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Commensal and Pathogenic Bacteria Associated with Farmed Rainbow Trout (*Oncorhynchus mykiss Walbum*) and Aquaculture Environment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The bacterial microflora associated with farmed rainbow trout (O*ncorhynchus mykiss Walbaum*) and its aquaculture environment was characterized. The counts of commensal, indicator and pathogenic bacteria were determined and bacterial colonies were characterized. The mean mesophilic counts were 4.5 log CFU g $^{-1}$ and 7.6 log CFU g $^{-1}$ in skin with muscle and intestine respectively. Significantly high densities of faecal coliform, *E. coli* and Enterococci was detected in pond sediment, fish intestine and feed when compared to pond water and trout tissues. A total of 110 randomly selected isolates from TSA were identified and characterized. Of the mesophiles, 70–75% were aerobic Gram-negative bacteria, both motile and non-motile, and members of the Enterobacteriaceae family. The skin microflora of trout was dominated by Gram-negative aerobic

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rods belonging to Enterobacteriaceae, Aeromonadaceae, *Pseudomonas/ Shewanella, Moraxella, Acinetobacter* and *Flavobacterium*. Psychrotrophic bacterial isolates of the genera *Pseudomonas, Morganella, Hafnia* were identified in trout. Pathogenic bacteria such as *Clostridium botulinum, Yersinia enterocolitica* could not be detected. However, *Aeromonas hydrophila, A. veronii* biovar *sobria, A. veronii* biovar *veronii* were prevalent in farmed trout. Presence of bacterial pathogens in the trout farm envisages a strict hygienic handling and processing of fish from such culture systems for ensuring public health safety.

Keywords: Rainbow trout; aquaculture environment; bacterial flora; indicator bacteria; pathogenic bacteria.

1. INTRODUCTION

"Fish are generally regarded as safe, nutritious and beneficial but aquaculture products have sometimes been associated with certain food safety issues" (WHO, 2007)." Numerous investigations have shown that *Streptococcus* sp. is one of the many bacterial species found in many fish that, in some circumstances, may be harmful (AL-Harbi, 1994). In addition to mortality, disease results in financial losses for the handler and eventual consumer of the affected fish due to treatment costs, missed or delayed sales opportunities, and zoonotic disease contraction.

"It is generally accepted that the environment can influence the microflora associated with the skin, gills and intestines of finfish" (Horsely, 1973). "In the aquatic environment, hosts and microorganisms share a similar ecosystem where bacteria can either colonise the host (intestinal tract, gills, or skin) or not" (Harris, 1993). "Therefore, fish grown in different aquaculture systems may harbor different species and number of bacteria. The culture practices such as pond fertilization, supplementary feeding with slaughter house waste or agricultural byproducts imposes a high probability of contamination on the aquacultured fish" (Toor et al., 1991; Surendran et al., 1995). "Freshly harvested aquaculture products, particularly those from tropical regions may harbor pathogenic bacteria, which forms a part of natural micro-flora of fish ponds" (Clucas & Ward, 1996). "The presence of human pathogenic bacteria in aquaculture products is dependent on a number of factors including method of production, rearing practices, environmental conditions and the methods used to harvest, process and distribute the products" (ICMSF, 1998; Reilly and Ka¨ferstein, 1999). "In recent times increased attention is given to the possibility of cultured fish as vector of human pathogenic bacteria" (Apun et al., 1999; Islam et al., 2000). Farmed fish not only transmit disease to man but are themselves subject to many diseases and capable of transmitting many of the established food borne microbial infections and intoxications (FAO/WHO, 1974).

