Original Research Article

Distribution and Antibiotic Resistance profile of Extended Spectrum Beta-lactamase Producing *Escherichia coli*from fish farm within Abakaliki Metropolis

ABSTRACT

Background and objectives: The emergence of antibiotic resistant determinant in fish farms and its spread is on the increase and has evolved into strains that are resistant to many classes of antibiotics. It is important to determine the distribution and antibiotic resistance profile of Extended Spectrum Beta-lactamase (ESBL) producing *Escherichia coli* from fish farm within Abakaliki Metropolis.

Methodology: Aseptically, fifty (50) milliliters of fishpond water was collected from twenty locations and were analyze using standard microbiological culture and identification of *Escherichia coli.* Detection of phenotypic extended spectrum β-lactamases production was performed usingDouble-Disk Synergy Test (DDST). Antibiotic susceptibility studies of extended spectrum β-lactamases producing *Escherichia coli* was determined using the Kirby–Bauer disk diffusion method and the results were construed using the Clinical Laboratory Standard Institute (CLSI) zone diameter breakpoints.

Results: Extended spectrum beta-lactamase producing *Escherichia coli* distribution from fishpond water revealed overall occurrence rate of 34(11.3 %). The proportion of ESBL producing *Escherichia coli* was 5(25.0 %) from fish farm L followed by Farm A, Farm E, Farm G, which both accounted for 20.0 % respectively while the least occurrence of 1(5.0 %) was recorded against Farm I. ESBL-producing *E. coli* were resistant to cephalosporin particularly Ceftriaxone (88.2%), Ceftazidime (91.2 %), Cefotaxime (94.1) and Cefepime (85.3 %). This was followed by Amoxicillin-Clavulanate (91.2 %), Azetronam(97.1 %). In all, Ciprofloxacin (82.4 %), Imipenem (97.1 %) and ~~meropenem~~ Meropenem(100 %) were the most effective antibiotic against ESBL-producing *E. coli* isolate.

Conclusion: This study reveals the prevalence of the ESBL phenotype in fish farming. The increasing prevalence of resistance to routinely used antibiotics in medical and veterinary therapies among the study isolates from aquaculture products poses a significant challenge to the treatment of human and animal diseases. As a result, adequate antibiotic intervention is essential to ensure the continued efficacy of antibiotics for aquaculture and human health, as well as the industry's viability.

Keywords: *Escherichia coli*, Extended Spectrum Beta-lactamase, Antibiotic Resistance, fish farm

Introduction

Fish farm (aquaculture) is a rapidly growing field of food production since the demand for fish is increasing worldwide, including Nigeria being the largest fish consumers in Africa and among the largest fish consumers in the world [1] with about 3.2 million metric tons of fish consumed annually [1, 2]. It has been projected that Nigeria needs an average annual increase of 3.8% in fish production to keep up with the demands of an ever-increasing population [3].According to Gazal*et al*. [4], population growth, rising incomes and urbanization are factors that contribute to the increase in production. However, the possible emergence of bacterial diseases and the need to treat sick fish also increase [4]. Gram negative bacteria especially members of the E~~e~~nterobacteriaceace family are the

main pathogens that cause diseases in fish [5, 6,7, 8]. These strains are responsible for different infectious diseases, such as skin lesions, abscesses, bleeding, and sepsis; these pathogens increase morbidity and mortality in fish and cause significant economic loss [4]. Worldwide, there is a massive increase in fish farming, which is associated with intensive use of antibiotics to combat bacterial infections [9].

