DOI: 10.53704/fujnas.v11i1.400

A publication of College of Natural and Applied Sciences, Fountain University, Osogbo, Nigeria.
Journal homepage: www.fountainjournals.com
ISSN: 2354-337X(Online),2350-1863(Print)

Antibiotic and Gene Profiles of Enterobacteriaceae from Selected Fish Ponds in Southwest, Nigeria

Joshua S. A., *Cajethan O. E., Toyosi F. O., Lawrence S. O.

Department of Microbiology, Babcock University, Ilishan, Ogun State, Nigeria.

Abstract

Fish is a significant part of human diet globally including Nigeria because of its economic and nutritional value. However, fish-associated infections caused by Gram negative bacteria of Enterobacteriaceae family have become a serious challenge with significant mortality in both aquaculture and healthcare institutions. With the increasing burden of antibiotics resistance in in this group of bacteria globally, the investigation of its resistance determinants and antibiotic profile was undertaken. A total of 64 fish pond water samples were collected from Lagos (16), Ogun (16), Oyo (16) and Osun (16). Enterobacteria were isolated by standard microbiological procedures and identified by 16S rRNA partial sequencing, after which antibiotic susceptibility test was performed using the Kirby-Bauer disc diffusion method. Resistance determinants were assayed by multiplex polymerase chain reaction. The family Enterobacteriaceae obtained were *Enterobacter kobei* (7), *Enterobacter mori* (9), *Enterobacter asburiae* (2), *Enterobacter tabaci* (2), *Enterobacter* sp. (27), and *Plesiomonas shigelloides* (5). Antibiotic susceptibility patterns revealed that all the (50) 100% isolates were resistant to ceftazidime, cefuroxime, cefixime and amoxicillin/clavulanic acid, while 28 (56%), 27 (54%) 17 (34%) and 15 (30%) were resistant to ciprofloxacin, nitrofurantoin, gentamicin and ofloxacin respectively. The resistance determinants; SHV (14), the integrons class 1 (14), QNRA (10), QNRS (9), TETB (9), QNRB (8) and TETA (7) were detected. The results revealed multi-drug resistance among the isolates. This poses a serious health challenge to the consumers of fish. Therefore, proper monitoring of pond management system is important to prevent infections caused by these opportunistic pathogens.

Keywords: Aquaculture, Antibiotic resistance, Enterobacteriaceae, Fish, Infection, Resistance determinant

Introduction

Fish is high in protein and minerals, and it plays an important role in human nutrition (Obire & Ariyo, 2015). The growing popularity of fish and fishery products in Nigeria has led to the construction of several fish ponds, with positive impact upon fish farming (Obire & Ariyo, 2015). With a global per capita consumption of fish of 19.2 kg, fish supply has increased by 3.2 percent. In Nigeria, aquaculture provides over 70% of the fish supply, particularly in Oyo State, where aquaculture

*Corresponding author:
Email address: onyezecajeth@yahoo.com

practices are prevalent. The most cultivated species of fish in Nigeria is *Clarias gariepinus*, which has a great economic importance (Adeshina et al., 2016). Fishing ponds range in size from modest to large-scale commercial systems (Sapkota et al., 2008). Fish pond aquaculture is becoming increasingly popular around the world, as well as in countries such as Nigeria (FAO, 2005). Furthermore, poor bacteriological quality of fish pond water has an important role in disease transmission in farmed fish, particularly Enterobacteriaceae (Arifin, Ni'Mahtuzzahro, & Suhariningsih 2013). The Enterobacteriaceae family is a non-spore-forming bacteria that includes a wide range of Gram-negative bacteria found in the gastrointestinal tracts in animals and humans (Rawash, Saad, Hassanin, Hassan, & Afifi, 2019). Enterobacteriaceae are opportunistic pathogens that cause common water-borne bacterial illnesses in fishes (Newaj, Mutani, Ramsubhag & Adesiyun, 2008). Kousar et al. (2020) reported fish ailments including saprolegniasis, lernaeasis, bacterial hemorrhagic septicemia, and anoxia. The prevalence of Enterobacteriaceae in fish and fish ponds has been linked to water-borne infections in humans, including typhoid fever, cholera, food poisoning, gastroenteritis, dysentery, and salmonellosis (Fakorede, Fatokun, Philip-Kantiok, Iwu, & Jaja, 2020; Kousar et al., 2020).

Some bacteria from the Enterobacteriaceae family, such as *E. coli, Enterobacter* sp*., Salmonella* sp*., Citrobacter freundii, Proteus* sp*.,* and *Klebsiella* sp., have been found to be the most often isolated pathogens from fish and its environments (Adewoye & Lateef, 2004a; Adewoye & Lateef, 2004b; Oliveira, Oliveira & Pelli, 2017; Rawash et al., 2019).