India is the second largest producer of freshwater fish in the world (FAO, 2005) and important farmed species include Indian major carps, exotic carps, Tilapia, murrels and catfish (magur, Singhi, Pangasius). One of the more recent species is rainbow trout (*Oncorhynchus mykiss* Walbaum) an exotic coldwater fish. Trout farming is gaining popularity in India. This species is primarily used for recreational fishing in India, but it has recently made its way into metro area stores as an exotic food item that the urban populace wants. In India today, trout is regarded as a very expensive fish. However, there is dearth of information on the bacterial load of farmed trout in India. The microbial profile and incidence of pathogen on rainbow trout (O*ncorhynchus mykiss Walbaum*) from temperate countries has been investigated by a number of workers and has been the subject of a number of reviews (Austin & Al-Zahrani, 1988; Cahill, 1990; Ringø et al., 1995; Gonzalez-Rodrguez et al., 2002; Mc Adams et al., 2005; Nam & Joh 2007; Kayis et al. 2009; Salgado–Miranda et al., 2010). However, the microbial profile of aquacultured rainbow trout from tropical countries has received little attention. Ignorance of the microbial profile of aquaculture products can also affect human health as evidenced by the recent transmission of Streptococcal infections due to S*. iniae* and *L. garvieae* from tilapia to humans, which resulted in several meningitis cases in Canadian fish processors (CDC, 1996). Therefore, in order to create safe farm management techniques for the production of safe fish, it is imperative to look at the bacterial flora connected to trout grow-out culture. The current study's main goals were to determine the prevalence of bacterial infections in farmed trout and to identify and describe the bacterial flora

associated with farmed rainbow trout and their farm environment.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Rainbow trout (*O. mykiss*) of weight in the range of 250±15g and length 270±10mm were collected from aquaculture farm located in Idukki district, Kerala (India) using a drag net. Aseptically transported to the lab in insulated polystyrene cartons with ice for analysis within six hours of harvesting. Fish were fed on animal protein sources like livestock processing waste. Feed collected from trout farm was also analyzed.

2.2 Microbiological Analysis

Bacteriological analysis was performed on muscle with skin, intestine and feed samples. A sterile physiological saline (NaCl, 0.85% w/v) solution (225 ml) was added to 25g of muscle and skin, which were then aseptically weighed and homogenised in a stomacher (Lab blender 400, Seward Medical, London) for 60 seconds at room temperature. A section of fish intestine was removed, weighed, and put in sterile bags with enough saline solution to create dilutions of 1:10. Ten-gram feed was aseptically weighed, homogenized in mortar and pestle using sterile physiological saline (NaCl, 0.85% w/v) solution (90ml). For the MPN method, decimal dilutions in saline solution were made and either put into tubes or plated onto agar.

2.3 Commensal Bacterial Flora

For mesophilic and psychrotrophic bacteria, 0.5 ml of appropriate serial dilutions was spread plated onto the surface of preset tryptic soy agar plates (TSA, Oxoid, U.K (Spanggaard et al., 2000; Huber et al., 2004) and incubated at 37°C and 7°C for 2 and 10 days respectively (Austin & Al-Zahrani 1988; AOAC, 2000). *Pseudomonas* were counted on Cetrimide-Fusidin-Cephaloridine (CFC) agar (Oxoid code CM 559, supplemented with SR 103; Oxoid U.K.) after 3 days of incubation at 20°C (Molin & Ternstrom 1982; Mead, 1985). *Brochothrix thermosphacta* was determined on Streptomycin sulfate-Thallous acetate – Actidione Agar (STAA Hi Media, India) after incubation at 20°C for 4days (Gardner, 1966).

Enterobacteriaceae and H_2S-producing bacteria (including *Shewanella putrefaciens*) were counted on violet red bile glucose agar (VRBGA, Oxoid code CM 485) and Iron Agar (IA, Oxoid code CM 867), respectively by pour plate method and plates were incubated respectively at 30°C for 24 h. (Mossel 1987) and 20°C for 5 days (Gennari & Campanini, 1991).