Many factors are known to favor the emergence of antibiotic resistant determinant in aquaculture and its spread to other sectors. This includes high stocking densities leading to elevated stress and infections in shrimp, widespread use of various chemicals (such as spawning aids, disinfectant and herbicide use in pond maintenance), nutrient rich environment in the ponds, occupational human exposure to Antimicrobial resistant bacteria, release of untreated water/waste to local environment [10]. Antibiotic-resistant bacteria, including human and zoonotic pathogens have been reported from various aquaculture settings [11, 12]. Among these, members of Enterobacteriaceae are of particular concern owing to their considerable ability to acquire resistance determinant to various antimicrobials and to disseminate widely. This in large part is due to the highly diverse and rapidly evolving group of beta-lactamase determinant such as extended-spectrum beta-lactamases (ESBLs). ESBLs, generally found in Enterobacteriaceae, are a class of enzymes conferring resistance to penicillins, first-, second- and third-generation cephalosporins, and aztreonam, and are usually inhibited by beta-lactamase inhibitors such as clavulanic acid [12, 13].

There is strong evidence that the other AR determinants and ESBL encoding genes are found on integron that has the ability to integrate, express and facilitate their transfer among bacteria of different genera and kingdoms [14, 15, 16].Although Nigeria is the world's largest consumers of farmed fish, no studies on the distribution of Eenterobacteriaceae with resistance determinant to critical antibiotics (extended-spectrum beta-lactam) in farmed fish environment exist to the best of our knowledge within our region. Identification of ESBL phenotype in the bacterial isolates could help to trace the prevalence among bacteria presence with fish farming environment.

Materials and Methods

Study Area

The study was carried out using fifteen (15) fish farms denoted by A, B, C, D, E, F, G, E, F, G, H, I, J, K, M, N and O in Abakaliki, Ebonyi State, Nigeria. Abakaliki town is the capital city of Ebonyi State. It is located in 6.32^{o}N latitude and 8.12^{o}E and longitude and is situated at an elevation of 117 meters above sea level. Abakaliki is populated and inhibited by indigenes and people from other parts of Nigeria. Ebonyi State shares border with Benue State to the north, Enugu State to the west, Imo and Abia to the south and Cross River to the east. The climate is characterized by a hot dry period which stretches from November-April, while the rainy season is from May - October. The maximum temperature during dry season is 37.6^{o}C while the minimum temperature is 27.1^{o}C [17]. The major occupations of people in Abakaliki are farming and trading, there are also civil servants and students and all these people engage in a busy activity of life.