When fishes get affected by environmental stress or damage, they develop significant disease epidemics and die. The initiation as well as degree of Enterobacteriaceae infection in fish are influenced by environmental factors (Zheng et al., 2004; Sekar et al., 2008). Overpopulation, biological pollution, hypoxia, stress, greater temperature, and low dissolve oxygen are all factors that contribute to disease outbreaks in fish farms (Njoku et al., 2015). According to Umeh et al. (2020), a fish pond with acceptable water condition will produce healthy fish, but a fish pond having poor water quality will result in fish death and the breakout of disease-causing Enterobacteriaceae (Rawash et al. (2019). Enterobacteriaceae members like *Salmonella serovars* or *E. coli* could be implicated for even more than 15% of yearly food poisoning cases, making it a cause for concern. The World Bank estimates that the loss from the fishery sector due to fish infections is US$ 3 million per year (Adeshina et al., 2016).

To reduce the impact of these infections, fish farmers have begun treating affected fish with antibiotic therapy. Antibiotic overuse, on the other hand, has led in a rapid rise in antibiotic resistance among microorganisms, posing a hazard not just to fish but also to humans (Meshref et al., 2021). Antibiotic resistance in bacteria belonging to the Enterobacteriaceae family has become a global threat, with 4.95 million deaths linked to antibiotic resistance in 2019, with Western Sub-Saharan Africa having the highest rate of 27.3 deaths per 100,000 and Australia having the lowest rate of 6.5 deaths per 100,000 (Murray et al., 2022).

Raising African catfish (*Clarias gariepinus*) in the South-West of Nigeria contributes to the provision of nutritious food (Ugwuba & Chukwuji, 2010). Nevertheless, bacterial infection in fishes may endanger its production, resulting in lower nutritional value, higher mortality rates, disease transfer to customers, and a decline in the fish's economic value. It is critical to examine the prevalence and resistance pattern of bacteria in order to monitor antibiotic use and decrease disease transmission to humans. Hence, the aim of this study was to determine the prevalence of Enterobacteriaceae in water samples collected from various fish ponds in South-West, Nigeria, as well as to identify the specific genes associated with the bacterial resistance.

Materials and Methods

Sources and Isolation Procedures

A total of 64 pond water samples were collected from selected areas in South-West of Nigeria: Lagos state {(3), Ijede (2), Ketu (3), Ibeshe (3), Berger (2) and Maya (3)}, Ogun state { Ota (4), Atan Ota (4), Alagbole (4) and Ilishan (4)}, Osun

state { Oshogbo (3), Ilesa west (2), Iree (2), Odo otin (1), Ila (1), Iwo (2), Kuta (1), Ede north (3) and Ejigbo (1)} and Oyo state { Otun agba akin (1), Ilora (1), Iwo road (2), Ido (1), Challenge (1), Gate (1), Idigba (1), Awotan (1), Ikereku (1), Kosobo (1), Apata (1), Akinmorin (1), Oluyole (1), and Moniya (2)}. Exactly 10 ml each of the samples was collected in a sterile universal bottle and transported to the Microbiology Laboratory, Babcock University, Ilishan-Remo, Nigeria for further analysis. Serial dilutions of all the samples were made and inoculated. These were incubated and observed for growth (Niyi-David, Ogbonna, Akani & Douglas, 2020). Representative colonies were sub-cultured to obtain pure cultures for further characterization. Discrete colonies were molecularly characterized to ascertain the identity of organisms.

Molecular characterization of the isolates

Ten (10) isolates were selected for species barcoding. The genomic DNA extracted from the bacteria was isolated using the methodology outlined in the Quick-DNATM miniprep plus kit (Zymo research, Biolab, USA). Prior to PCR amplification, the quality of the DNA was checked using a 1.5 percent agarose gel electrophoresis. On a commercial basis, the amplicons were purified as well as sequenced using the Sanger sequencing technique by Inqaba Biotech in South Africa. Standard techniques were used to perform unidirectional sequence reads, and thus the contigs were assembled using the BioEdit (version 7.2.5.0) sequence tool (Ezeamagu, Harry, Ama & Barns, 2021). Molecular evolutionary genetics analysis 11.0 MEGA11 was used to determine the evolutionary relationship.

Antibiotic Susceptibility Test

Bacterial isolates were tested for their susceptibility to 8 antibiotics and performed using a Kirby-Bauer disc diffusion assay. The antibiotic discs used were ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), cefixime (5 µg), ofloxacin (5 µg), amoxicillin/clavulanic acid (30 µg), nitrofurantoin (300 µg), and ciprofloxacin (5 µg). A 24-hpure culture of the isolates was inoculated into Mueller -Hinton agar using sterile swab sticks; the antibiotic discs were placed aseptically on the plates with sterile forceps, and incubated at 37 ^{o}C for 24 h. After incubation, the diameter of the zones of inhibition were measured using a ruler. The results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020) to determine susceptibility or resistance to the antibiotics.