2.4 Indicator Bacteria

Enterococci and *Staphylococcus aureus* counts were determined respectively on KF Streptococci Agar (Oxoid code CM 701) after incubation at 37°C for 2 days and on Baird Parker Agar (Oxoid code CM 275) incubated at 37°C for 2 days and typical colonies were confirmed (FDA, 2001). Coliforms, faecal coliforms and *Escherichia coli* counts were determined by a 3-replicate tube MPN (Most Probable Number) procedure (APHA, 1998). Positive EC tubes were confirmed by streaking onto Eosine Methylene Blue Agar (EMB Agar, Difco, Detroit, MI) and incubated at 37ºC for 24 h. Characteristic *E. coli* colonies were isolated and confirmed by biochemical tests as described by APHA (1998).

2.5 Pathogenic Bacteria

Clostridium perfringens numbers were determined by the three tube MPN method using Lactose Sulphite Broth (West, 1989) and confirmed by streaking on to Tryptose Sulfite Cycloserine (TSC) agar and 3-4 characteristic colonies were confirmed by biochemical reactions as described by FDA (2001). Samples weighing around 5.0g were added to 25ml of water in order to detect Clostridium botulinum. Sterile paraffin oil and Cooked Meat Media (CMM) were added. For three to six days, inoculated tubes were incubated at 30°C. The intestine and muscle parts of the fish's skin/shell were examined independently. Following a 6-day incubation period at 30°C, cultures were centrifuged at 10,000 x g and 4°C for 20 minutes. Each supernatant was then brought to pH 6.2 using 1N HCl and stored at -20°C until the presence of C. botulinum toxin was detected. The methodology outlined in the U.S. Food and Drug Administration Bacteriological Analytical Manual (Solomon & Lilly, 2001) was employed for trypsinization, toxicity test by mouse bioassay and toxin neutralization tests using type specific monovalent antitoxins A-E obtained from National Institute for Biological Standards and Control (NIBSC) Herts, UK.

Aeromonas spp. were counted on starch ampicillin (SA) agar (Hi Media, India) containing 10ug/ml of ampicillin incubated at 28°C for 48 h

(Palumbo et al., 1985) and 25 characteristic colonies were confirmed by biochemical tests as described by Kirov (2001) and API 20 NE system (Bio Merieux, France).

For detection of *Y. enterocolitica,* 10g muscle tissue was aseptically weighed into 90 ml Peptone sorbitol bile broth (PSBB; pH 7.6), homogenized for 30 s in a stomacher (Lab blender 400, Seward Medical) and incubated at 4^0C for 21 days (Singh et al., 2003). After 7-, 14- and 21-days incubation, alkaline post enrichment treatment was performed as described by FDA (2001), followed by spread plating on CIN agar (Oxoid CM 653 and Sr 109). Plates were incubated overnight at 30^0C. Small (1-2 mm diameter) red "bull's eye" colonies having deep red center with sharp border surrounded by clear colorless zone with entire edge were isolated into LAIA slant (FDA, 2001). Twenty-five presumptive isolates were confirmed using biochemical tests prescribed by FDA (2001) and API 20E system (Bio Merieux, France).

2.6 Characterization of Commensal and Pathogenic Bacterial Flora

By separating and identifying 20% of the colonies from TSA (30°C and 7°C) plates, the dominating aerobic microflora at the final sample points were determined. Twenty colonies were randomly picked from TSA plates sampled from feed samples, and 66–80 colonies were randomly selected from TSA plates sampled from rainbow trout skin with muscle and intestinal samples. Each and every colony from a section of the plate or from the entire plate was separated, purified, and given morphological and biochemical characteristics. 20 typical colonies each were isolated from IA and VRBGA sampled from rainbow trout skin with muscle and intestine samples and subsequently identified biochemically. A total of 220 bacterial cultures were isolated from trout muscle with skin, intestine and feed samples. The strains were examined for motility, oxidative/fermentative metabolism, spore presence, catalase and oxidase responses, and Gram reaction. They were then grouped according to the taxonomic schemes of Bergey's Manual of Systematic Bacteriology (Krieg & Holt, 1984; Sneath et al., 1986), further tested for the most relevant characteristics of each group and identified using schemes proposed by several authors for identification (Allen et al., 1983; Krieg and Holt, 1984; Sneath et al., 1986; Austin, 1988; Andrew & Mitchell, 1997; Kirov, 2001). The API 20E and 20 NE system (Bio Merieux, France) was employed for the confirmation of the isolates.

