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# Study On Microflora In Freshwater Grass Carp (Ctenopharyngodon Idella)

**K.Sundaravalli[1] ,K.Revathi[2*], R.Devi[2], A.Anitha[2]**
[1] Meenakshi Academy of Higher Education and Research, KK Nagar, Chennai 600078, India
*Corresponding author Email: reva63@rediffmail.com

**Abstract**
Increasing threat of pollution in recent times may have a direct or indirect effect on the microbial flora of fish which in turn may cause a threat to human health due to frequent consumption of the fish. Grass carp (*Ctenopharyngodonidella*) were collected and analyzed for the presence of different microbial organisms from their gut content. Microbial counts were analyzed in the fish skin, gills and gut. The intestinal gut showed the highest biome of bacteria. *Pseudomonas spp., Aeromonas spp., Salmonella spp.,* and *Clostridium botulinum* were identified in 55%, 25%, 12% and 8% respectively from the gut biome. We found the occurrence of human pathogenic *Pseudomonas spp*in the gut of grass carp were confirmed to be in higher count, which may affect the human health on consumption. The result analyzed were found to be statistically significant $P<0.05$.

## 1.Introduction

Human beings consume fish which acts as an extensive source of protein. Yet, it may cause serious effect in human on consumption of contaminated or spoilt fish due to existence of pathogenic and opportunistic microorganisms (1). There is an utmost health risk for consumers since food borne pathogens may result in serious disease outbreaks. Appearance of such causative food borne pathogens may be due to external sources like environmental pollution, water quality and damage caused by human, animal or plants (2). Determining the micro flora in the different parts of fish organ is vital to understand the contamination and quality of fish. Contamination is also associated with rigorous microbial growth which may detoriate the quality of fish which has natural habitat may harbor abundance of microorganisms especially bacteria (3). Settlement of bacteria in the gill and skin is due to continual subjection to infected water whereas the alimentary canal infection may happen due to contaminated feed and water. Infection in the muscle of fish may also occur if the immunological resistance is low in the fish (4). Antibiotic resistant bacteria associated with food chains have been found to cause untreatable, prolonged and complicated infections and sometimes death. Low economic, retarded sanitation, lack of hygiene and use of broad spectrum of antibiotic has been a bane with potential adverse effect in the population of fish found in the fresh waters (5).

Overcrowding of fish population and opportunistic contamination with pathogenic bacteria result with close interaction between pathogenic bacterial contaminations. It is therefore mandatory to analyze the ubiquity of pathogens present in fish which remains to be the major food source for a wide population (6). Study on the bacterial pathogens present in skin and gills are prevalent whereas infection in gut becomes a necessity to analyze and study since it may contaminate the muscles of the fish(4). Our study focuses on the analyzing the existence of varied pathogens in the gut of routinely consumed fish named Grass carp

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*Ctenopharyngodonidella*.The Glass carp is highly cultivated freshwater fish species in the world (7). There are more than 34 different types of bacteria including 125 different bacterial species found to associate with the fish disease in the world (8). This study was conducted to enlighten the bacterial infection in the gut region of the glass carp.

## 2.Materials and Methods

2.1 Study sample collection

The Green carp fishes were collected from Poondi reservoir and were collected in a sterile polythene bag. The collected fish were of various body weight (50gms to 250gms) and length (10cm to 30 cm). The collected fishes were transferred to Central Laboratory of MAHER for further microbiological analysis.

**Grass carp collected from Poondi Lake**

2.2 Clinical Examination

The collected fishes were examined clinically to detect any abnormality in external changes of the fish. The fish was dissected aseptically using sterile scalpel and forceps. Different parts of fish were dissected and cut into pieces to obtain 10 grams and were homogenized. Homogenized samples were examined for the presence of Microbial contents.

2.3 Postmortem examination

**Gill and Skin:** For examination in Gill and skin, the fish was washed using double distilled water to remove the unwanted debris. A sterile Cotton swab was rubbed against the samples and inoculated in nutrient broth and MacConkey agar broth.