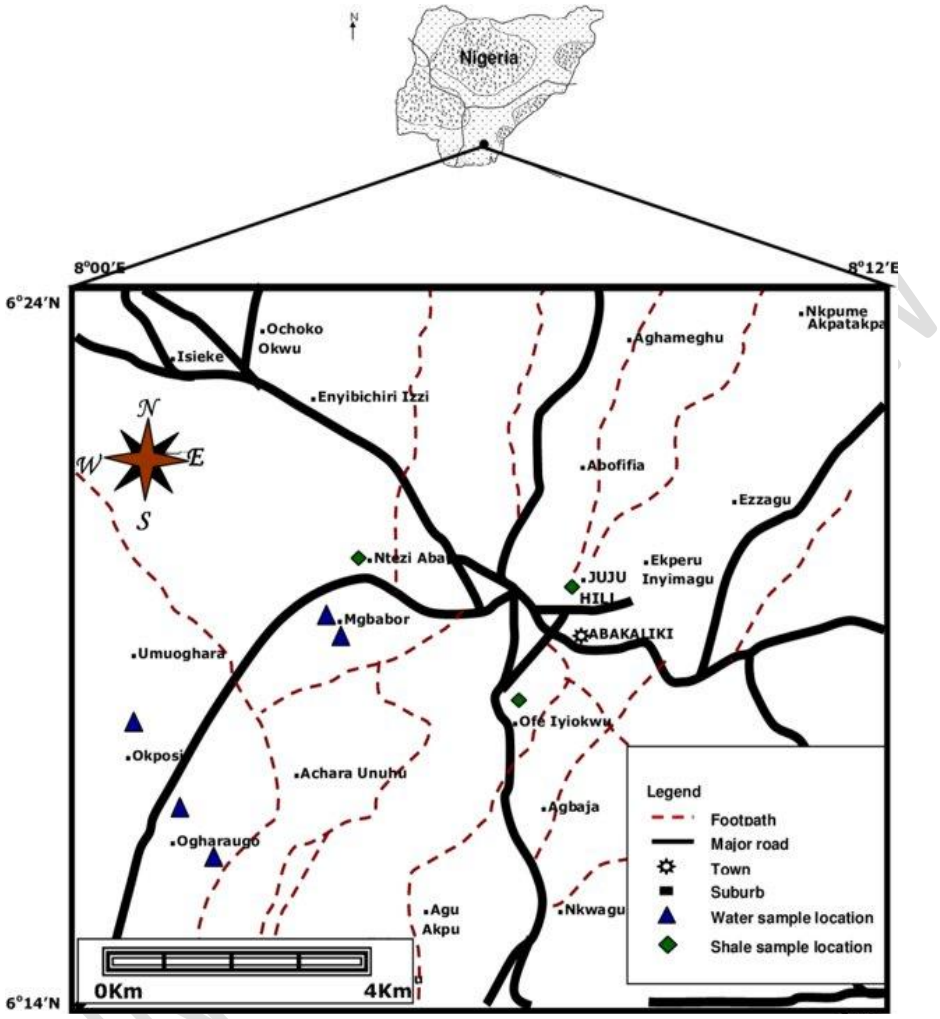

Figure 1: Map showing Abakaliki in Ebonyi State the study area [17].

Sample collection and processing

Aseptically, fifty (50) milliliters of fishpond water was collected from twenty locations and each pond was subjected to ten folds serial dilution. Exactly 0.5ml of dilution factor three for each randomly serially diluted water samples were spread plated on plate count agar and incubated at 37 °C for 24 hrs. After 24 hrs of incubation, colonies were counted using Colony counter (Techmel and Techmel, USA) and a loopful of each colony were aseptically streaked on solidified eosin methylene blue agar plate. The plates were incubated aerobically for 18-24 hours at 37 ^{o}C. Bacterial colonies with greenish-metallic sheen on eosin methylene blue agar plate for were infer as the presence of *Escherichia coli*. All discrete colonies were purified by plating onto nutrient agar (Hi-Media, India). The sub-cultured plates were incubated at 37 ^{o}C for 24 hrs. Discrete colonies were purified by plating onto nutrient agar (Hi-Media, India). Isolates was characterized based on their colonial morphology (color, consistency, texture), microscopic techniques (Gram staining and motility test) and biochemical characteristics, including oxidase, indole,

citrate utilization, triple sugar iron test, methyl red, Voges-Proskaeur test, coagulase test, catalase and carbohydrate fermentation tests such as mannitol, sucrose, glucose and lactose [18, 19].

Detection of extendedspectrum beta-lactamase producing *E. coli*

ESBL production was phenotypically confirmed in only the bacteria isolates that showed reduced susceptibility to the 3rd-generation cephalosporins (such as cefotaxime and ceftriaxone) using the double disk synergy test (DDST) technique. Standardized inoculum of the isolate (adjusted to 0.5 McFarland turbidity standards) were aseptically swabbed on MH agar plates; and amoxicillin/clavulanic acid disc (20/10 µg) was placed at the center of the plate while cefotaxime (30 µg) and ceftazidime (30 µg) discs each was placed at adjacently distance of 15 mm away from the amoxycillin-clavulanic acid disc. The plates were incubated at 37^{o}C for18 - 24 hrs; and ESBL production was phenotypically inferred by expansion of the zone of inhibition of either cephalosporin in the presence of amoxicillin-clavulanic acid than in its absence giving a dumbbell shape [20].

