



Diversity of Bacteria and Fungi Associated with Freshwater Fishes from Mijawal River, Nasarawa, Nigeria

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ABSTRACT: Understudying the presence of bacteria and fungi population on freshwater fishes, provides the knowledge and understanding of their diversity and potential roles they may play in the health of the aquatic and terrestrial ecosystems. The present study was conducted to evaluate the diversity of bacteria and fungi species of identified fish samples from River Mijawal, Nasarawa State. The fishes were sampled from the only landing for three months (October to December, 2019) for this study. During the sampling periods, thirteen (13) different fish species were identified; they were swabbed with sterilized swab stick from the skin surface for bacteria and fungi identification. The Dominant fish species were *Tilapia zillii*, *Labeo senegalensis*, *Mormyrus rume* and *Mormyrus tapines*. The total number of thirteen (13) bacterial and five (5) fungi species were isolated and identified. Eight (8) of the bacteria isolates, were gram negative bacteria (*Pseudomonas sp*, *Aeromonas sp*, *Serratia mercerscenes*, *Proteus sp*, *Salmonella spp*, *Escherichia coli*, *Klebsiella sp*, and *Enterobacter sp*.); whilst the other five (5) were gram positive bacteria (*Bacillus sp*, *Micrococcus sp*, *Staphylococcus sp*, *Enterococcus sp* and *Listeria monocytogenes*). The five isolated fungi species were *Aspergillus niger*, *Rhizopus sp*, *Penicillium sp*, *Trichoderma viridae* and *Geotrichum candidum*. The frequency of occurrence of the isolated bacteria indicated that *Staphylococcus sp* had the highest frequency of occurrence (16.67%) while *Serratia mercerscenes* had the least occurrence (2.78%). The highest number of occurrence of the isolated fungi species was observed in *Rhizopus sp* while the least was recorded in *Geotrichum candidum*. It was observed that *Tilapia zillii* and *Mormyrus rume* had the most frequent bacteria and fungi isolates. It can therefore be deduced from the results that fish samples identified from River Mijawal has high bacteria and fungi diversity that may be pathogenic to man. The occurrence of these microbes may be attributed of human and animal faeces in the River

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Fishes are the most important protein sources for people of many countries (Hussain *et al.*, 2011); however, they are faced with severe decline globally due to economic and human population growth (Limburge *et al.*, 2011). The natural aquatic systems have witnessed changes in stock diversity and abundance, genetic structure and age composition of stocks resulting from structural changes in habitat, food composition and uncontrolled exploitation. The presence of foodborne pathogens in fish is related to environmental conditions and microbiological quality of the water at the fishing site. On the other hand, the microbial diversity of freshwater or Rivers and Lakes may consist of complex flora of microorganisms, including genuinely aquatic pathogens and other components introduced from human, animal and plant sources (Adams and Tobias, 1999); as the scale of human activities has been demonstrated to exert some detrimental effects on coastal waters. Diseases in freshwater fishes are of great threat in achieving

optimum production and have become a limiting factor to economic success of aquaculture. Bacterial infections are the major constraint for rapid cultivation of these species as it is believed that contaminated water is one of the root causes for the origin of bacterial-borne infection in fishes. Several studies have shown that bacteria belonging genera *Aeromonas*, *Corynebacterium*, *Myxobacterium*, *Pseudomonas* and *Vibrio* cause infectious diseases in fish (Cahill, 1990). FAO (2009), reported that the level of contamination of a fish at the time of its capture, depends on the environment and the microbiological quality of the water in which it was harvested. Studies have also shown that fish are host to bacteria species as they are the most causative agents of fish diseases (Musefiu *et al.*, 2011; Jimoh *et al.*, 2014; Anyanwu *et al.*, 2015; Olugbojo and Ayoola, 2015). Fungi on the other hand, are known to be responsible for a number of diseases in freshwater fishes (West, 2006). Fungi can infest fishes of all ages and can also prevent

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successful hatching when it invades fish eggs. Among the numerous aquatic fungi species, Oomycetes have gained more attention because of their effect on fish health (Qureshi et al., 2006). Even though every freshwater fish is exposed to at least one species of fungi during its lifetime, only stressed and poorly fed fishes are more susceptible to fungal infection. Studies of fungi diversity in aquatic environment has been conducted in water and sediment (Doi et al., 2018); on plants (Adamu et al., 2017) and on fishes (Ali, 2015; Angahar, 2016; Atef et al., 2016) as they have reported to cause serious diseases in freshwater fishes. Therefore, the present study was conducted to determine the presence of bacteria and fungi associated with freshwater fishes sampled from River Mijawal, Nasarawa, Nasarawa State Nigeria.

