated at 28°C. After 2 and 5 days of incubation, 0.5 ml of Kovac's Reagent was added into each tube and shook gently. A dark red colour in the surface layer was taken as positive for indole production.

#### 2.4.2 Methyl red test:

The Methyl red test was used to check the ability of the organisms to perform mixed acid fermentation. Loop full of test cultures A1 and A2 were inoculated aseptically into test tubes containing MRVP medium. The uninoculated broth served as control. All test tubes containing MRVP medium were incubated for 24-48 hours at 37°C and after incubation, five drops of methyl red indicator were added to the incubated test tubes and examined for the colour change.

**2.4.3 Voges-Proskauer test:** The VP test is used to detect the formation of acetyl methyl carbinol (acetoin). Acetoin is formed as a product of an alternative pathway of glucose fermentation. It is detected by the addition of 5% alpha naphthol followed by 40% KOH to the MRVP medium previously inoculated and incubated with the test organisms. The tubes were shaken gently and allowed to remain undisturbed for 10-15 minutes and then the results were noted.

#### 2.4.4 Nitrite reduction test:

The ability of the isolates to reduce nitrate to nitrite was evaluated in a test medium that contains KNO3, 1g peptone, 5g yeast extract, 3g agar and 3g in 1 litre distilled water and sterilized at 121°C for 15 minutes in tubes. Each isolate was inoculated by stabbing and sealed with 3ml sterilized molten agar to avoid false positives and incubated at 28°C. Observation was made after 3,5 and 7 days of inoculation. Bubble formation beneath the upper agar layer was taken as positive result for nitrate reduction.

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**2.4.5 Citrate utilization test:**
Simmons citrate medium was prepared dispensed in test tubes, sterilized at 121°C for 15 minutes and allowed to set as slopes. The agar slopes were inoculated with a saline suspension of the organisms to be tested. The tubes were incubated at 37°C for 96 hours and the results were noted.

**2.4.6 Urease test:**
Urease production was tested with the medium composed of monopotassium phosphate 9gm, dipotassium phosphate 9g, yeast extract 0.1g, phenol red 0.01g pH (at 25°C), 6.8±0.2 in one 950 ml of distilled water. The medium was heated to dissolve completely and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. The media was cooled to 55°C and aseptically 50 ml of sterile 40% urea solution was added, mixed well and distributed in 10 ml amounts into sterile tubes. The test cultures were inoculated into urea broth using sterile techniques. A control was maintained without inoculating culture into urea broth. The test tubes were kept for incubation at 37°C for 24-48 hours and after incubation results were recorded.

**2.4.7 Triple sugar test:**
Using sterile technique, the test organism was inoculated by means of a stab and streak method in triple sugar iron agar slants. The sterile straight needle was first inserted deep into the butt and withdrawn. The needle was then used to inoculate on the slant of the medium. Uninoculated slant was used as control. The inoculated TSI slants were then incubated for 18-24 hours at 37°C and the results were recorded.

**2.4.8 Carbohydrate Fermentation Test:**
Prepare media containing Peptone-1 gm, Meat extract -0.3 gm, NaCl -0.5, Distilled water – 100ml, Indicator (such as Andrade's solution, Bromocresol purple (BCP), Bromothymol blue (BTB) or Phenol red) -0.008 gm, Sugar(glucose/lactose/sucrose) -0.5 gm then Take three different tubes containing three different types of sugar broth and invert a Durham's tube in it and screw cap the tubes. Further, the three sugar broth tubes are sterilized by autoclaving. After sterilization, the tubes are cooled down to room temperature and inoculated with a cell suspension in aseptic condition. The tubes are incubated at 37°C for 24 hours. After incubation, the tubes are examined for acid and gas production and results are noted down.

## 3. Results and Discussion:
Pigeon keeping / breeding, conducted for thousands of years in practically every thousands of years in practically every corner of the world, has grown into a personal or commercial industry for the purposes of industry for the purposes of aesthetic satisfaction, recreation, entertainment and food. Pigeon droppings, combined with the growing pigeon population, are becoming a major environmental concern. Furthermore, pigeon droppings are a major public health problem because they are repositories and carriers of opportunistic and pathogenic microbes such as fungus, bacteria, and viruses (Abulreesh *et al.*, 2019; Santos *et al.*, 2020).