**Gut and muscles:** The dissected and cut samples were performedwith the help ofscalpel and further inoculated aseptically in the nutrient and MacConkey broth.

**2.4 Isolation and Identification of bacteria:**

Isolation and identification procedure was followed according to *Cheesbourgh 1984* .MPN technique was followed to enumerate the microbial counts.MPN technique was preferred since it is effective for even low population levels than Plate count method. This quantitative analysis was performed in order to project the count of bacteria. $10^{-7}$ dilutions were taken. The quantity of 10 microlitre of sample was poured into the Nutrient agar, MacConkey agar, Salmonella /Shigella agar. After pouring the samples spread plate method is followed. All the plates are marked and placed at 37°C for 24-48 hrs incubation. After incubation bacterial isolates were identified by their morphology and cultural characters.

2.5 S**tatistical analysis**

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Statistical analysis was performed using independent T test. The result were found to be statistically significant $P<0.05$

**3.Results**

Bacterial infections and diseases are serious problem in aquaculture. Bacterial pathogens are common in fish associating the natural surroundings, during certain environmental factors fish mortalities are caused. *Pseudomonas* infection is implicated highly among the freshwater fish diseases.*Pseudomonas* sp., *Aeromonas* sp., *Salmonella* sp., and *Clostridium* sp., were isolated from the organs of *C.idella* collected from Poondi Lake. All these four organisms are said to affect in humans on consumption of bacterial pathogen infected fish due to ignorance or lack of awareness.The bacterial count values were obtained for three different dilution factors such as $10^{-3}$, $10^{-5}$, and $10^{-7}$. Table 1,Table 2 and Table 3 shows the counts of *Pseudomonas* sp., *Aeromonas* sp., *Salmonella* sp., and *Clostridium* sp., in $10^{-3}$,$10^{-5}$,$10^{-7}$ dilution factor respectively in three different organs namely Skin, Gill and Gut . Among these $10^{-7}$ dilution factor showed values feasible for counting which was further taken for replicate values. Three replicate counting were done for the dilution factor of $10^{-7}$ and named as R1, R2, R3 and the mean value was obtained. The *Pseudomonas* sp., overall showed higher counts than the other organisms. The *salmonella* sp., and *clostridium* sp., was comparatively high in the Skin of fish yet not more than the value of *Pseudomonas* sp., *Aeromonas* sp., expressed elevated counts in Gill than that of Skin and Gut however not more than the *Pseudomonas* sp., On the whole *Pseudomonas* sp., were found to be abundant in all the organs of the fish. The resultswere analyzed with independent T test and the results were found to be statistically significant ($P>0.05$)

Clostridium species

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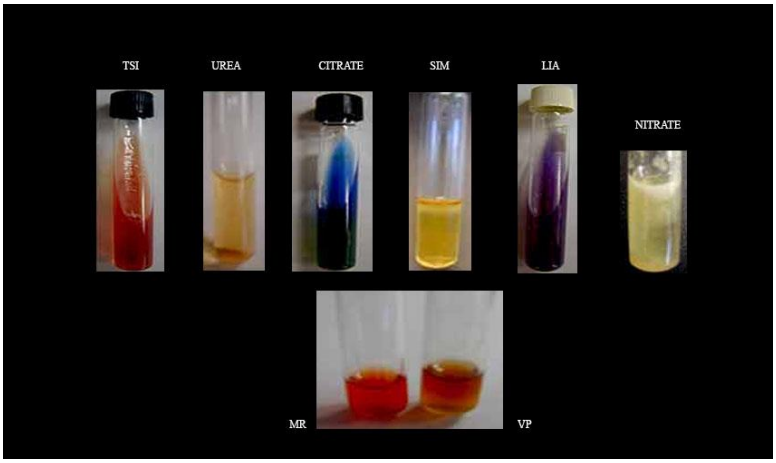
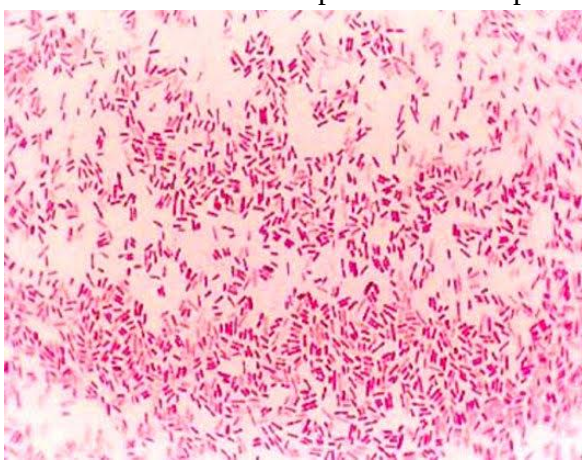
Salmonella in Mac Conkey agar