MATERIALS AND METHODS

The study was carried out at Mijawal River, Nasarawa, Nasarawa State (Plate 1); which is located in the western senatorial district of Nasarawa State. The fish samples were collected aseptically from the landing site of the River and where identified with fish identification key (Olaosebikan and Raji, 2004). The fishes were swabbed with sterile swab stick on the skin; the swab stick was placed in a normal saline solution and transported to Departments of Zoology and Microbiology Laboratories, Nasarawa State University Keffi for further analyses. Sampling was conducted for three months (October to December, 2019).

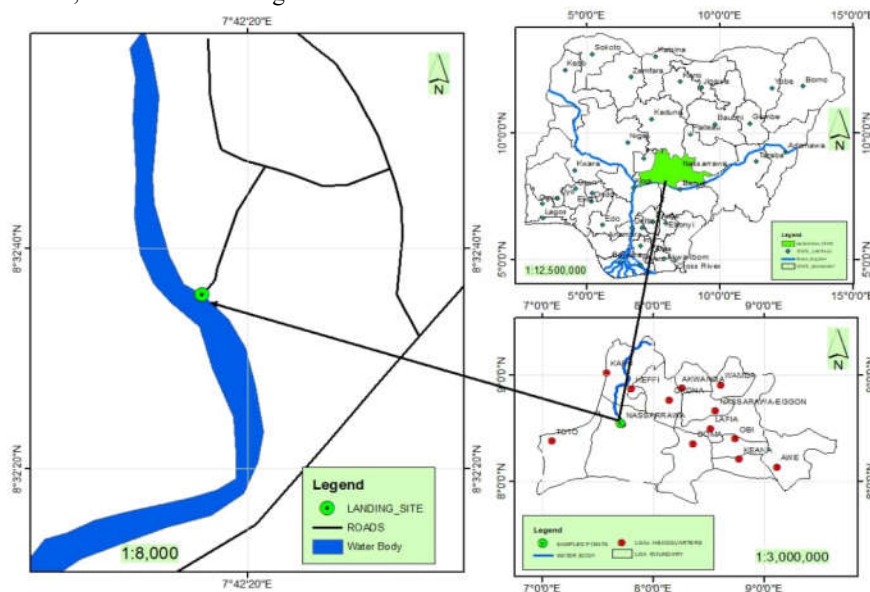


Plate 1. Pictorial representation of the location and sampling site in River Mijawal, Nasarawa State

Bacteriological Analysis: Ten (10) fold serial dilution of the sample was prepared from which 0.1ml was plated into a sterile Petri dish. Nutrient agar (Titan Biotech) which was prepared according to the manufacturer's specification was poured onto a sterile Petri dish containing 0.1ml of the sample using pour plate technique.

The plates were then incubated at 37°C for 24hours to obtain mixed culture. Isolated colonies were recorded and purified to obtain pure culture by repeated sub-culturing on fresh media used for primary isolation as described by Chikozie (2015). Pure stock cultures obtained were inoculated on nutrient agar slant and preserved in the refrigerator at 4°C until needed for further characterization and identification.

Characterization and identification of bacterial isolates: Bacterial isolates were characterized by their morphological/macrosopic and microscopic characteristics and identified further by biochemical tests (catalase, coagulase, urease, citrate, oxidase, indole, methyl-red, glucose, lactose and sucrose) as described by Cheesebrough (2000) and Adeoye, (2007).

Isolation and Identification of Fungi: Potato Dextrose Agar (PDA) was used and prepared in accordance to the Manufacturer's instruction (Accumix®- Tulip Diagnostics (P) Ltd). Dilution factors 10^{-2} and 10^{-3} were used as stock solution. One ml of each dilution was aseptically taken from the suspension and transferred into sterile Petri dishes. Then Potatoes Dextrose Agar (PDA) was poured into the Petri dishes

containing the suspension and 1ml of chloramphenicol. The plates were swirled gently to allow even distribution of the sample in the medium and were incubated at room temperatures ($28 \pm 2^\circ\text{C}$) for 24 hours. From the culture obtained, sub-culturing was made to get the pure culture of each fungus isolated. Fungi isolated were identified using Fungi Families of the world mycological monographs by Samson et al. (2004). Isolates were studied macroscopically by colony shape, size, colour and growth pattern. Slides were prepared from each colony and stained with 0.05% Trypan blue in lacto phenol. The slides were observed under microscope. The existing septate wall, sexual organ structure, size and arrangement of spores were also examined and recorded. The fungi species were identified with the help of available fungi identification keys and literature (Willoughby, 1994).