Resistance Gene Detection

Multiplex PCR was used to detect integron class 1, Sulfhydryl variable gene, Quinolone genes (QNRA, QNRB, and QNRS), Tetracycline genes (TETA and TETB) and beta-lactamase (SHV). Primer mixtures (25 µl) contain 12.5 µl solution of master mix, 0.5 µl of each primer used, 3.0 µl of DNA template and 8.0 µl of sterilized distilled water to bring the volume reaction to 25 µl. Amplification were carried out in a thermal cycler (GeneAmp PCR System 2700) with a programmable period of 30 cycles and amplicons were verified by gel electrophoresis.

Statistical Analysis

All data were analyzed by descriptive statistics using IBM Statistical product and service solutions (SPSS) version 20 (Adeshina et al., 2016).

Ethical Consideration

The study was conducted after ethical approval was obtained from Babcock University Health Research Ethics Committee (BUHREC: 032/22).

Results

Species distribution

A total of 50 Enterobacteriaceae were obtained (Table 1) consisting of *Enterobacter asburiae* 1 (2%), *Enterobacter kobei* 7 (14%), *Enterobacter mori* 9 (18%), *Enterobacter sp.* 27 (54%), *Enterobacter tabaci* 1 (2%) and *Plesiomonas shigelloides* 5 (10%). The isolates were deposited in GenBank and have the accession numbers ranging from ON359949 to ON359958.

Antibiotic Susceptibility

All the isolates were resistant to ceftazidime, cefuroxime, cefrixime and amoxicillin/clavulanic acid, while 34% were resistant to gentamicin, 30% were resistant to ofloxacin, 54% were resistant to nitrofurantoin and 56% were resistant to ciprofloxacin.

Table 1: Distribution of Enterobacteriaceae isolated from fish ponds across the four states.

Bacteria isolated	Sample location				
	Lagos state	Ogun state	Osun state	Oyo state	Prevalence percentage
Enterobacter kobei	Nil	Nil	1	6	14%
Enterobacter mori	2	3	4	Nil	18%
Enterobacter asburiae	Nil	Nil	Nil	1	2%
Enterobacter tabaci	Nil	Nil	Nil	1	2%
Enterobacter sp.	6	3	10	8	54%
Plesiomonas shigelloides	Nil	Nil	Nil	5	10%
Total	**8**	**6**	**15**	**21**	**100%**
	(16%)	**(12%)**	**(30%)**	**(42%)**	