Twenty colonies randomly selected from TSA Plates incubated at 7°C were processed for 16S rRNA gene sequence analysis for identification. Crude DNA template was prepared using sterile Milli Q water in a single cycle of boiling for 10 min and freezing at -20ºC and stored until further use. Amplification of 16s rDNA gene (Partial sequence) was performed with the combination of 27F (GAGTTTGATCCTGGCTCAG) and 1544 R (AGAAAGGAGGTGATCCAGCC) as per Shigematsu et al. (2009). Polymerase chain reaction was performed in 50µl reaction mix with 1x concentration of Onetaq Quick load master mix with GC buffer (NEB, Cat.No.M0487), 0.5µM concentration of each primer (Sigma), 5µl of crude DNA prepared by cell lysate method and the Cycling conditions were standardized for one taq master mix as 94°C for 2min initial denaturation, followed by 30 to 35 cycles of denaturation (94°C for 30sec), annealing (50°C for 1 min) and extension (68°C for 1.5 min) and a final extension of 68°C for 7 min. Amplicon of size 1500bp were purified using Gen Elute gel extraction kit (Sigma, NA1111) and sent for sequencing for the same primers. Blast analysis was performed in public domain NCBI to determine the homology and isolates were identified.

2.7 Statistical Analysis

The number of bacteria in the MPN g-1 or cfu g-1 samples was converted to log10 values. The Microsoft Excel 97 software's statistical tool package was used to conduct a statistical analysis of the bacterial parameters. The significance of the variations between the means of microbial counts conducted at 30°C and 7°C was assessed using Student's t-test analysis. The threshold for statistical significance was set at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1 Bacterial Load on Farmed Trout and Farm Environment

The bacterial population associated with trout skin with muscle, intestine and feed samples was shown in Table 1. In trout skin with muscle and intestine, the highest counts were shown by mesophilic aerobic bacteria. Their population was 4.5 $\log_{10}$ cfu g^{-1} in trout muscle

and 7.6 log_{10} cfu g^{-1} in intestine. The population of psychrotrophic aerobic bacteria, H_2S producing bacteria, Enterobacteriaceae, *Aeromonas, Pseudomonas,* LAB and *B. thermosphacta* counts ranged between 10^2 and 10^3 cfu g^{-1}. The intestine samples of trout harboured high bacterial loads; the highest counts were noted for mesophilic aerobic bacteria which were in the range of 10^7- 10^8 cfu g^{-1}. Trout were fed with livestock processing waste and analysis of feed showed high counts of mesophilic aerobic bacteria (10^8 cfu g^{-1}), H_2S producing bacteria, Enterobacteriaceae (10^5 cfu g^{-1}) and *B. thermosphacta* (10^4 cfu g^{-1}). *Pseudomonas* count was 10^3 cfu g^{-1}. H_2S producing bacterial flora contributed 10-13% of the total flora of fresh trout. The mesophilic counts on skin with muscle of trout were close to or lower than the m value ($5x10^5$ cfu/g) recommended by the International Commission of Microbiological Specification for Foods (ICMSF, 1998) for whole fresh water fish and indicates good quality of trout. The results confirmed the earlier reports from Spain (Gonzalez-Rodriguez et al., 2001; González et al., 1999), Greece (Chytiri et al., 2004; Savvaidis et al., 2002), U.K (Ozogul & Ozogul 2002), USA (Mc Adams et al., 2005) and Iran (Rezaei et al., 2008) on aquacultured fresh trout. It is widely accepted that the initial microbial load of fresh water fish varies depending on water conditions and temperature.