3.2.13 Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out using the Kirby-Bauer disc diffusion method as outlined in the current Clinical and Laboratory Standards Institute (CLSI) guidelines. In brief, overnight culture of the test bacterial suspension (1x10^{6} colony forming unit per milliliter (cfu/ml) was adjusted to 0.5 MacFarland turbidity standard and was spread over the entire surface of solidified Mueller-Hinton agar using a sterile cotton-tipped swab stick. This was allowed to stand for 15 mins to enable the inoculated organisms to pre-diffuse. The following antibiotics:-ceftazidime (30 µg), ceftriaxone (30 µg), cefotaxime(30 µg),-,cefepime (30µg), imipenem (10µg), amoxicillin-clavulanic acid (20/10µg), ciprofloxacin, (5µg), aztreonam (10µg), gentamicin (10µg), imipenem (10µg), meropenem (10µg), ofloxacin (5µg), tetracycline (30), trimethoprim-sulfamethoxazole (25) was aseptically placed onto the surface of solidified Mueller-Hinton agar plates with a sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37°C for 18-24 hrs and zones of inhibition after 24 hrs of incubation was taken. The inhibition zone diameters (IZD) around each antibiotic disk were measured using a calibrated transparent ruler and recorded in millimeters. A standardized table was used to determine if each bacterium was 'resistant', 'intermediate,' or 'sensitive.' For analysis, isolates with intermediate or resistant results was merged as resistant [21, 22].

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RESULT AND DISCUSSION

***E. coli* distribution from fishpond water**

E. coli distribution from fishpond water revealed overall occurrence rate of 97(32.3 %) consisting of high occurrence rate of 9(45.0 %) in both fish farm H and L followed by Farm A, Farm D, Farm E, Farm N, Farm M, which both accounted for 40.0 % respectively while the least occurrence of 20.0 % was recorded against Farm B and Farm G respectively as presented in Table 1.

Distribution of extended spectrum beta-lactamase producing *Escherichia coli* from different fishpond water from fishpond within Abakaliki Metropolis

Extended spectrum beta-lactamase producing *Escherichia coli* distribution from fishpond water revealed overall occurrence rate of 34(11.3 %) consisting of high occurrence rate of 5(25.0 %) from fish farm L followed by Farm A, Farm E, Farm G, which both accounted for 20.0 % respectively while the least occurrence of 1(5.0 %) was recorded against Farm I. Non- Extended spectrum beta-lactamase producing *Escherichia coli* accounted for 63(21.0 %) as presented in Table 2.

The ESBL-producing *E. coli* from fish farm showed varying percentage rate of resistance and susceptibility to the test antibiotic. From the chart section (Figure 1), a high rate of resistance by ESBL-producing *E. coli*was shown against cephalosporin particularly Ceftriaxone (88.2%), Ceftazidime (91.2 %), Cefotaxime (94.1 %) and Cefepime (85.3 %). This was followed by Amoxicillin-Clavulanate (91.2 %), Azetronam(97.1 %). In all, Ciprofloxacin (82.4 %), Imipenem (97.1 %) and meropenem (100 %) were the most effective antibiotic against ESBL-producing *E. coli* isolate in this study (Figure 2).

Table 1: Distribution *E. coli*from fishpond water

Aquaculture	No. Sampled	*E. coli* (%)
Farm A	20	8(40.0)
Farm B	20	4(20.0)
Farm C	20	6(30.0)
Farm D	20	8(40.0)
Farm E	20	8(40.0)
Farm F	20	5(25.0)
Farm G	20	4(20.0)
Farm H	20	9(45.0)
Farm I	20	7(35.0)
Farm J	20	7(35.0)
Farm K	20	4(20.0)
Farm L	20	9(45.0)
Farm M	20	5(25.0)
Farm N	20	8(40.0)
Farm O	20	5(25.0)
Total	**300**	**97(32.3)**

Table 2: Distribution of extended spectrum beta-lactamase producing *Escherichia coli* from different fish water from fishpond within Abakaliki Metropolis