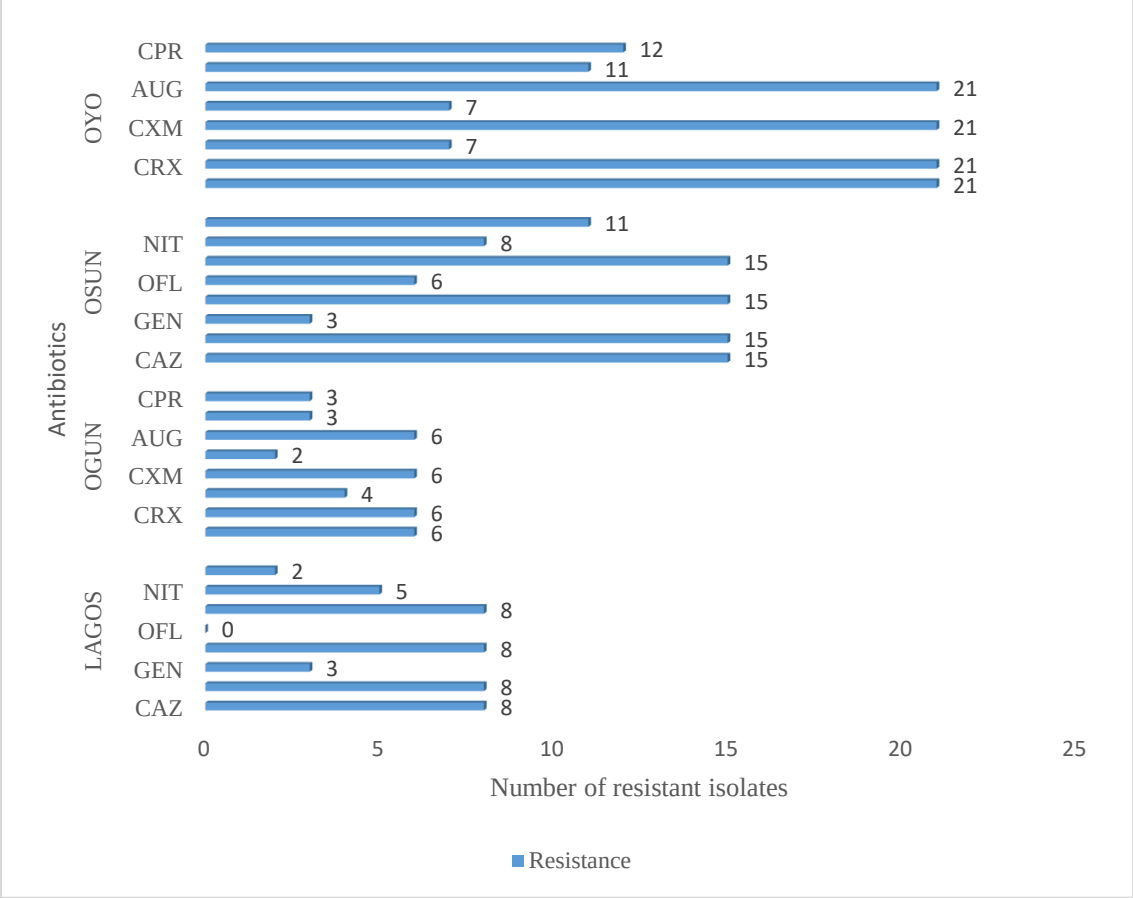

Figure 1: Resistance Pattern of Isolates According to Each State

Key: CAZ-Ceftazidime, CRX-Cefuroxime, GEN-Gentamicin, CXM-Cefixime, OFL-Ofloxacin, AUG-Amoxicillin/clavulanic acid, NIT-Nitrofurantoin, and CPR-Ciprofloxacin **Gene Detection**

The result as presented in Figure 2 shows the resistance determinants in Enterobacteriaceae that were isolated. The resistance determinants were SHV (14), the integrons class 1 (14), QNRA (10), QNRS (9), TETB (9), QNRB (8) and TETA (7).

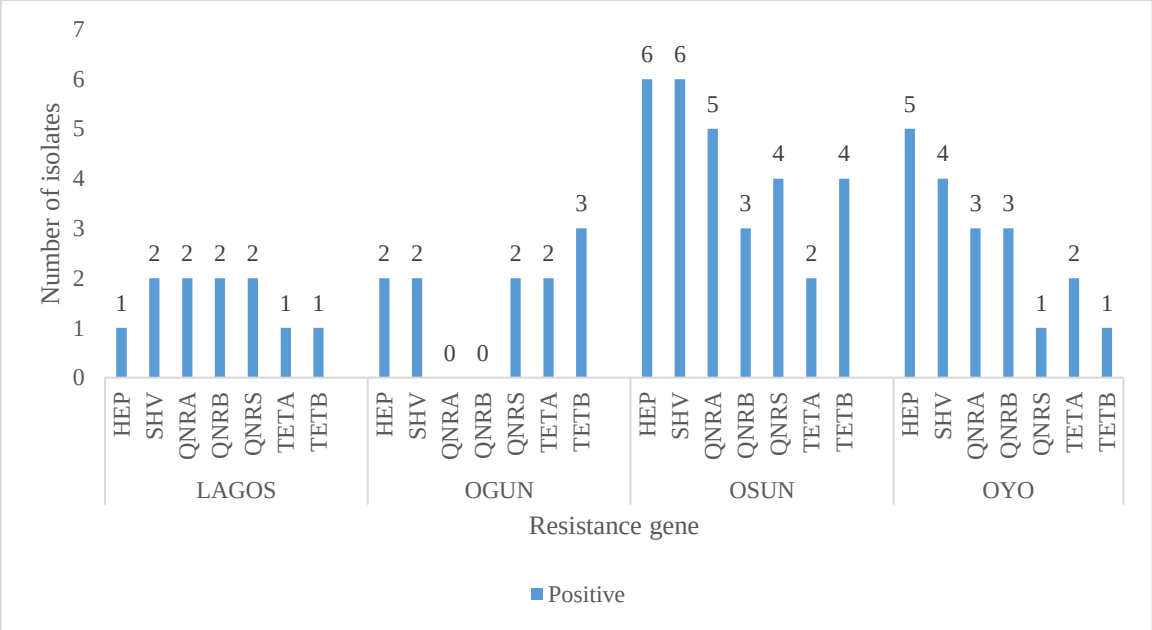

Figure 2: Resistance Gene Detection According to Each State

Key: HEP - Integron class 1, SHV – Sulfhydryl reagent variable gene, QNRA – Quinolone resistance gene A, QNRB Quinolone resistance gene B, QNRS – Quinolone resistance gene S, TETA – Tetracycline resistance gene A, TETB – Tetracycline resistance gene B.