RESULTS AND DISCUSSION

The data obtained from the sampling periods, revealed the dominant fish species as *Mormyrus tapirus*, *Mormyrus rume*, *Labeo senegalensis* and *Tilapia zillii*. While *Chysichthys aluensis*, *Chelathiops bibie*, *Barbus callipterus*, *Synodontis vermiculatus*, *Clarias*

angularis and *Mormyrus hasselgusti* in two sampling periods. However, the species of *Mormyrus senegalensis*, *Clarias gariepinus* and *Raiamas senegalensis* were only present once during the sampling period as presented in Table 1. The composition of fish species in this study was lower than those reported by Mandal et al. (2009). However, the decline may be attributed to increased fishing pressure and habitat destruction as reported by Emmanuel and Ikusemiju (2005). Similarly, the decline in catch, may also be attributed to the season (dry) of the catch which is characterized by poor water quality, reduced water level and high temperature (Lawson and Olusanya, 2010). Under these conditions, migrations of the species of fish may be responsible for the low catch. However, the findings of this study was in line with the reports of Austin, (2006) who reported eleven (11) species belonging to seven (7) families. Higher abundance of the family Cichlidae may be due to their high level of adaptability to a high range of conditions. According to Terentjeva et al. (2015), Cichlidae are rare in areas where they are competitively disadvantaged especially in areas where they co-exist with larger families (Okomoda et al., 2013).

Table 1: Fish species sampled from River Mijawal during the sampling periods

Families	Species	Sampling Period		
		October	November	December
<i>Mormyridae</i>	<i>Mormyrus tapirus</i>	+	+	+
	<i>Mormyrus senegalensis</i>	+	-	-
	<i>Mormyrus rume</i>	+	+	+
	<i>Mormyrus hasselquisti</i>	+	+	-
<i>Clariidae</i>	<i>Clarias gariepinus</i>	-	+	-
	<i>Clarias angularis</i>	+	-	+
<i>Mochokidae</i>	<i>Synodontis vermiculatus</i>	-	+	+
<i>Cyprinidae</i>	<i>Barbus callipterus</i>	-	+	+
	<i>Labeo senegalensis</i>	+	+	+
	<i>Raiamas senegalensis</i>	+	-	-
	<i>Chelathiops bibie</i>	+	-	+
<i>Cichlidae</i>	<i>Tilapia zillii</i>	+	+	+
<i>Claroteidae</i>	<i>Chysichthys aluensis</i>	+	+	-

+Present; - Absent

A total number of thirteen (13) bacterial isolates were identified from the fishes during the sampling period as shown in Table 2. Amongst these bacteria, eight were gram negative and the other five were gram positive. From the gram positive bacteria isolated, it was observed that *Staphylococcus* sp occurred more frequent while *Enterococcus* sp and *Micrococcus* sp occurred less frequent. Also amongst the gram negative bacteria isolated, *Proteus vulgaris*, *Klebsiella* sp and *E. coli* appeared more frequent while *Serratia*

mercescens appeared least during the sampling periods as shown in Table 3.

The number of occurrence of bacterial isolates (Fig 1) revealed that *Staphylococcus* spp was recorded to be the highest, followed by *Bacillus* spp while the lowest was recorded in *Serratia mercescens*. Based on the frequency of occurrence, it was observed that *Tilapia zillii* recorded the highest value of 16.67% while the least was recorded in *Raiamas senegalensis* (2.78%) as shown in Fig 2.

Table 2. Identification of bacterial isolates from sampled fishes in River Mijawal during the sampling period.