Biochemical tests of pseudomonas sp.



Gram stained Pseudomonas sp.

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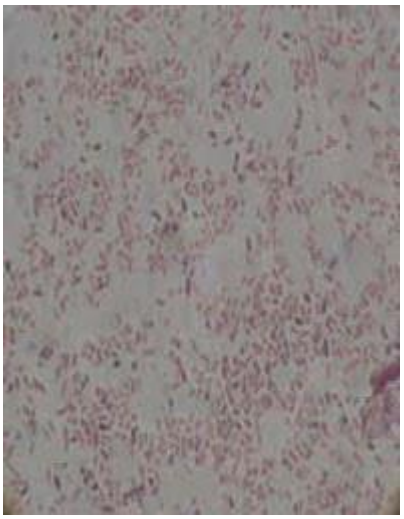
Pseudomonas in Cetrimide agar

Clostridium sp., in Blood agar



Gram stained Aeromonas sp.,

**Table: 1 Bacterial count of Pseudomonas sp., Aeromonas sp., Salmonella sp., and Clostridium sp., in Dilution factor $10^{-3}$**

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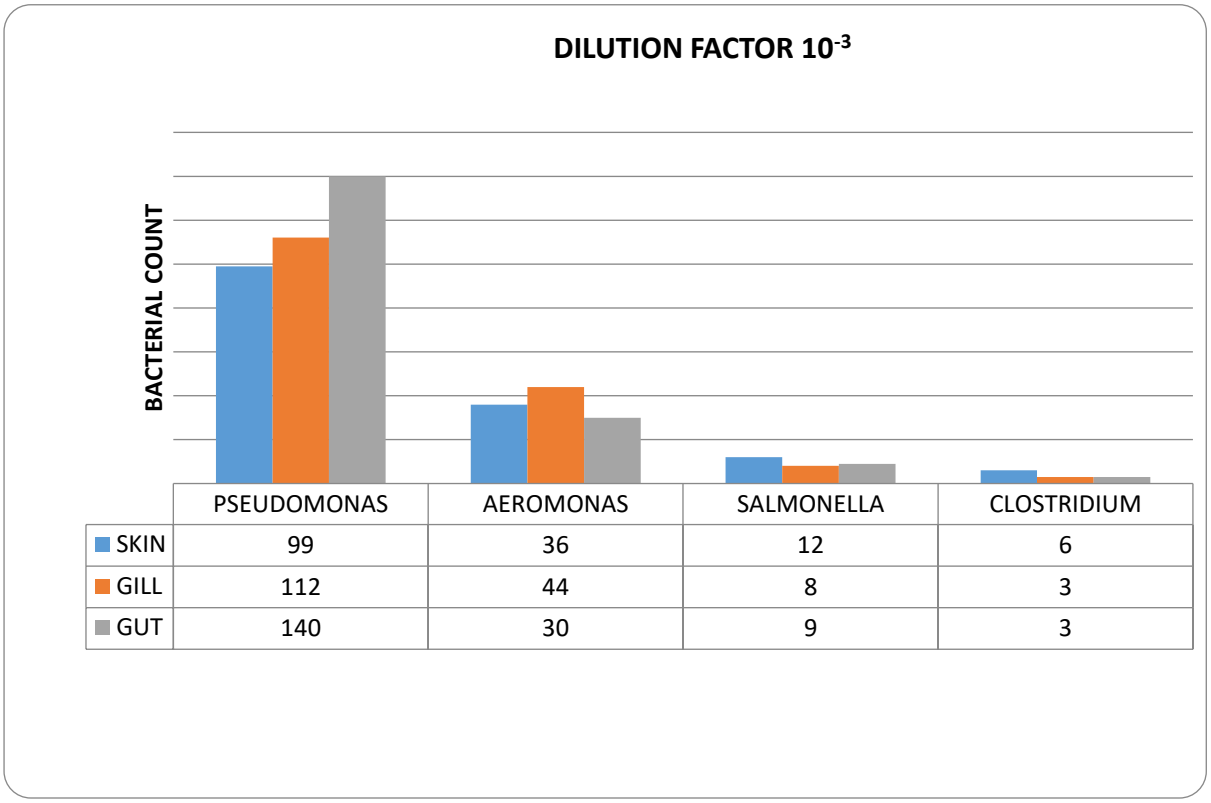