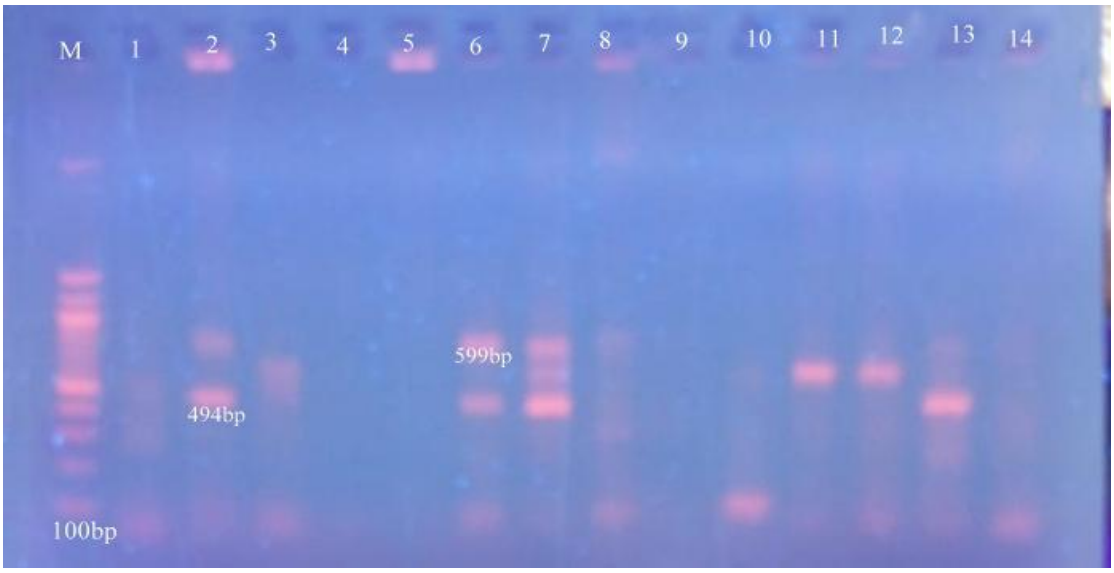

Figure 3 Electrophoresis pattern showing the QNRS and TETA bands.

Lane M is a 100bp ladder as a molecular size standard. Lane 2 and 6 represent positive isolates of QNRS at 599bp and TETA at 494.

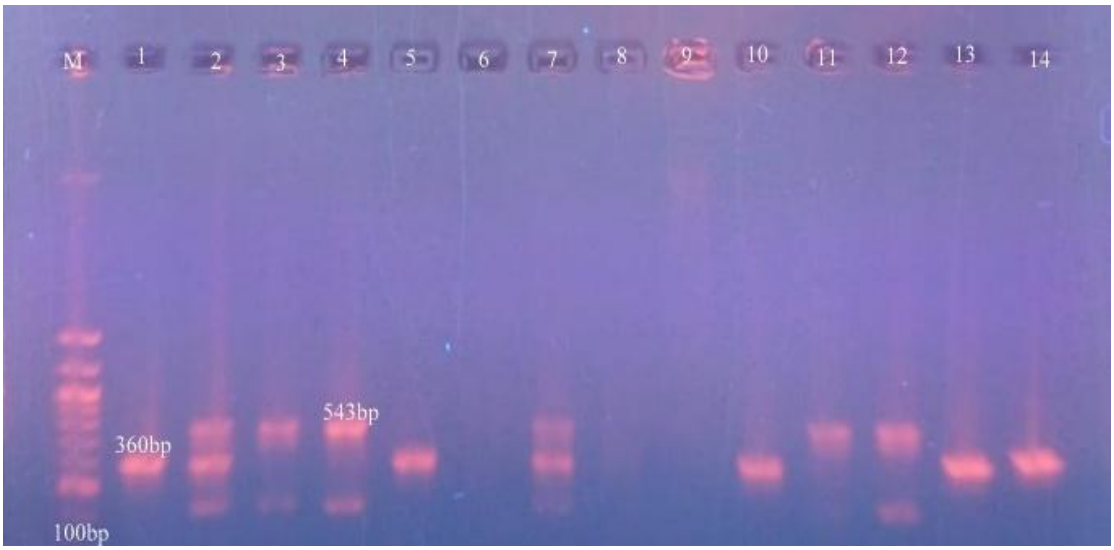

Figure 5 Electrophoresis pattern showing the QNRB and QNRA bands.

Lane M is a 100bp ladder as a molecular size standard. Lane 1 and 4 represent positive isolates of QNRB at 360bp and QNRA at 543.

Discussions

Several gram-negative bacteria belonging to Enterobacteriaceae family have been discovered from fish ponds in recent years, and have been linked to diseases in both fish and people (Behera, et al., 2018). *Enterobacter* is highly prevalent in this study with 54% and the predominance was similar to earlier studies on enteric bacteria associated with farmed fish and fish environment (Surendraraj et al., 2009; Elsherief et al., 2014). *Enterobacter* causes diseases such as gastroenteritis, cholera and food poisoning, nosocomial infection and urinary tract infection. They have been isolated from urine, blood, stool, wounds, skin, gallbladder and skin (Hoffman et al., 2005; Zhu et al., 2011; Duan et al., 2015; Zhu et al., 2017; Hartl et al., 2018; Fakorede et al., 2020). *Enterobacter* isolated from the fish ponds in this study could be as a result of human and animal activities in the environment where the ponds are located (Fakorede et al., 2020). *Plesiomonas shigelloides* which is also a member of Enterobacteriaceae in the ponds is known to cause diseases such as septicemia, pneumonia, endocarditis and urinary tract infections (Behera, et al., 2018; Cortes-Sanches, Espinosa-Chaurand, Diaz-Ramirez & Torres-Ochoa, 2021). Although not predominant in this study with 10%, it agrees with the findings of Surendraraj et al. (2009) that reported 4.6%. The variance could be as a result of sample size.