Microorganisms	Biochemical Tests							Sugar fermentation		
	Gram reaction	Catalase	Oxidase	Urease	Citrate	Methyl red	Indole	Glucose	Lactose	Sucrose
<i>Bacillus</i> spp	+	+	+	+	+	-	-	+	+	+
<i>Micrococcus</i> sp	+	+	-	+	-	-	-	-	-	-
<i>Staphylococcus</i> sp	+	+	-	+	+	+	-	+	+	+
<i>Enterococcus</i> sp	+	-	-	-	-	-	-	+	+	+
<i>L. monocytogenes</i>	+	+	-	-	-	+	-	+	+	+
<i>Pseudomonas</i> sp	-	+	+	-	+	-	-	-	-	-
<i>Aeromonas</i> sp	-	+	+	-	+	-	-	+	-	-
<i>Serrate mercescens</i>	-	+	-	+	+	-	-	+	-	+
<i>Proteus</i> sp	-	+	-	-	+	+	-	+	-	-
<i>Salmonella</i> sp	-	+	-	-	-	+	-	+	-	-
<i>E. coli</i>	-	+	-	-	-	+	+	+	+	-
<i>Klebsiella</i> sp	-	+	-	+	+	-	-	+	+	+
<i>Enterobacter</i> sp	-	+	-	-	+	-	-	+	-	-

+Present; - Absent

Table 3: Total number of Bacterial isolated during the sampling periods

Bacteria isolates	Sampling Period (Monthly)			Number of occurrence
	October	November	December	
<i>Bacillus</i> spp	+	+	+	8
<i>Micrococcus</i> sp	+	+	+	5
<i>Staphylococcus</i> sp	+	+	+	12
<i>Enterococcus</i> sp	+	+	+	3
<i>L. monocytogenes</i>	+	+	+	5
<i>Pseudomonas</i> sp	+	+	+	9
<i>Aeromonas</i> sp	+	+	+	9
<i>Serrate mercescens</i>	+	-	+	12
<i>Proteus</i> sp	+	+	+	3
<i>Salmonella</i> sp	+	+	+	7
<i>E. coli</i>	+	+	+	10
<i>Klebsiella</i> sp	+	+	+	12
<i>Enterobacter</i> sp	+	+	+	5

+present, - = absent

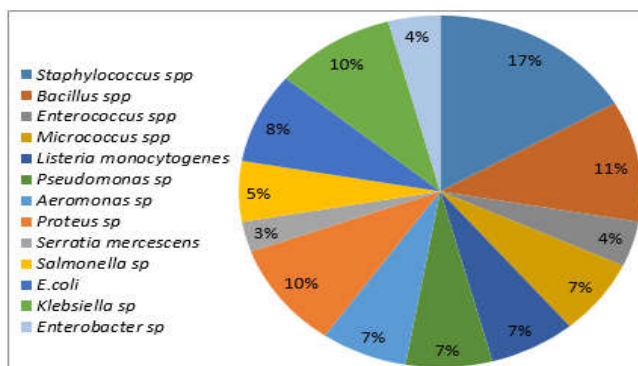


Fig. 1: Percentage frequency of bacterial isolates during the sampling period in River Mijawal, Nassarawa

It was observed in this study, that *Tilapia zillii* and *Mormyrus rume* recorded the most frequent bacterial isolate which was similar to the work of Musefiu et al. (2011) on bacteria flora of *Clarias gariepinus* and *Oreochromis niloticus*. In most cases fresh fish flora are mainly bacteria. Among all the bacteria species isolated, *Pseudomonas* sp, *Serratia* sp, *Salmonella*, *Aeromonas* sp, *Proteus* sp and *Klebsiella* sp and *E. coli* from the freshwater fishes sampled from River

Mijawal; *Pseudomonas* are well-known fish specific spoilage bacteria and their abundance in fish may led to rapid fish spoilage processes that may causes changes in fish meat quality and makes fish unfit for human consumption (Austin, 2006). Studies have shown that *Serratia* sp. is present in the environment, plant surfaces, soils and water (Blackburn, 2006). Thus their isolation in fish samples. The bacteria are able to grow in an environment that is unsuitable for