Aquaculture	No. Sampled	*E. coli* (%)	ESBL (%)	Non-ESBL (%)
Farm A	20	8(40.0)	4(20.0)	4(20.0)
Farm B	20	4(20.0)	3(15.0)	1(5.0)
Farm C	20	6(30.0)	2(10.0)	4(20.0)
Farm D	20	8(40.0)	2(10.0)	6(30.0)
Farm E	20	8(40.0)	4(20.0)	4(20.0)
Farm F	20	5(25.0)	3(15.0)	2(10.0)
Farm G	20	4(20.0)	4(20.0)	0(0.0)
Farm H	20	9(45.0)	2(10.0)	7(35.0)
Farm I	20	7(35.0)	1(5.0)	6(30.0)
Farm J	20	7(35.0)	0(0.0)	7(35.0)

Farm K	20	4(20.0)	0(0.0)	4(20.0)
Farm L	20	9(45.0)	5(25.0)	4(20.0)
Farm M	20	5(25.0)	1(5.0)	4(20.0)
Farm N	20	8(40.0)	3(15.0)	5(25.0)
Farm O	20	5(25.0)	0(0.0)	5(25.0)
Total	**300**	**97(32.3)**	**34(11.3)**	**63(21.0)**

Key: ESBL Extended Spectrum Beta-lactamase

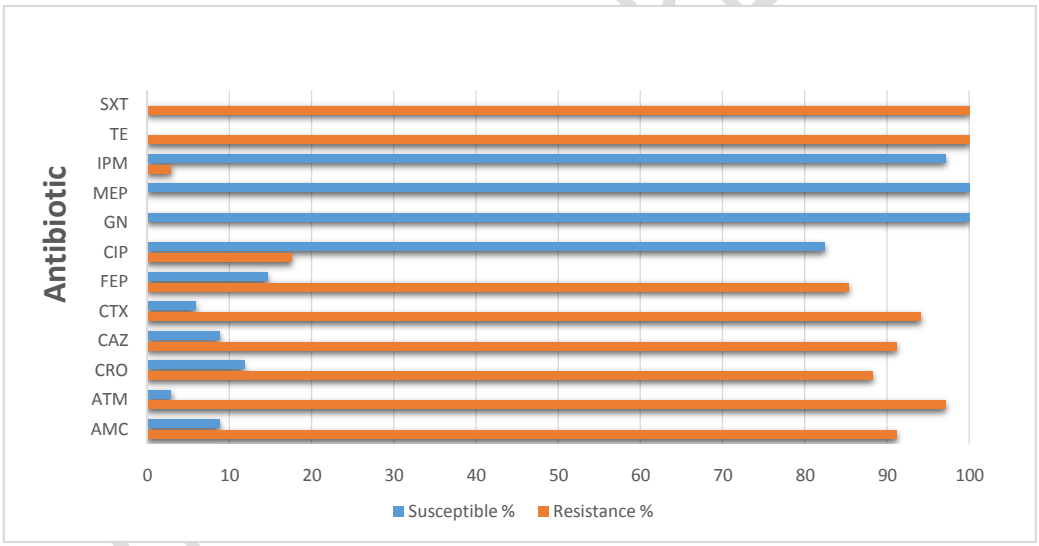

Figure 2: Chart showing Antibiogram of ESBL-producing *E. coli*

Key: Amoxicillin-Clavulanate =AMC, Azetronam = ATM, Ceftriaxone = CRO, Ceftazidime = CAZ, Cefotaxime = CTX, Cefepime = FEP, Ciprofloxacin = CIP, Gentamicin = CN, Meropenem = MEP, Imipenem = IPM, Tetracycline = TE, Trimethoprim-Sulfamethoxazole= SXT

DISCUSSION

E. coli distribution from fishpond water revealed overall occurrence rate of 97(32.3 %). The major findings of this study reiterate with report in Northern Ethiopia [23], Nigeria [24], Bangladesh [25], Northwest of Borneo [26], Malaysia [27] were these bacteria was isolated in fishpond. *Escherichia coli* has been traditionally recognized as an indicator organism of fecal contamination of water and fish [23, 28]. The source for the occurrence of the organism might have come from the point of distribution, poor water treatment, flies (*Musca domestica*) that wander around the pond and poor handling practice by farm keepers and visitor operating in the study sites through the introduction of contaminated materials.