The results showed that all the isolates in this study were resistant to ceftazidime, cefuroxime, cefixime and amoxicillin/clavulanic acid. This report is similar to that of Fakorede et al. (2020) that reported 100% resistance to ceftazidime, cefuroxime and amoxicillin/clavulanic acid. The results also corresponded with the findings of Ng et al. (2018) in Singapore where 97% of the isolates were resistant to ceftazidime. The least resistance was reported in ofloxacin and this is similar to report of Singh et al. (2020) that presented lowest resistance of Enterobacteriaceae isolates to ofloxacin. The possible reason behind the development of higher level of resistance to the antibiotics could be as a result of misuse of antibiotics due to easy availability and affordability (Shakya et al., 2013). Highest level of resistance was observed in the isolates obtained from Oyo state.

In this study, the presence of Quinolone resistance gene (QNRA, QNRB and QNRS) and Tetracycline resistance gene (TETA and TETB) were consistent with the findings of Zhou et al. (2019) that reported QNRA, QNRB, QRNS and TETA as bacterial resistance genes isolated from fish ponds. The detection of tetracycline gene in this study was consistent with the work of Ezeamagu et al. (2020) that reported bacteria from pond water carrying TETA and TETB genes. The detection of SHV gene was in line with the findings of Niyi-David et al. (2020) which reported members of Enterobcteriaceae carrying the SHV gene.

In this study, PCR showed that 14 (28%) isolates possessed integron class 1. The integron gene was reported by Barns et al. (2021) that documented 40.8% of the isolates as carriers of integron class 1 gene. The presence of the integron class 1 gene is as a result of strong selective pressure while using antibiotics, and it is one of the major worries of health workers as well as practitioners due to their potentials to spread resistance rapidly. Thus, increasing pathogenicity and virulence in bacteria (Sabbagh et al., 2020). The variation in prevalence of this genetic element reported in this study could be as a result of sample size, sample location, antibiotic pressure as well as stains encountered.

Isolates from Osun State carried the highest number of resistance genes in this study while the lowest number of resistance genes carried by the isolates was recorded in Lagos State and Ogun State. As shown in Figure 2, QNRA and QNRB were not found in any of the isolates obtained from Ogun State. The differences in the prevalence of resistance gene and their distribution could be as a result of variation in sample location and/or antibiotic pressure. An increasing antibiotic pressure has been implicated in lingering problem associated with emergence and development of resistant genes in bacteria. The bacteria with resistant genes can easily transfer these genes to other bacteria via horizontal gene transfer.

Conclusion

The results in this study showed that the ponds were contaminated with high level of multidrug resistant Enterobacteriaceae which are potential pathogens. These potential pathogens could affect the health of fish and humans and also lead to economic loss. Therefore, proper monitoring of pond management system is important to prevent infections caused by these opportunistic pathogens and to ensure the safety of the fish and humans.

References

Adewoye, S. O. and Lateef, A. (2004). Evaluation of the Microbiological characteristics of Oyun river – A polluted river in North-Central, Nigeria. *Pollution research,*23(4), 587- 591.

Adewoye, S. O. and Lateef, A. (2004). Assessment of the Microbiological Quality of Clarias gariepinus Exposed to an Industrial Effluent in Nigeria *Environmentalist* 24, 249–254. https://doi.org/10.1007/s10669-005-1000-7

Adeshina, I., Abdurahman, S. A. and Yusuf, A. A. (2016). Occurrence of Klebsiella species in cultured African catfish in Oyo state, South-west of Nigeria. *Nig. Vet.J,* 37(1), 24-31.

Ampofo, J. A. and Clerk, G. C. (2010). Diversity of bacteria contaminants in tissues of fish cultured in organic wastefertilized ponds: health implications. *The Open Fish Science Journal,* 3, 142-146.

Barns, J. N., Ezeamagu, C. O., Nkemjike, M. E. and Akindele, T. S. (2021). Prevalence of Enterobacteriaceae obtained from clinical samples. *Journal of Microbiology and Antimicrobials,* 13(1), 1-10.

Behera, B. K., Bera, A. K., Paris, A., Das, A., Parida, P. K., Kumari, S., Bhowmick, S. and Das, B. K. (2018). Identification and pathogenicity of *Plesiomonas shigelloides* in Silver Carp. *Aquaculture,* 493, 314-318.