| | PSEUDOMONAS | AEROMONAS | SALMONELLA | CLOSTRIDIUM |
|---|---|---|---|---|
| SKIN | 99 | 36 | 12 | 6 |
| GILL | 112 | 44 | 8 | 3 |
| GUT | 140 | 30 | 9 | 3 |

**Table : 2 Bacterial counts of Pseudomonas sp., Aeromonas sp., Salmonella sp., and Clostridium sp., in Dilution factor $10^{-5}$**

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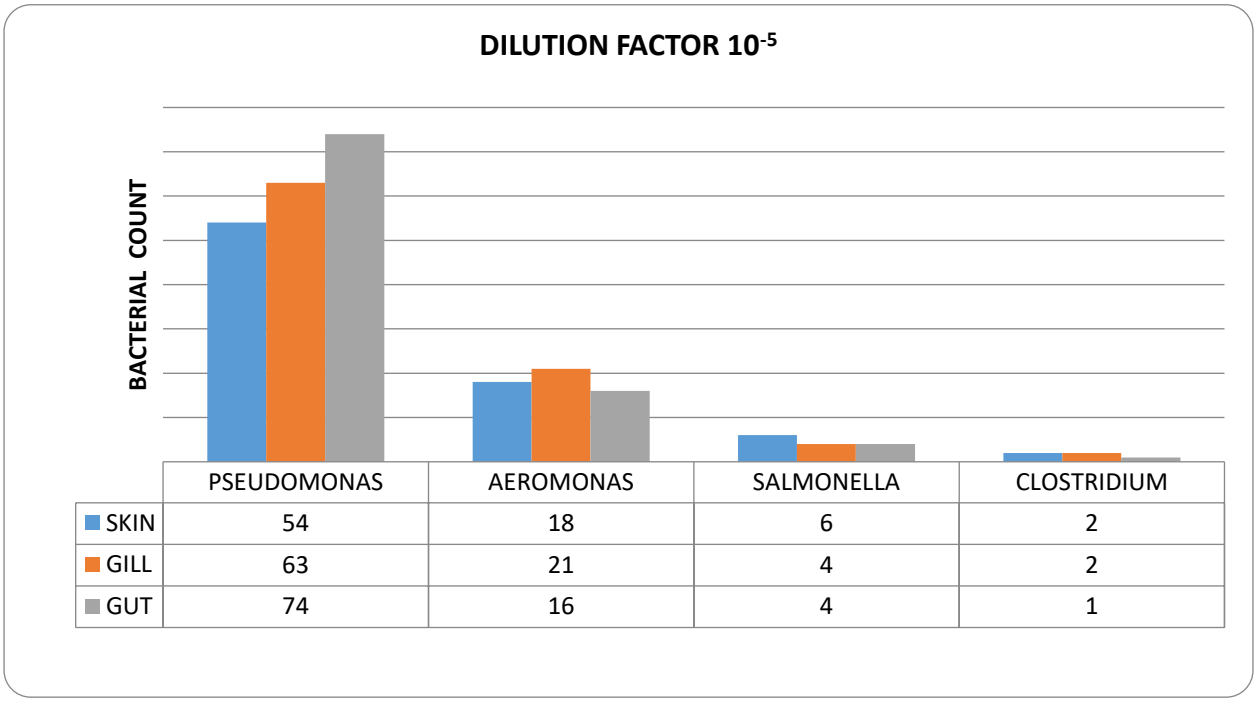
