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Pathogenic and zoonotic potential of bacterial flora of commercial fish ponds in Ibarapa Central Local Government Area of Oyo State, Nigeria

[1]Obisesan, O.M., [3]Oladosu, G.A., [1]Abegunde, P.T., [2]Ajibade, A.O., [4]Adah, A.D., [1]Oladejo, A.O.
[1]*Animal Health Technology Dept., Oyo State College of Agriculture and Technology, Igboora.*
[2]*Fisheries Technology Dept., Oyo State College of Agriculture and Technology, Igboora.*
[3]*Veterinary Medicine Department, University of Ibadan.*
[4]*Veterinary Medicine Department, University of Ilorin.*
Corresponding author: dolapoobisesan09@yahoo.com

Abstract
Pathogenic and non-pathogenic bacteria are normal microflora of the aquatic ecosystem. The bacterial organisms isolated in this study are from fish and pond water samples collected from 3 farms and they belong to the following families: Streptococcaceae, Staphylococcaceae, Enterobacteriaceae, Psedomonadaceae, Vibrionaceae and Aeromonadaceae. Seven of the 13 bacterial species isolated namely; *Citrobacter freundii, Aeromonas hydrophila, Vibrio vulnificus, Pseudomonas aeruginosa, Aeromonas schubertii, Klebsiella pneumoniae* and *Edwardsiella tarda* are pathogenic to fish in freshwater habitat, while almost all the 13 species are pathogenic to humans. These organisms are therefore of economically significant to commercially important farmed fishes, have zoonotic potential and are of public health importance to man. Strict hygiene and biosecurity measures become very important on fish farms to avoid disease outbreaks. Consumers should also avoid eating raw and lightly cooked aquatic products.

Keywords: Pathogen, zoonotic, bacteria, microflora, fish pond

Introduction
Fish and fish products are not only important from the nutritional point of view, but are also items of international trade and foreign exchange earner for many countries in the world (Danba *et al*., 2014). Catfish dominates Nigeria aquaculture, and they are found nationwide, eaten by most tribes, resistant to harsh environmental conditions, commands good price, tasty and can be kept alive for days during marketing (Anetekhai, 2013).

However, fish are host to various bacteria organisms with both pathogenic and non-pathogenic bacterial population existing in the natural freshwater and marine sources. Fish diseases are caused by pathogenic bacteria (Kabir *et al*., 2018), the preponderance of which are short, Gram-negative rods belonging to the families Enterobacteriaceae, Pseudomonadaceae and Vibrionaceae. Typically, they cause septicaemic and ulcerative disease conditions. While the long, Gram-negative, myxobacteria of the family Cytophagaceae, causes heavy mortality in fish stocks, the Gram-positive bacteria especially acid-fast are less frequently encountered, but can cause severe losses in certain species of fish under particular conditions. Sources and modes of infection among fish are variable, many pathogens are either normal inhabitants in or on fish, or saprophytes present in soil or water or invertebrate hosts. Spread of fish infection is through direct and indirect exposure of cultured fish to pathogens, enhanced by poor management of fish health. Fish disease occurs due to interaction between pathogen, fish host and environment. Also, majority of infection are stress related; poor water quality is a main contributor, making the challenge very complex (Salah, 2003). Besides, circumstances that lead to spread of pathogens are unpredictable and can result in sudden onset of disease in an obviously healthy population (Ikpi, and Offem, 2011). This study is therefore an investigation of the pathogenic and zoonotic potential of the bacterial flora of farmed catfish.

Materials and Methods
Three commercial farms and ten ponds located in Ibarapa Central Local Government Area of Oyo State were sampled randomly. Five fish and water samples were randomly collected from ten different ponds in each of the 3 farms. The fish were aseptically dissected and the following tissues were collected; blood, liver, kidney, gut, skin. These tissues and water samples were taken to the laboratory for bacteriological analysis. The samples collected were inoculated into nutrient broth at 37^{0}C for 18 hours, after which they were streaked on sterile blood agar, nutrient agar and tryptone soy agar and incubated at 37^{0}C for 24 hours. Organisms were identified based on colony and cellular features as well as biochemically characterization, according to Bergy's manual of Systematic Bacteriology.

Results

The organisms isolated in this study are represented in table 1. *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus spp., Vibrio cholerae, Escherichia coli, Citrobacter freundii, Proteus mirabilis, Aeromonas hydrophila, Vibrio vulnificus, Pseudomonas aeruginosa, Aeromonas schubertii* and *Edwardsiella tarda*; with prevalence of 26.25%, 1.25%, 8.75%, 2.50%, 11.25%, 15.00%, 7.50%, 2.50%, 16.25%, 3.75%, 2.50%, 1.25%, and 1.25% as shown in the table. *S. aureus* and *E. tarda* has the highest and lowest prevalence respectively. Pond water isolates were *Klebsiella pneumoniae, V. cholerae, V. vulnificus, A. schubertii, A. hydrophila and Aeromonas spp.* with prevalence of 9.2%, 9.2%, 45.4%, 9.2%, 18.2% and 9.2% respectively.