Bacterial levels in farm water, feeder canal water and sediment samples are given in Table 2. Aerobic mesophilic counts at 37°C and 7°C for water and sediment samples differ significantly ($P< 0.05$). Enterobacteriaceae population in water and sediment samples was in the range of 10^2 -10^3cfu g^{-1}. Mesophilic counts (mean log cfu g^{-1} at 30 ºC) were significantly higher in sediment samples than in water ($P<0.01$) as reported earlier for trout farms in Denmark (Schmidt et al., 2000). Schmidt et al. (2000) observed total bacterial counts of 10^3-10^4 culturable bacteria per ml of water, while sediment samples yielded counts of 10^6 -10^8 CFU $g–^1$. Similar results were obtained in this study. The bacterial numbers in the intestine samples were 7.657 log_{10} cfu $g–^1$, a value much higher than that of the skin with muscle of trout and the surrounding waters ($P<0.01$). Generally, this result is consistent with those in the previous studies reported by Spanggaard et al. (2000) and Kim et al. (2007) for trout intestine.

Table 1. Mean Microbial count on farmed trout (*Onchorynchus mykis*) from farm located in Idukki district, Kerala (India)

		Microbial Count log_{10} cfu/g *Oncorhynchus mykiss*		
Microbial parameters		**SM**	**Int**	**Feed**
TPC	30^0C	4.529 ± 0.109	7.657 ± 0.283	8.282 ± 0.55
	7^0C	3.771 ± 0.128	6.086 ± 0.081	NT*
H2S Producing bacteria		3.964 ± 0.208	5.562 ± 0.159	5.679 ± 0.697
Aeromonas		2.692 ± 0.763	5.601 ± 0.504	NT
Enterobacteriaceae		3.613 ± 0.011	6.226 ± 0.17	5.358 ± 0.316
Pseudomonas		2.34 ± 0.287	4.281 ± 0.232	3.602
Lactic acid bacteria		3.166 ± 0.117	5.764± 0.314	NT
Brochothrix thermosphacta		2.281 ± 0.134	4.775 ± 0.439	4.512 ± 0.47

** Not Tested*

Table 2. Mean Microbial count of water and sediment from farm located in Idukki district, Kerala (India)

		Microbial Count log_{10} cfu/g		
Microbial Parameters*		**Farm water**	**Feeder canal water**	**sediment**
TPC	30^0C	3.70 ± 0.091	2.849 ± 0.151	7.322 ± 0.009
	7^0C	2.428	NT*	6.085
H2S Producing bacteria		3.756	NT	5.439
Enterobacteriaceae		2.799	2.113	3.519

** Not Tested*

Table 3. Indicator and pathogenic bacteria in water, sediment, feed and trout from farm located in Idukki district, Kerala (India)

Bacteriological parameters	Mean bacterial count (log_{10} cfu g-1)					
				Trout		
	Farm water*	Feedercanal water	Sediment	SM	Intestine	Feed
Total Coliforms [a]	3.041	3.041	3.146	2.041	3.146	3.146
Fecal coliforms[a]	1.653	1.397	3.146	1.397	3.041	3.146
Escherichia coli[a]	1.653	1.176	3.041	0.977	3.041	3.146
Faecal streptococci [b]	ND	ND	2.204	2.216	5.068	5.531
Staphylococcus aureus[b]	ND	ND	2.7	2.389	3.623	2.34
Clostridium perfringens[a]	ND	ND	0.544	ND	1.397	ND
Yersinia enterocolitica[c]	ND	ND	ND	ND	ND	ND
Clostridium botulinum[c]	ND	ND	ND	ND	ND	ND

*[a]MPN; [b] cfu/g; c – present / absent * for water samples MPN/100ml, for sediment, fish and feed MPN/g; ND not detected*