### 3.1 Pigeon dropping suspension and its characterization:
The suspension of pigeon droppings carrying large numbers of bacteria and fungi. Serial dilution technique was used for microbial load analysis. That involves spreading a suspension over the surface of Nutrient agar for the isolation of bacteria and Sabouraud Dextrose Agar media (SDA) for fungus isolation from pigeon droppings. Further characterization of isolated bacterial and fungal colonies was performed according to standard protocols.

### 3.2 Isolation of bacteria by serial dilution of pigeon droppings:
The collected samples of pigeon droppings were enumerated for their bacteria in microbial load of pigeon dropping were calculating cfu/gm of samples. Serially diluted pigeon droppings suspension was plated of nutrient agar plates. The maximum number (TNTC) of bacterial population was exhibited in initial dilution, then $10^{-4}$ to $10^{-7}$ showed which ranged from $76\times10^4$ to $3\times10^8$cfu/ml and minimum concentration (TNFC) was exhibited in dilution$10^{-9}$. Table (1) and Figure (1) shows the microbial count of pigeon dropping in initial dilution large numbers of microbes are present as further dilution shows isolated pure colonies

| S. No | Dilution | Methods used | CFU Value(cfu/ml) |
|---|---|---|---|
| 1 | $10^{-1}$ | Serial Dilution method | TNTC |
| 2 | $10^{-2}$ | | TNTC |
| 3 | $10^{-3}$ | | TNTC |
| 4 | $10^{-4}$ | | $76\times10^5$ |
| 5 | $10^{-5}$ | | $10\times10^6$ |
| 6 | $10^{-6}$ | | $5\times10^7$ |
| 7 | $10^{-7}$ | | $3\times10^8$ |
| 8 | $10^{-8}$ | | TFTC |
| 9 | $10^{-9}$ | | TFTC |

(**Table 1**) Microbial count of the Bacterial isolates

**TNTC**–Too Numerous to Count **TFTC** – Too Few to Count

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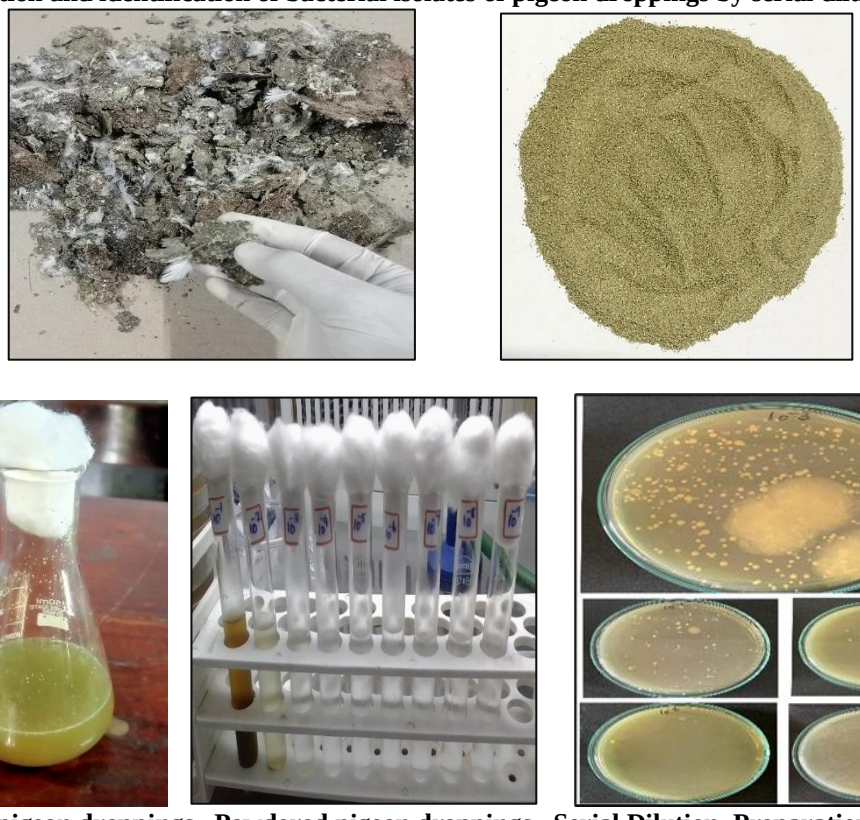
**Figure 1 Isolation and identification of bacterial isolates of pigeon droppings by serial dilution methods.**