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other microbial growth and also contribute to spoilage of foods (Austin, 2006). Among the 13 bacterial species, 8 species were gram negative while 5 were gram positive as Austin (2006) reported 3 species of gram negative bacteria; *Micrococcus*, *Staphylococcus* and *Bacillus* sp. with five (5) genera including *Acinetobacter* sp, *Aeromonas* sp, *Enterobacter* sp, *Alcaligenes* sp, *Moraxella* sp and *Pseudomonas* sp which is similar to the findings of the present study. Similarly Amande and Nwaka (2013), observed the presence of bacteria species such as; *Streptococcus*, *E. coli*, *Salmonella* sp, *Staphylococcus* sp, *Vibrio* sp, *Pseudomonas* sp, *Serratia* sp, *Klebsiella* sp, *Enterococcus* sp and *Proteus* sp which is also in conformity with bacteria isolated in this work. Based on the percentage frequency of occurrence, the presence of *Salmonella* sp indicates faecal contamination of the water from which the fishes were harvested which is in line with the work of Elsherif (2004) who reported the isolation of some pathogenic organism such as *Salmonella* sp, *E. Coli* and potential pathogenic organisms such as *Klebsiella* sp, *Citrobacter* sp and *Proteus* sp. Abdelhamed et al. (2006) reported that the isolation of *Salmonella* sp and *E. coli* from the fish samples indicates faecal contamination of the water resulting from the livestock manure that is being added to the fish ponds as feed.

Bacillus spp on the other hand are known to cause a wide range of infectious diseases including abscesses, bacteremia/Septicimia, wound and food borne infections, ear infections, endocarditis, meningitis, ophthalmitis, Osteomyelitis, peritonitis and respiratory and urinary infections, (Morales et al., 2004). *Listeria monocytogenes* is widespread in the environment and humans can be exposed to the bacteria in various ways, though many persons remain symptomless. The diversity of potential pathogens from the samples of fish is of concern, particularly at a time when many in our communities are immunologically compromised as a result of various illnesses. The fungi isolated during the sampling periods are presented in Table 4. Five (5) isolates were identified from the sampled fishes based on their morphological examination (Table 5). The highest cumulative average of fungi load on the identified fishes was recorded in *Chysichthys aluensis* as 4.00×10^5 cfu/ml, while the least values (1.00×10^5 cfu/ml) were recorded in *Synodontis vermiculatus* and *Mormyrus senegalensis* as presented. Table 6 reveals the occurrence of different fungal isolates per fish and it was observed that *Rhizopus* sp was the most frequent isolate whilst *Chysichthys aluensis* recorded the highest number of fungal isolates on the skin.

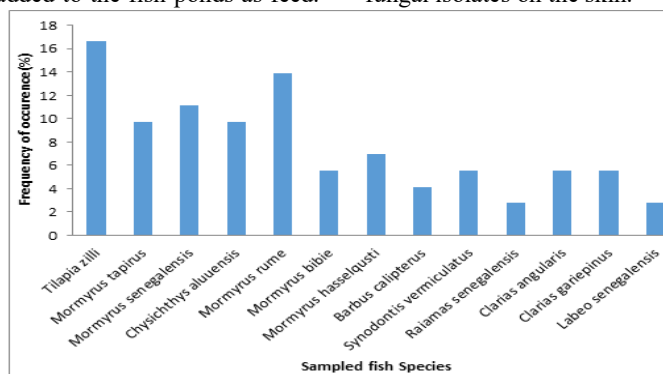


Fig. 2: Frequency of occurrence of bacteria isolated from identified fishes in River Mijawa Nassarawa

Table 4: Fungal load on the skin of identified fishes from River Mijawal, Nasarawa.

Fish Sample	Sampling periods (monthly)			Cumulative Average CFU/ml x 10 ⁵
	Fungi Colony in CFU/ml x 10 ⁵			
	October	November	December	
Tilapia zillii	3.00	4.00	-	3.50
Mormyrus tapirus	2.00	2.00	-	2.00
Mormyrus senegalensis	-	-	1.00	1.00
Chysichthys aluensis	-	-	4.00	4.00
Mormyrus rume	3.00	-	4.00	3.50
Chelathiops bibie	2.00	-	4.00	3.00
Mormyrus hasselquisti	-	2.00	-	2.00
Barbus calipterus	2.00	3.00	-	2.50
Synodontis vermiculatus	-	1.00	-	1.00
Raiamas senegalensis	-	-	3.00	3.00
Clarias angularis	2.00	-	-	2.00
Clarias gariepinus	-	-	3.00	3.00
Labeo senegalensis	3.00	3.00	-	3.00

Table 5: Characterization and identification of fungi isolates from identified fishes in River Mijawal, Nassarawa