H_2S producing bacterial flora contributed 10-13% of the total flora of fresh trout. Significantly higher counts of Enterobacteriaceae, *Aeromonas, Pseudomonas,* LAB and *Brochothrix thermosphacta* were obtained in the intestine samples compared to skin with muscle sample ($P<0.01$). The results of this study confirmed the findings of González et al. (1999); Savvaidis et al. (2002); Arashisar et al. (2004) and Chytiri et al. (2004) that Enterobacteriaceae, *Brochothrix thermosphacta* and lactic acid bacteria were also found to be members of the microflora of fresh rainbow trout. In this Study, significantly higher counts of Enterobacteriaceae, LAB and *Brochothrix thermosphacta* was obtained which indicates that they are able to persist and multiply in the environment provided by the intestinal tract as reported earlier (Sugita et al. 1988; Cahill, 1990). Mexis et al. (2009) also reported that LAB and Enterobacteriaceae were part of the microflora of fresh rainbow trout. Naviner et al. (2006) reported counts in the range of 10^2 – 10^5 for LAB and Enterobacteriaceae in trout intestine samples from French farms. LAB is not considered as belonging to aquatic environments, but certain species (i.e. *Carnobacterium*, *Vagococcus*, *Lactobacillus*, *Enterococcus*, *Lactococcus*) have been found in freshwater fish and their surrounding environment (González et al., 1999, 2000; Ringø et al., 2000). Several species of *Streptococcus* and *Lactococcus* bacteria including *S. iniae, S. agalactiae, S. dysagalactiae, S. parauberis, S. feacalis, L. garvieae* and *L. lactis* have been so far discriminated as the cause of Streptoococcosis/lactococcosis outbreaks in trout aquaculture (Austin & Austin, 2007; Haghighi Karsidani et al. 2010; Bekker et al., 2011).

3.2 Characterization of Microflora

3.2.1 Commensal bacterial flora

A total of 110 bacterial isolates selected from TSA plates were identified from rainbow trout skin with muscle and intestine. Of the 56 isolates from trout skin with muscle, 21.4% Enterobacteriaceae (*Enterobacter, Citrobacter, Klebsiella, Hafnia*), 14.3% Aeromonadaceae, 39.4% Gram-negative aerobic coccobacilli and rods (*Pseudomonas/ Shewanella, Moraxella, Acinetobacter, Flavobacterium*), 21.4% Gram positive cocci - *Micrococcaceae* (*Micrococcus* and *Staphylococcus*) and *Streptococcaceae* and 3.5% Gram positive spore forming rods (*Bacillus*). Identification of the 54 intestinal isolates showed that Enterobacteriaceae (30%) and Aeromonadaceae (18.5%) dominated the microflora followed by Gram-negative aerobic coccobacilli and rods (*Pseudomonas/ Shewanella, Moraxella, Acinetobacter, Flavobacterium*) constituting 33% of the flora. Gram positive bacteria were identified as Streptococcaceae (7.4%) and Bacillaceae (11.1%).

16s *rDNA* partial sequencing analysis of predominant psychrotrophic bacterial isolates found in Rainbow trout revealed that isolates belonged to *Pseudomonas fragi*, *Morganella morganii*, *Hafnia alvei* and *Aeromonas* spp.

The isolates from peptone iron agar plates (20 isolates) were identified as Aeromonadaceae (*A. hydrophila, A. veronii* biovar *sobria*) Enterobacteriaceae (*Enterobacter intermedius*, *Citrobacter*) and *Shewanella*. The VRBGA isolates (20 isolates) were identified as

Enterobacter agglomerans, E. intermedius, E. aerogenes, Providencia rettgeri, Klebsiella spp. *Citrobacter* and *Hafnia*. Of the 20 isolates from feed collected from trout farm, the bacteria identified were Enterobacteriaceae (30%) Aeromonadaceae (25%), Gram-negative aerobic coccobacilli and rods 25% (*Pseudomonas/ Shewanella, Flavobacterium*), Gram positive cocci (Streptococcaceae) 10% and Gram-positive spore forming rods (*Bacillus*) 10%.