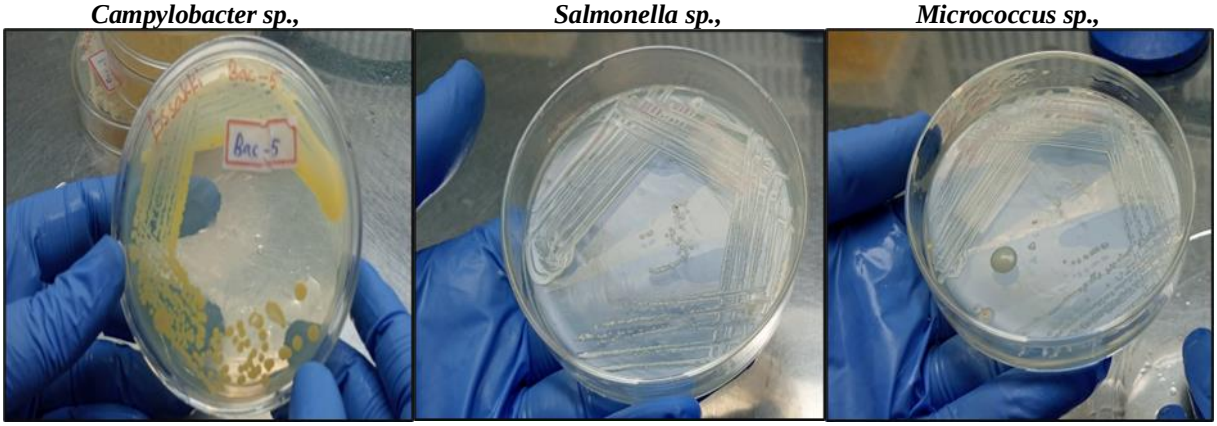
**Collection of dry pigeon droppings Powdered pigeon droppings Serial Dilution Preparation of culture plates**

**3.3 Morphological and microscopical characterization of bacterial isolates:**

Microorganisms create colonies that exhibit traits known as cultural features that are visible to the human eye. The culture characteristics were observed on nutrient agar after incubation. These morphological traits were noted in several forms, including colony form, colony elevation, colony surface, and colony colour (Figure 2). The morphological and microscopical characteristics of these isolated bacteria were shown in Table (2) Figure (2). Bacteria were classified as Gram-positive and Gram-negative based on Gram's staining, as demonstrated in Figure (2). *Campylobacter sp., and Micrococcus sp.,* is Gram positive and *Salmonella sp.,* is Gram negative respectively. As a result, three bacteria, *Campylobacter sp., Salmonella sp., and Micrococcus sp.,* were isolated from pigeon droppings to evaluate their effects separately and in consortium. Various authors reported different bacteria species from pigeon droppings. Similarly, (Tanaka *et al*., 2005) reported the presence of pathogens such as *Escherichia coli, Campylobacter, Salmonella, Listeria, Chlamydia,* isolated from feral pigeon droppings in Japan.

**Figure 2 Morphological observation of bacterial colonies of pigeon droppings**

***Campylobacter sp.,*** ***Salmonella sp.,*** ***Micrococcus sp.,***



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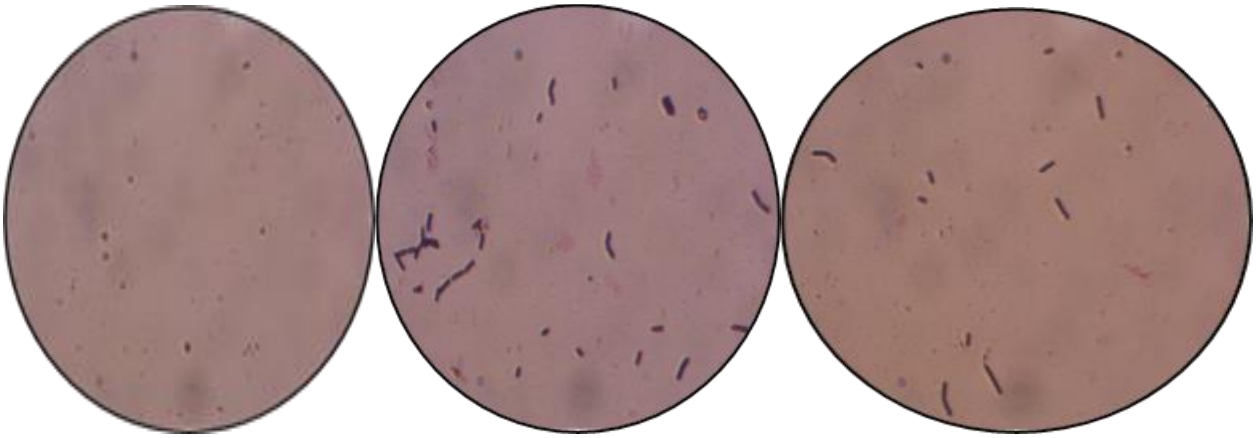