Prevalence of ESBL strain accounted for 34(11.3 %) among samples from fishpond water. Although there is paucity of information on phenotypic ESBL occurrence in aquaculture but few studies have reported numeric presence of ESBL-producing *E. coli* in fish guts [25, 29] and fish pond [30]. Unfortunately, as with other livestock, antimicrobials usage in the aquaculture industry is not monitored and, therefore, accurate data are not available. The occurrence of ESBL phenotype could stern from frequent incorporation of antimicrobial agent into the fish pellet as well as being used as a prophylactic agent in healthy fish which may facilitate prolonged selective pressure to bacteria in the aquatic environment. Actually, water ESBL strain could be indigenous to aquatic environments, or exogenous, transiently and occasionally present in the water as a result of shedding from animal, vegetal, or soil surfaces. The occurrence of the ESBL strains could also be explained by the possibility of the heavy metal use of these compounds in aquaculture, several of which are non-biodegradable, thus increasing antibiotic selective pressure in water, facilitating the transfer of antibiotic-resistant determinants between aquatic bacteria, including fish and human pathogens, and allowing the presence of residual antibiotics in commercialized fish and products.

The findings highlighted the high resistance level of *E. coli* isolated towards antibiotics categorized as a priority and critically important for human use and as veterinary critically important drugs for food-producing animals, indicating important risk to public and animal health. Indeed, tetracycline resistant accounted for 100 % in all isolate. Few studies has reported similar pattern of resistant from aquaculture; 26 ESBL-producing isolates from fish demonstrated 61.5% resistant to tetracycline [31]; Gufe*et al.* [32] reported 63%, Dewl*et al.* [27] reported tetracycline resistant in fish: 31.2% and water 53.3%, in Tanzania, Extended-Spectrum β-Lactamase-Producing *Escherichia coli* in the Aquatic Environment and Nile Perch (*Latesniloticus*) of Lake Victoria showed resistance to tetracycline 90.9% (10/11) [33] while tetracycline 100% susceptibility has been reported [26]. Clearly the findings of Lihan*et al.* [26] have shown that the force driving tetracycline resistant in aquaculture may differ between two settings. However, oxytetracycline are frequently incorporated into the fish pellet for Streptococcosistreatment in fishery as well as being used as a prophylactic agent in healthy fish [34]. Most of the isolate found were conferring resistance to tetracyclines, which could indicate the history of oxytetracycline use in the aquaculture. However, the presence of tetracycline resistance genes has been previously observed also in fish farm, pond sediment environments [33, 35, 36].

ESBL-producing *E. coli* from aquaculture 66.7-100 % resistant to beta-lactam antibiotic such as aztreonam, ceftriaxone, cefotaxime and cefepime has not change from report from existing literature; Sapugahawatte *et al.* [29] reported cefepime (35.6%), ceftriaxone (100%), and cefotaxime (100%); also in Tanzania, extended spectrum beta lactamase producing *Escherichia coli* in integrated agro-aquaculture in Morogoro, showed 70.0 % resistivity to Cefotaxime (70%) [37]; also in Ghana, antibiotic sensitivity patterns of microbial isolates from fish ponds showed that 15 isolates of *Escherichia coli* showed resistance to Cefuroxime 70% [38] and in Abakaliki, phenotypic screening of multidrug-resistant *E. coli* from water and fish collected from different fish farms revealed that the Isolates exhibited resistance (54% - 100%) to ceftazidime, aztreonam, cefuroxime, and ceftriaxone [39] while aquaculture and fishery in Asian revealed the mean resistance to third-generation and fourth-generation cephalosporins 69.6% (95% CI 65 to 75%)[40]. High prevalence of ESBL-producing *E. coli* with resistant traits in fish and water samples in our study area is a serious public health concern as this will make the treatment of infections, especially *E. coli*-associated foodborne diseases very difficult, thus leading to increase in health care cost, morbidity, and mortality. Clearly, antibiotics resistance arises quickly and spreads rapidly, especially when resistance genes are horizontally transferred via plasmids and integrons among individuals, among species, and even among bacterial kingdom [41]. Much of the problem of antimicrobial resistance has been shown to be due to the presence of transferable plasmids encoding MDR and their dissemination among different enterobacterial species and it is common for a single plasmid to simultaneously mediate resistance to multiple antimicrobials and to be shared among different bacterial genera [41, 42, 43, 44]. Also, important consideration should be paid to the spread