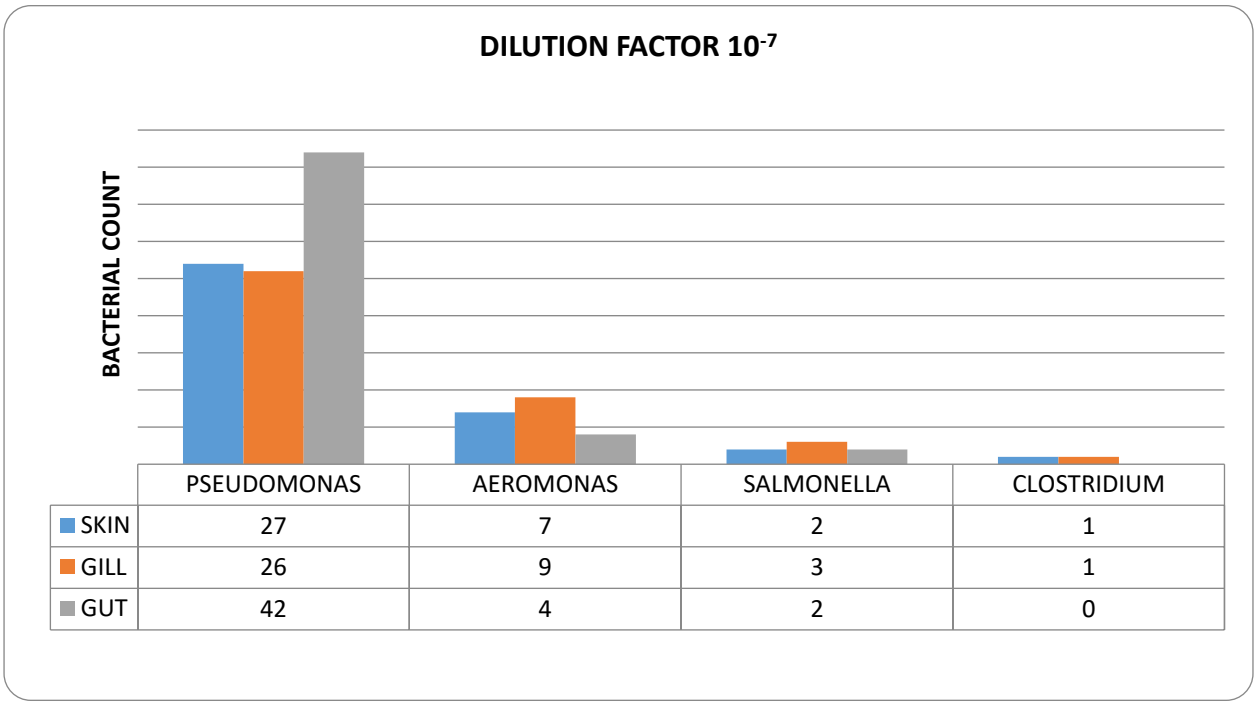



**Table : 3 Bacterial counts of Pseudomonas sp., Aeromonas sp., Salmonella sp., and Clostridium sp., in Dilution factor $10^{-7}$**




| | Pseudomonas sp., | | | |
|---|---|---|---|---|
| Parts of fish | R1 | R2 | R3 | Average |
| Skin | 20 | 23 | 27 | 23.33333 |