Morphological Characterization		Probable Fungi
Macroscopic	Microscopic	
Surface-colonies like yellow to white hyphae, turning black with the formation of hyphae	Hyphae are septate, hyaline and conidiophores are long and globose at the tip	<i>Aspergillus niger</i>
Surface-texture velvety to powdery, green, blue-green or gray green on surface.	Hyphae septate, hyaline, conidiophores simple or branched phialides grouped in brush like clusters (penicilli) at the ends of the conidiophores.	<i>Penicillium sp</i>
Reverse; usually white to yellowish sometimes red or brown.	Hyphae broad, not or scarcely septet	<i>Rhizopus sp</i>
Surface-texture deeply cottony white becoming gray brown on surface		
Reverse:pale white		
Surface-o-white to cream coloured colonies with butyrous texture with a velvety, suede like or ground glass/matt appearance	Clear hyaline, septate hyphae. Produce chains of arthroconidia.	<i>Geotrichum candidum</i>
Surface-fluffy white tufts, green tufts may develop within the colony,	Septate hyaline hyphae, conidiophores are short and branching. Phialides are flask shaped. Conidia are ellipsoidal.	<i>Trichoderma viridae</i>
Reverse; typical light tan to yellow or pale orange.		

Table 6: Occurrence of Fungal isolates identified from freshwater fishes sampled from River Mijawal, Nasarawa.

Fish Samples	<i>Aspergillus niger</i>	<i>Rhizopus sp</i>	<i>Penicillium sp</i>	<i>Trichoderma viridae</i>	<i>Geotridum candidum</i>
<i>Tilapia zillii</i>	+	+	-	+	-
<i>Mormyrus tapirus</i>	-	-	+	-	+
<i>Mormyrus senegalensis</i>	-	+	-	-	-
<i>Chysichthys aluensis</i>	+	+	-	+	+
<i>Mormyrus rume</i>	+	-	-	+	-
<i>Chelathiops bibie</i>	+	-	+	-	-
<i>Mormyrus hasselquisti</i>	-	+	+	-	-
<i>Barbus calipterus</i>	-	+	-	+	-
<i>Synodontis vermiculatus</i>	-	+	-	-	-
<i>Raiamas senegalensis</i>	-	+	+	+	-
<i>Clarias angularis</i>	+	-	+	-	-
<i>Clarias gariepinus</i>	+	-	+	+	-
<i>Labeo senegalensis</i>	+	+	+	-	-
Total Number of isolates	7	8	7	6	2

+Present; - Absent

Pathogenic Fungi isolates such as *Aspergillus niger* was isolated in this study which are higher than that reported by Ali, (2015). Reports have revealed that the fungi are mostly frequent in fishes (Ali, 2015; Doi et al., 2018). De Hoog et al. (2000) had described *Aspergillus niger* in water as causative agents of kidney, liver disorder, allergy, burns, otitis media and increase risk of invasive infections. *A. niger* is a common allergen and may cause opportunistic invasive respiratory infection in hospitalized immunized patients (De Hoog et al., 2000). According to Doctor, (2007), fungi are reported to infest fish when bacteria infestations is present; thereof, they are reported as opportunistic parasites that are able to take advantage of damaged or stressed fishes. The high quantum of fungal pathogen recorded in this study may be an indication of high bacteria or other

pathogens present on the surface of the identified sampled fishes which as supported by researcher (Junaid et al., 2010). The source of these fungal pathogens may be associated with anthropogenic activities that occur around the water as well as the presence of bacterial isolates. The presence of *Penicillium sp* in the sampled fishes had shown that the fungal is most associated with fish as supported by the study conducted by Momeni et al. (2014), who reported that *Penicillium sp* caused 22% mortality of fish eggs which is supported by the report of Iqbal and Saleemi (2013) as an indication that as these parasites increase in their number, they are likely to affect fish production in the wild, thereby affecting fish harvest from the river. The isolation of yeast from this study is revealed that they are used for supplementation based diets.

Conclusion: In conclusion, this research has brought to light, the bacterial and fungal species associated with fishes at River Mijawal Nasarawa. The presence of these microbes in large population indicates high levels of faecal contamination in the River which are also potentially pathogenic to human. Hence, adequate measures should be taken in the examination of fish, especially for the presence of pathogens, during handling, storage and up to the very point of consumption is needed for the protection and maintenance of community health by keeping foodborne diseases to a minimum.

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