Acinetobacter, Pseudomonas, Staphylococcus, Enterococcus, and Bacillus were shown to be predominant in Spanish rainbow trout (González et al., 1999). Kayis et al. (2009) isolated *Pseudomonas/ Shewanella, Flavobacterium, Aeromonas, Enterobacter, Citrobacter, Hafnia* and *Yersinia* from rainbow trout from farms in Turkey. Bacterial groups *Aeromonas* and *Shewanella* were reported to be the specific spoilage organism (SSO) in trout from temperature and tropical waters (Chytiri et al. 2004; Ninan et al., 2011). Naviner et al. (2006) reported abundance of *A. hydrophila* and *A. sobria* in the intestinal microbiota of trout in France. The results of the present study agree well with that of Spanggaard et al. (2000) who reported predominance of Enterobacteriaceae (*Citrobacter, Hafnia, Proteus, Serratia*) and Aeromonadaceae in the intestinal microflora of rainbow trout from Denmark farms. They also reported predominance of other Gram-negative bacteria of the genera Acinetobacter, *Flavobacterium*, *Shewanella*, *Pseudomonas* in some farms. Gram positive bacteria were identified as *Arthrobacter, Carnobacterium, Streptococcus* and *Staphylococcus.* In contrast, Huber et al. (2004) reported *Acinetobacter*, *Aeromonas*, *Flavobacterium*, *Shewanella*, *Pseudomonas* and *Carnobacterium* as the common bacterial genera in the microbiota of rainbow trout intestine from Denmark farms. It has been shown that the microbiotas of the fish intestine are highly dependent on the bacterial colonization during early development, environmental conditions and dietary changes (Ringø et al., 1995; 2006; Olafsen, 2001).

3.2.2 Indicator bacteria

Among the indicator organisms, significantly higher levels of faecal coliform, *E. coli* and faecal streptococci were detected in pond sediment, fish intestine and feed (Table 3). Faecal Streptococcal (2.3 $\log_{10}$ cfu g^{-1}) and *S. aureus* (1.3 $\log_{10}$ cfu g^{-1}) populations were low in fresh trout and were within the acceptable limit. *C. perfringens* was detected in sediment and intestine samples in low numbers. High levels of faecal coliforms and faecal streptococci were previously reported for fish farms in India (Surendran et al., 1995; Lalitha & Surendran 2004; 2006). *E. coli* count in trout muscle was below the m limit (9.5 g^{-1}) recommended by the ICMSF (1998) for good quality fish.

3.2.3 Pathogenic bacteria

Motile aeromonads are a part of the microflora of fresh trout and their population was 2.692 $\log_{10}$ cfu g^{-1}. and 5.6 $\log_{10}$ cfu g^{-1} respectively in skin with muscle and intestine samples. Among pathogenic bacteria, *A. hydrophila, A. veronii* biovar *sobria* and *A. veronii* biovar *veronii* were isolated from farmed trout. *Y. enterocolitica* and *C. botulinum* were not detected in farmed trout samples. Naviner et al. (2006) reported Aeromonas counts of 10^2-10^6 cfu g^{-1} intestine sample from farmed trout in France and *A. hydrophila* and *A. sobria* were identified in the samples. Nam & Joh (2007) observed *Aeromonas* counts of 10^2 - 10^3 cfu/ml of water from trout farms in Korea and found dominance of *A. sobria* in water and trout intestine. In the same study, they isolated *A. hydrophila* from farm water. *A sobria* was identified as the causative agent of Epizootic Ulcerative Syndrome (EUS) in fish farms in South East Asian countries such as Bangladesh and India (Chacon et al., 2003). *A. hydrophila*, *A. veronii* biovar *veronii* and *A. veronii* biovar *sobria* are the strains more often associated with gastroenteritis in humans.