| Characteristics | Bacterial Isolates | | |
|---|---|---|---|
| | *Campylobacter sp.,* | *Salmonella sp.,* | *Micrococcus sp.,* |
| From of colony | Circular | Circular | Circular |
| Opacity | Opaque | Opaque | Opaque |
| Elevation of colony | Convex | Convex | Convex |
| Surface of colony | Smooth | Smooth | Smooth |
| Pigmentation | Creamy white | Creamy white | Dark yellow |
| Margin | Entire | Entire | Entire |

**Table 2 Morphological characteristics of bacteria isolates of pigeon droppings**

**3.4 Biochemical test:**

Biochemical test such Indole test, Methyl red test, Voges- Proskauer test, Nitrite reduction test, Citrate utilization test, Urease test, Triple sugar test, Carbohydrate fermentation test was performed for identification of bacterial isolates as shown in a Figure (3) Identification of the bacterial isolates was made based on gram staining ,colony morphology cultural, physiological, biochemical characteristics of bacterial isolates by using Bergey's Manual of Determinative Bacteriology and the Api Kit profiles (Holt *et al* .,1994). All the bacterial isolates positive results for methyl red test, nitrate test, catalase test, gelatin hydrolysis test and negative results for indole test, NP test, citrate test, TSI test, $H_2S$ test results shown in Figure (3) and Table (3).

**Figure 3** Occurrence of bacterial isolates of pigeon droppings powder

**Table 3**: Biochemical test of bacterial isolates of pigeon droppings powder.

| Biochemical test | Bacterial isolates | | |
|---|---|---|---|
| | *Campylobacter spp.,* | *Salmonella spp.,* | *Micrococcus spp.,* |
| Gram staining | + | - | + |
| MOT | + | + | - |
| IND | - | - | - |
| MR | + | + | + |

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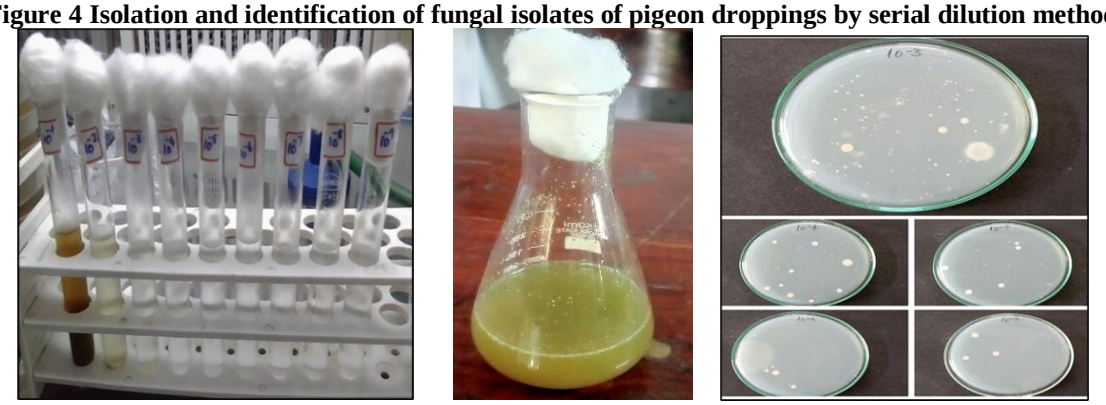
| | | | |
|---|---|---|---|
| VP | - | - | - |
| NIT | + | + | + |
| CIT | - | - | - |
| Urease | + | - | - |
| Catalase | + | + | + |
| Oxidase | + | - | + |
| TSI | - | - | - |
| $H_2S$ | - | - | - |
| GAS | - | - | - |
| STA | - | - | - |
| GEL | + | + | + |
| Glucose | - | + | - |
| Sucrose | - | + | - |
| Maltose | + | + | + |