Table 1: Prevalence of bacterial organisms observed in fish tissue and pond water samples

Isolated organisms from tissues	Prevalence (%)	Isolated organisms from ponds	Prevalence (%)
Staphylococcus aureus	26.25	*Klebsiella pneumoniae*	9.2
Streptococcus pneumoniae	1.25	*Vibrio cholerae*	9.2
Streptococcus pyogenes	8.75	*Vibrio vulnificus*	42.4
Streptococcus spp.	2.50	*Aeromonas schubertii*	9.2
Vibrio cholerae	11.25	*Aeromonas hydrophila.*	18.2
Escherichia coli	15.00	*Aeromonas spp.*	9.2
Citrobacter freundii	7.50		
Proteus mirabilis	2.50		
Aeromonas hydrophila	16.25		
Vibrio vulnificus	3.75		
Pseudomonas aeruginosa	2.50		
Aeromonas schubertii	1.25		
Edwardsiella tarda	1.25		

Discussion

The bacterial isolates in this study is close to those reported by Efuntoye *et al.*, 2012, who isolated *Edwardsiella, Escherichia, Proteus, Pseudomonas,* and *Staphylococcus* from 3 farms. Another study by Danba *et al.*, 2014 reported isolating *E. coli, S. aureus and P. aeruginosa* in some fish farms in Kano State. These isolated organisms (*C. freundii, A. hydrophila, A. schubertii, V. vulnificus, P. aeruginosa, K. pneumoniae* and *E. tarda*) are pathogenic to fresh water fishes, while almost all the species isolated are zoonotic in nature i.e. *S. aureus, S. pneumoniae, S. pyogenes, V. cholerae, E. coli, C. freundii, V. vulnificus, P. aeruginosa, A. hydrophila* and *E. tarda*. Pathogenic bacteria of fish mentioned above have been reported among important fish pathogens by El-Refaey, 2013. *A. hydrophila* causes dermal ulceration, haemorrhagic septicaemia, red sore, red rot and scale protrusion diseases (Alain, 2009). *A. schubertii* causes multiple, white nodules (0.8–2.2 mm) in the liver, spleen and kidney of diseased *O. niloticus* and snake head fish with mortality (Liu *et al.*, 2018; Liu and Li, 2012). In 2003, Jeremić *et al.*, reported *C. freundii* infection in rainbow trout characterized by gastroenteritis and high mortality. Isolate of *C. freundii* from infected *O. niloticus* by El-Barbary and Hal (2017) was challenged on *O. niloticus*, fin erosions, body haemorrhage, skin ulcer, congestion on the liver, spleen, kidneys and gall bladder, fatty gut, 60% mortality and alteration in liver and kidney histology were observed. Lesions caused by Pseudomonads are septicaemia, skin ulceration, tail and fin haemorrhage etc. Species involved are Nile tilapia and African catfish, silver carp and grey mullet (Amrevuawho *et al.*, 2014). *E. tarda* infection in fish causes severe losses in fish farms of many countries (Kebede and Habtamu, 2016). *E. tarda* recovered from pond water was observed to cause severe mortality in experimentally infected *C. gariepinus* (Oladosu *et al.*, 2016).

Human infection has been linked to fish and its environs; Seasons, contact with fish and related environment, dietary habits and immune status of the exposed individual may be responsible. These pathogenic organisms for both fish and human beings have been isolated from fish without symptoms of disease. The following bacteria i.e. *Streptococcus iniae, Photobacterium damselae, Vibrio alginolyticus, V. vulnificus, V. parahaemolyticus, V. cholerae, E. coli, Aeromonas spp., Salmonella spp., Staphylococcus aureus,* and *E. tarda* isolated from fish and aquatic environs has been associated with human infections and intoxications (Novotny *et al.*, 2004); of which some were observed in this study.

A. hydrophila causes human enteritis, septicaemia, eye infection in immuno-compromised patients and pneumonia in a 5-year old child (Novotny *et al.*, 2004).

Vibrio cholerae, an inhabitant of aquatic ecosystems, colonizes human host and cause gastrointestinal infections which manifest as profuse watery diarrhoea called cholera. Other vibrios of human health concern are *V. vulnificus*, *V. paraheamoluticus* and occasionally *V. alginolyticus*. (Sakib *et al*., 2018). *V. vulnificus* infection may manifest as necrotizing fasciitis, septicaemia and gastroenteritis (Tao *et al*., 2012). *Citrobacter freundii* is a food-borne and hospital-acquired pathogen, related infections are diarrhoea, urinary tract infections, peritonitis, bacteraemia, pneumonia, neonatal meningitis and brain abscesses (Sabrina *et al*., 2017). Human infections by *E. tarda* manifest as mild gastroenteritis, typhoid-like illness, colitis and extra intestinal infections. (Jnaneshwara *et al*., 2016). *Staphylococcus aureus* causes enteric infections to humans; poor sanitation, disposal of human and animal wastes are source of entry to the aquatic biosphere (Tulay *et al*., 2014).

Conclusions and Recommendations

This study revealed the importance of bacterial isolates observed in the economic viability of commercially important fishes and their zoonotic potential. One of the sources of bacterial infection/contamination in fish is the aquatic biosphere, through feed materials, tools, personnel, run-off water and pests. In summary, strict biosecurity measure should be observed. Majority of these potentially zoonotic organisms are life threatening, consumers desist from consuming raw/ lightly cooked aquatic products as is the custom some parts of the world.

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