Fish are susceptible to a wide variety of bacterial pathogens. *S. putrefaciens, A. hydrophila* and *A. sobria*, potential pathogens of fish, have been isolated from the skin and intestine of rainbow trout *Oncorhynchus mykiss* as reported earlier (Austin & Austin, 2007; Huber et al., 2004; Koziñska & Pêkala, 2004; Pond et al., 2006). Pond et al. (2006) proposed that fish digestive tract is a reservoir for many pathogens. The prevalence of *A. hydrophila* and *A. sobria*, which are generally recognized as agents of gastroenteritis, wound infections, septicemia and endocarditis (Kirov, 2001) in humans, are of concern in the culture environment because it may pose threat to public health. Motile aeromonas Septicaemia, sometimes known as hemorrhagic septicaemia, is likely the most prevalent bacterial illness affecting freshwater fish and has been linked to a number of Aeromonas species, including A. hydrophila, A. sobria, A. caviae, A. schuberti, and A. veronii. (Austin & Austin, 2007). *A. hydrophila* was

reported in diseased trout from Korea (Lee et al., 2000). Salgado–Mirranda et al. (2010) isolated Aeromonads most frequently from gills and intestine of farmed trout in Mexico. Rehulka (2002) reported severe skin lesions in Rainbow Trout (*Oncorhynchus mykiss*) caused by *A. sobria* and *A. caviae. As psychrotrophic pathogens, A. hydrophila and A. sobria* are able to create distinct virulence components at both optimal growth temperature and refrigeration temperature. For food items that are kept in the refrigerator, which typically have a long shelf life at this temperature, this might be significant. While proper boiling can render these bacteria inactive, handling and cross-contamination may pose a health risk, particularly for vulnerable groups such as children, the elderly, immunocompromised individuals, and sick adults.

In this study, *C. botulinum* and *Y. enterocolitica* could not be detected in farmed trout skin with muscle and intestine samples. In trout samples, majority of the *Yersinia* strains isolated belonged to *Y. intermedia.* Kapperud & Jonsson (1976) detected *Y. enterocolitica* in brown trout from Norway. Mc Adams et al. (2005) did not isolate *Y. enterocolitica* from aquacultural rainbow trout from farms in Virginia. Lalitha & Gopakumar (2000) reported distribution of *C. botulinum* in sediments and fish from farms located in and around Cochin (Kerala, India). Mc Adams et al. (2005) identified C. *botulinum* in trout farms in Virginia. In a study on *C. botulinum* from Finnish trout farms, Hielm et al. (1998) found *C. botulinum* type E in 68% of the farm sediment samples, in 15% of the fish intestinal samples, and in 5% of the fish skin samples. An outbreak of botulism by *C. botulinum* type E was reported in rainbow trout, *Salmo gairdneri* Richardson, farmed in Denmark (Huss et al., 1974) and in Britain (Cann & Taylor, 1982). Botulism outbreak was not reported in fish farms in India.

4. CONCLUSION

The study provides the first insight on the commensal and pathogenic bacteria associated with trout from aquaculture farm located in Idukki district, Kerala (India). The study confirms that farmed rainbow trout carry significant numbers of faecal coliforms and enterococci in the intestine. Significant numbers of faecal coliforms and enterococci were also found in the farm sediment and feed. However, water does not contain high numbers of these organisms. The rearing practices such as feeding and pond fertilization could have influenced the microflora of trout. The study reveals the presence of pathogenic bacteria as part of the natural microflora of farmed trout. *A. hydrophila* and *A. sobria* that are potential pathogens of both rainbow trout and humans are prevalent in farmed trout. These pathogens are potential hazards to fish handlers and consumers. The results of the present study have practical significance in trout husbandry with regards to feed management and control of pathogens. Further study on the antibiotic resistance profile of these bacterial pathogens could establish the real threat posed by these organisms.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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