Behera, U. K., Panigrahi, P. and Sarangi, A. (2012). Multiple water use protocols in integrated farming system for enhancing productivity. *Water Resources Management,* 26, 2605-2623.

Centers for Disease Control and Prevention (CDC). (2013). Surveillance for foodborne disease outbreaks-United States, 2009-2010. *Morb. Mortal. Wkly. Rep*., 62(3), 41-47.

Clinical and Laboratory Standards Institute (CLSI). (2020). Performance standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, USA.

Cortes-Sanchez, A. D., Espinosa-Chaurand, L. D., Diaz-Ramirez, M. and Torres-Ochoa, E. (2021). *Plesiomonas:* A Review on Food Safety, Fish-Borne Diseases and Tilapia. *The Scientific World Journal*, 2021, 1-10

Duan, Y., Zhou, X., Zhang, Y, Qui, L., Nimaichand, S. and Li, W. (2015).
Enterobacter tabaci sp. Nov., a novel member of the genus Enterobacter isolared from a tobacco stem, Antonie Leeuwenhoek, 108(5), 1161-1169.

Elsherief, M. F., Mousa, M. M., El-Galil, H. A. and El-Bahy. E. F. (2014). Enterobacteriaceae associated with farm fish and retailed ones. *Alexandria journal of Veterinary science*, 42, 99-104.

Ezeamagu, C. O., Dada, O. G., Omohoro, M. U. and Mokoshe, W. N. (2020). Evaluation of resistance determinants in gram-negative bacteria obtained from fish ponds and animal-based wastes in South-West, Nigeria. *Journal of Life and Physical Sciences*, 12(2): 23-26.

Ezeamagu, C., Imanatue, I., Dosunmu, M., Odeseye, A., Baysah, G., Aina, D., Odutayo, F. and Mensah-Agyei, G. (2018). Detection of methicillin resistant and toxin-associated genes in *Staphylococcus aureus. Beni-Suef University Journal of Basic and Applied Sciences*, 7, 92-97.

Fakorede, C. N., Fatokun, E. N., Philip-Kantiok, B., Iwu, C. J. and Jaja, I. F. (2020). Bacteriological Quality and Antibiotics' Susceptibility Profile of Small-medium Scale Commercial Fish Farms in Nigeria. *The Open Agriculture Journal*, 14, 198-208.

FAO. (2005). FAO yearbook fishery statistics: Aquaculture production. Vol. 96/2. FAO, FIDSU, Rome, Italy, pp:195.

Hartl, R., Kerschner, H., Gattringer, R., Lepuschitz S., Allerberger, F., Sorschag, S., Ruppitsch, W. and Apfalter, P. (2018). Whole-genome analysis of a human Enterobacter mori isolate carrying a blalM1-2 carbapenemase in Austria. *Resist* https://doi.org/10.1089/mdr.2018.0098

Hoffman, H., Stindl, S. Ludwig, W. (2005). Three new subspecies of clinical importance. *J. Clin. Microbiolo*, 43, 3297-3303

Kousar, R., Shafi, N., Andleeb, S., Ali, N. M Akhtar, T., & Khalid, S. (2020). Assessment and incidence of fish associated bacterial pathogens at hatcheries of Azad Kashmir, Pakistan. *Brazilian Journal of Biology*, 80(3), 607-614.

Madhuri, S., Shrivastav, A. B., Sahni, Y. P. and Govind, P. (2012). Overviews of the treatment and control of common fish diseases. *International Research Journal of Pharmacy*, 3(7), 123-127.

Meshref, A. E., Eldesoukey, I. E., Alouffi, A. S., Alrashedi, S. A., Osman, S. A., & Ahmed, A. M. (2021). Molecular Analysis of antimicrobial Resistance among Enterobacteriasceae isolated from Diarrhoeic Calves in Egypt. *Animals*, 11, 2-11.

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinsky, L., Aguilar, G. R., Gray, A., Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*, 399(10325), 629-655. https://doil.org/10.1016/50140-6736(21)02724-0

Newaj, A., Mutani, A., Ramsubhag , A., Adesiyun, A. (2008). Prevalence of bacterial pathogens and their anti-microbial resistance in Tilapia and their pond water in Trinidad. *Zoonoses Public Health*, 55(4), 206-213.

Ng, C., Chen, H., Goh, S. G., Haller, L., Wu, Z., Charles, F. R., Trottet, A. and Gin, K. (2018). Microbial water quality and the detection of multidrug resistant *E.coli* and antibiotic resistant genes in aquaculture sites of Singapore. *Mar Pollut. Bull*. 135, 475-480.

Niyi-David, C. C., Ogbonna, D. N., Akani, N. P., & Douglas, S.I. (2020). Characterization of Isolates and Antibiotic Resistance Genes Associated with Fish Pond. *International Journal of Research and Innovation in Science*, 5(8)

Njoku, O. E., Agwa, O. K. and Ibiere, A. A. (2015). An investigation of the microbiological and physicochemical profile of some fish ponds water within Niger Delta of Nigeria. *African Journal of Food Science*, 9(3), 155–162.