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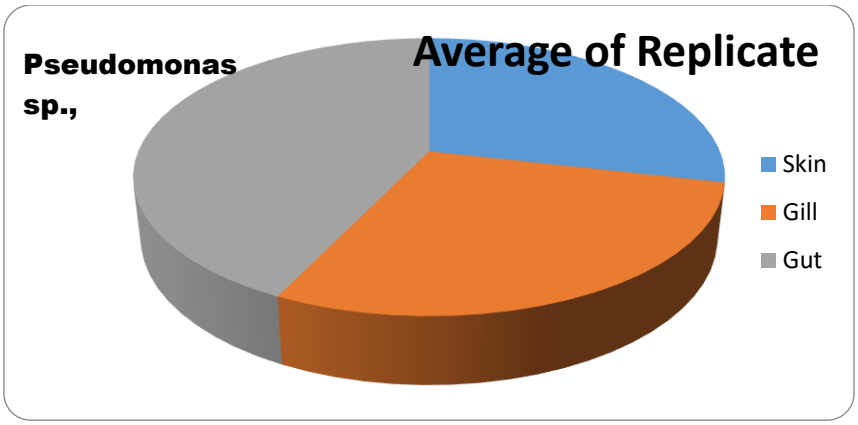
| | | | | |
|---|---|---|---|---|
| Gill | 29 | 24 | 26 | 26.33333 |
| Gut | 38 | 27 | 42 | 35.66667 |

**Table 4 : Replicates of dilution factor $10^{-7}$ in pseudomonas sp.,**




| | Aeromonas sp., | | | |
|---|---|---|---|---|
| Parts of fish | R1 | R2 | R3 | Average |
| Skin | 7 | 6 | 8 | 7 |
| Gill | 8 | 7 | 6 | 7 |
| Gut | 3 | 3 | 2 | 2.666667 |

**Table 5 : Replicates of dilution factor $10^{-7}$ in Aeromonas sp.,**

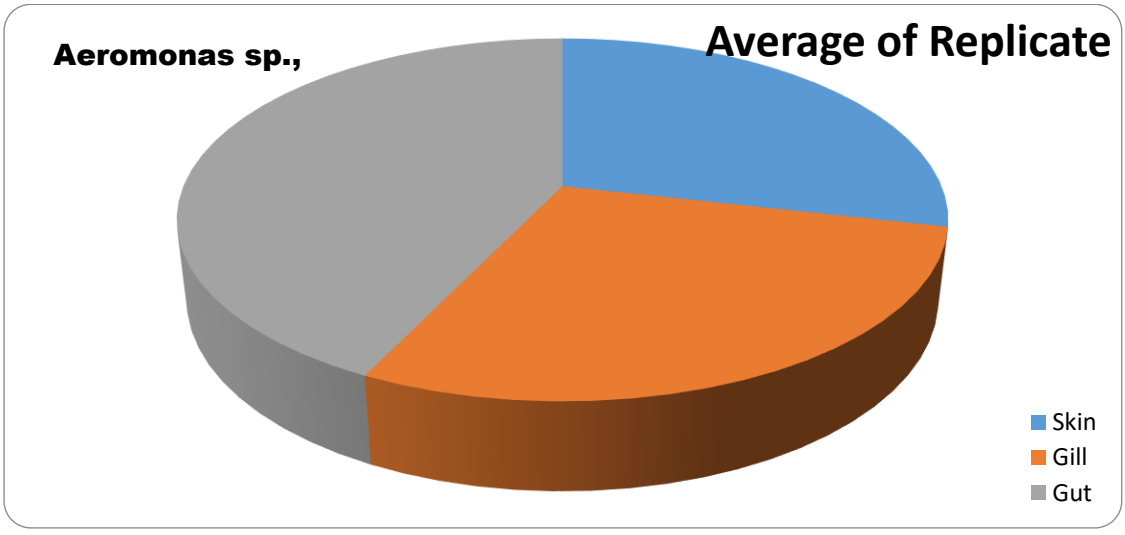



**Table 6 : Replicates of dilution factor $10^{-7}$ in Salmonella sp.,**

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| Parts of fish | R1 | R2 | R3 | Average |
|---|---|---|---|---|
| Skin | 2 | 3 | 3 | 2.666667 |
| Gill | 3 | 2 | 2 | 2.333333 |
| Gut | 2 | 1 | 0 | 1 |