Key: **+**, Positive, -, Negative, **MOT**; Motility test, **IND**; Indole test, **MR**; Methyl red test, **VP**; Voges Proskauer test, **NIT**; Nitrate reduction test, **CIT**; Citrate utilization test, **TSI**; Triple sugar test, **H2S**; Hydrogen sulfide test, **STA**; Starch hydrolysis test, **GEL**; Gelatin liquefaction test, **STA**; Starch hydrolysis test; **GEL**; Gelatin liquefaction test.

**3.5 Isolation of fungi by serial dilution of pigeon droppings:**

The collected samples of pigeon droppings were enumerated for their microbial load of total fungi. The serial dilution plating method was used to make suspension of pigeon droppings in distilled ware purpose to minimizing the fungi in the droppings in each dilution. The sample of pigeon droppings was diluted 9 times and labelled as $10^{-1}$ to $10^{-9}$ dilution. Serially diluted pigeon dropping suspension was plated of Sabouraud Dextrose Agar media (SDA). Microbial load of pigeon droppings is expressed in cfu/gm of sample using colony counter. The maximum number (TNTC) of fungal population was exhibited in initial dilution, $10^{-3}$ to $10^{-7}$ of range from $155\times10^4$ to $9\times10^8$ cfu/ml and minimum concentration (TNFC) was exhibited in dilution $10^{-9}$. The TNTC and TFTC microbial counts of fungal isolates are shown in (Table 4).

**Figure 4 Isolation and identification of fungal isolates of pigeon droppings by serial dilution method**



**Preparation of culture plates** **Serial Dilution** **Plates**

(**Table 4**) Microbial count of the Fungal isolates

| S. No | Dilution | Methods used | CFU Value(cfu/ml) |
|---|---|---|---|
| 1 | $10^{-1}$ | Serial Dilution method | TNTC |
| 2 | $10^{-2}$ | | TNTC |
| 3 | $10^{-3}$ | | $155\times10^4$ |
| 4 | $10^{-4}$ | | $18\times10^5$ |
| 5 | $10^{-5}$ | | $16\times10^6$ |
| 6 | $10^{-6}$ | | $11\times10^7$ |
| 7 | $10^{-7}$ | | $9\times10^8$ |
| 8 | $10^{-8}$ | | TFTC |
| 9 | $10^{-9}$ | | TFTC |

TNTC– Too Numerous To Count TFTC– Too Few To Count

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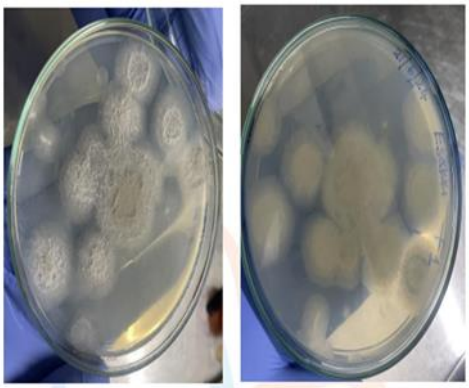
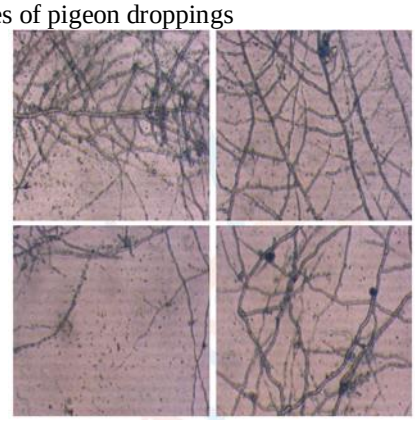
**3.6 Morphological and microscopical characterization of fungi isolates of pigeon droppings**