Noor El-Deen, A. E., Atta, N. S., Abd El Aziz, M. A. (2010). Oral vaccination of Nile Tilapia (*Orechromis niloticus*) against motile Aeromonas septicemia. *Nat. Sci*, 8(2), 21-25.

Oliveira, R. V., Oliveira, M. C. and Pelli, A. (2017). Disease infection by Enterobacteriaceae family in fishes: A review. *J Microbiol Exp,* 4(5), 119-122.

Onyango, M. D., Wandili, S., Kakai, R. and Eliud, N. W. (2009). Isolation of Salmonella and Shigella from fish harvested from the Winam Gulf of Lake Victoria, Kenya. *J Infect Developing Countries,* 3(2), 99-104

Rawash, R., Saad, S. M., Hassanin, F. S., Hassan, M. A. and Afifi, M. A (2019). Incidence of Enterobacteriaceae in some freshwater fishes. *Benha Veterinary Medical Journal*; 37, 64-68.

Sabbagh, P., Rajabnia, M., Maali, A. and Ferdosi-Shahandashti. (2020). Integron and its role in antimicrobial resistance: A literature review on some bacterial pathogens. *Iranian Journal of Basic Medical Sciences,* 24(2), 136-142.

Sekar, V., Santiago, T., Vijayan, K., Alavandi, S., Raj, V., Rajan, J., Sanjuktha, M. and Kalaimani, N. (2008). Involvement of Enterobacter cloacae in the mortality of the fish, Mugil cephalus. *Lett. Appl. Microbiol.,* 46(6), 667-672.

Shakya, P., Barrett, P., Diwan, V., Marothi, Y., Shah, H. & Chhari, N. (2013).

Antibiotic resistance among Escherichia coli isolates from stool samples of children aged 3 to 14 years from Ujjain, India. *BMC infect. Dis*, 13, 1-6

Shender, L. A. and Spraker, T. R. (2009). Salmonellosis in a free-ranging population of javelinas (Pecari tajacu) in south central Arizona. *J. Wild Dis*., 45(4), 941-51.

Singh, A. K., Das, S., Kumar, S., Gajamer, V. R., Lepcha, Y. D., Tiwari, H. K. and Singh, S. (2020). Distribution of antibiotic-resistant enterobacteriaceae pathogens in portable spring water of Eastern Indian Himalayas: Emphasis on virulence gene in *Escherichia coli*. *Front. Microbiol*. 11, 1-10.

Surendraraj, A., Sabeena, F. K. H., Yathavamoorthi, R. and Thampuran, N. (2009). Enteric bacteria associated with farmed freshwater fish and its culture environment in Kerala, India. *Research journal of Microbiology*, 4(9), 334-344.

Ugwuba, C. O. A. and Chukwuji, C. O. (2010). The economics of catfish production in Anambra state Nigeria: A profit function Approach. *Journal of Agricultural Society of Science*, 6(4), 105-109.

Umeh, O. R., Chukwura, E. I. and Ibo, E. M. (2020). Physicochemical, Bacteriological and Parasitological Examination of selected fish pond water samples in Akwa and environment, Anambra state, Nigeria. *Journal of Advances in Microbiology*, 20(3), 27-48

Wamala, S. P., Mugimba, K. K., Mutoloki, S., Evensen, O., Mdegela, R., Byarugaba, D. K. and Sorum, H. (2018). Occurrence and antibiotic susceptibility of fish bacteria isolated from *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) in Uganda. *Fisheries and Aquatic Sciences,* 21, 6

Zheng, D., Mai, K., Liu, S., Limin, C., Liufu, Z., Xu, W., Tan, B. and Zhang, W. (2004). Effect of temperature and salinity on virulence of Edwardsiella tarda to Japanese flounder, Paralichthys olivaceus (Temminck et Schlegel). *Aqua. Res*., 35, 494–500.

Zhu, B., Lou, M. M., Wang, G. F., Zhou, Q, Wang F., Fang, Y., Su, T., Li, B. & Duan, Y. P. (2011). *Enterobacter mori* sp. nov., associated with bacteria wilt on Morus alba L. *Int J Syst Evol Microbiol*, 61, 2769-2774

Zhu, B., Wang, S, Li, O., Hussain, A., Shen, J. & Ibrahim, M. (2017). High-quality sequence of human pathogen *Enterobacter asburiae* type strain 1497-78T. *J Glob Antimicrob Resist*, 8:104-105

Zhou, Q., Wang, M. & Zhong, X. (2019). Dissemination of resistance genes in duck/fish polyculture ponds in Guangdong Province: correlations between Cu and Zn antibiotic resistance genes. *Environ Sci Pollut Res*, 26, 8182-8193.