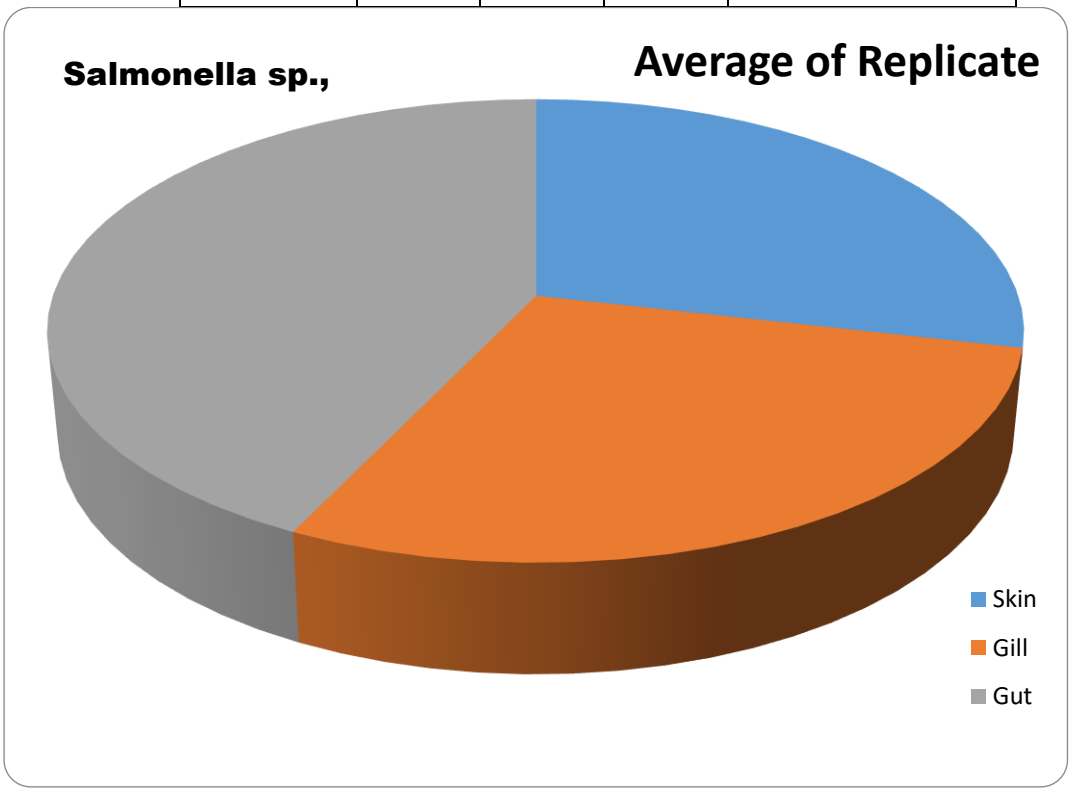


**Table 7 : Replicates of dilution factor $10^{-7}$ in Clostridium sp.,**

| | Clostridium sp., | | | |
|---|---|---|---|---|
| Parts of fish | R1 | R2 | R3 | Average |
| Skin | 1 | 1 | 0 | 0.666667 |
| | | | | |
| Gill | 0 | 1 | 1 | 0.666667 |
| Gut | 1 | 1 | 1 | 1 |