The isolation of fungi from pigeon droppings was carried out in order to identify each fungal species. The cultural traits of fungal colonies were observed on Sabouraud Dextrose Agar media (SDA) agar medium plates after incubation. The morphological examinations of the isolates were determined by using standard procedure. The fungal morphology was studied macroscopically by observing the colony features (color, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue stained slide mounted with a small portion of the mycelium (Gaddeyya *et al*., 2012). These morphological traits, which included colony form, colony elevation, colony surface, and colony colour, were noted in various forms. It was noted that distinct pure colonies of the same fungus were isolated. (Shown in figure 5). This isolated fungus was characterized on the basis of morphological cultural characteristics such as form of colony, colour of colony, texture of colony, aerial hyphae, pigmentation, margin, and covers the entire plate, and microscopically like conidia pattern, hyphae, conidia are present in Figure 5 and Table 5. *Fusarium sp., and Candida sp.,* were isolated from pigeon droppings. Various authors reported different fungi from pigeon droppings. For example, Soltani *et al.*, 2013 reported the presence of *Candida sp., Cryptococcus sp., Aspergillus sp., Rhizopus sp., Mucor sp., and Penicillium sp.,* from pigeon droppings collected from urban areas of Isfahan. In other hand, Maryam *et al.,* 2013, who proved that pigeon droppings are associated with different fungi species, including *Fusarium sp., Rhizopus sp., Mucor sp., Paecilomyces sp., Aspergillus sp.,* isolated from pigeon droppings.

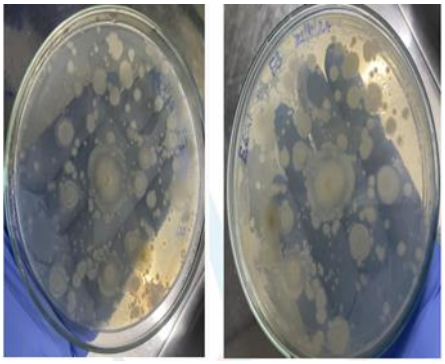
**Figure 5** Morphological and microscopic observation of fungal isolates of pigeon droppings



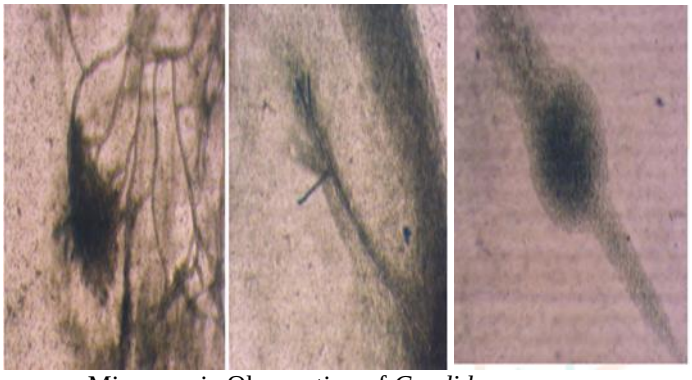
***Fusarium sp.,***



Microscopic Observation of *Fusarium sp.,*



***Candida sp.,***



Microscopic Observation of *Candida sp.,*

**Table 5 Morphological and microscopical characterization of fungi isolates of pigeon droppings**

| **Colony Morphology** | **Microscopic Morphology** | **Probable Identify** |
|---|---|---|
| Colonies were circular white cream colour, round surface with raised margin, serrated edge and white to pigment. | Macroconidia, branched separate hyphae frequently in cluster conidiaphores are long and curl at the tip. | *Fusarium sp.,* |
| White cream colour smooth, irregular spreading, white yellowish colour pigment. | Conidia in a branching pattern, dark round sclerotia, thick wall separate hyphae. | *Candida sp.,* |

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## 4. Conclusion

This study has been able to show that the isolation and identification of microorganisms from homing pigeon droppings. Isolated microorganisms were identified on the basis of their morphology, colony characteristics, Gram's staining and microscopically. The maximum number of bacterial population was exhibited in $10^{-4}$ which ranged from $76\times10^5$ cfu/ml. Among them free bacterial isolates were identified as *Campylobacter sp.*, *Salmonella sp.*, and *Micrococcus sp.*, respectively on the basis of morphology and microscopically. The maximum number of fungal population was exhibited in dilution $10^{-3}$ which ranged from $155\times10^4$ cfu/ml. Different fungal colonies of *Fusarium sp.*, were *Candida sp.*, observed on the basis of morphology and microscopically. The present results provide evidence that pigeon droppings can serve as an environmental reservoir of multiple bacteria and fungi.

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**Reference**

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