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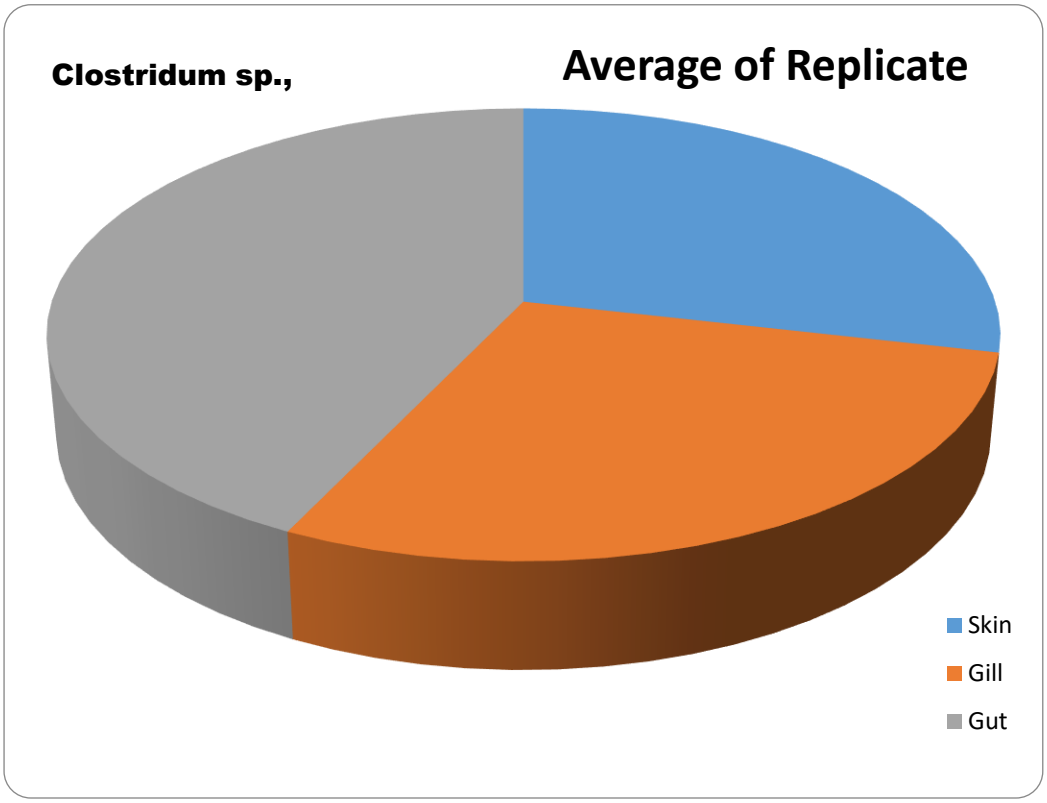


## 4.Discussion

Fish plays a vital role as a major dietary protein source yet they are prone to infection to microbes due to their polluted living environment especially in recent days. It also plays a role in the healthy living of humans who consume it regularly. Grass carp available commonly in local markets and freshwater pond was examined for the presence of microbe, in particular the bacterial flora which is a threat to human on consumption with ignorance(9).

*Pseudomonas* is a major organism affecting the fish of various species.*Pseudomonas* shows resilience surviving in brackish water and also in the temperatures below 50$^{o}$F (10$^{o}$C) and above 100$^{o}$F (38$^{o}$C) (10). *Pseudomonas* and *Aeromonas* are major pathogens affecting fish. dropsy, hemorrhagic septicemia, gill rot disease, fin and tail rot diseases(11). Ulcerative syndromes are caused by these microbes in fish (12).*Clostridium* species causes dangerous diseases namely necrotic enteritis to wound infection and gas gangrene which threatens life majorly(13).Gastro intestinal problems are to be caused by *clostridium* species and its high toxic occurrence in fresh fish is proved (14). Total of 1.3 billion cases of Human gastroenteritis annually were recorded which were due to the ingestion of Fish and Shell fish affected by *Salmonella*sp(15). Seepage of Sewage water into the fresh lakes or fish farm may contaminate the water and the fish gradually(16). Fish contamination could also happen through improper transportation or improper handling or washing with contaminated water.

## 5.Conclusion

The current study demonstrated the fundamental basis of bacterial infection in grass carp, this will help as an initial point for the diagnosis of bacterial infections, prevention and control of pathogenic bacterial infections in the poondi reservoir region. *Pseudomonas* and

*Aeromonas*were found to be highly prominent microbial agents affecting the grass crap. This could be further implicated with pathogenic infection of human after consumption of the fish leading to disease complication and mortalities.

**Competing interest**

The author declares no conflict of interest.

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