of resistant in aquaculture through potential sources of product contamination in the supply chain at the nursery periods of fingerlings and fry linked to humans, where poor or inappropriate personal hygienic practices during transport, methods of breeding and the potential implications of treated wastewater used for growth/nursery phase in the dissemination of antibiotic-resistant also should not be neglected

CONCLUSION

This study highlights the occurrence rate of ESBL phenotypein fish farming. The presence of ESBL contribute to phenotypicantibiotic resistance in bacteria. The increasing level of resistance among the study isolate from aquaculture product to commonly used antibiotics in medical and veterinary therapies poses a great challenge to the treatment of human and animal diseases. Hence, appropriate intervention of antibiotic use is required to ensure the continuous efficacy of antibiotics for aquaculture and human health and the sustainability of the industry.The isolated ESBL producing bacteria pose high risk to the environment, human and animal health. Strict guidelines and supervision of aquaculture activities, as well as food safety training for farm owners/breeders on many elements of excellent hygiene standards, are strongly advised.

REFERENCES

1. Adelesi OO. Economic analysis of small holder aquaculture Farmers: The case of Nigeria. 2019.
2. The Embassy of the Kingdom of the Netherlands. *Aquaculture in Nigeria fact sheet*. https://www.agroberichtenbuitenland.nl/landeninformatie/nigeria. 2019.
3. Kaleem O, Sabi ABS. Overview of Aquaculture Systems in Egypt and Nigeria, Prospects, Potentials and Constraints. *Aquaculture and Fisheries*. 2021; **6**:535–547.
4. Gazal LE, Tagliari de Brito KC, Kobayashi RKT, Nakazato G, Cavalli LS, Otutumi LK, Guimarães de Brito B. Antimicrobials and Resistant Bacteria in Global fish farming and the possible risk for public health. *Arquino Institute Biol*. 2019; **87**:1-11.
5. Kilonzo-Nthenge A, Liu S, Hashem F, Millner P, Githua S. Prevalence of Enterobacteriaceae on Fresh Produce and Food Safety Practices in Small-acreage Farms in Tennessee, USA. *J VerbrauchLebensm.* 2018; **13**:279–287.
6. Al-Kharousi ZS, Guizani N, Al-Sadi AM, Al-Bulushi IM. Antibiotic Resistance of Enterobacteriaceae Isolated fromFresh Fruits and Vegetables and Characterization of their *AmpC* Beta-lactamases. *J Food Protection*. 2019;**82**:1857–1863.
7. McDaniel C, Jadeja R. A Review of Fresh Produce Outbreaks, Current Interventions, Food Safety Concerns and Potential Benefits of Novel Antimicrobial Sodium Acid Sulfate. *MOJ Food Processing and Technol.* 2019;**7**:59–67.
8. Motlagh AM, Yang Z. Detection and Occurrence of Indicator Organisms and Pathogens. *Water and Environ Res.* 2019; **91**:1402–1408
9. Hamza E. Emergence of β-lactamase- and carbapenemase-producing Enterobacteriaceae at Integrated Fish Farms. *Antimicrobial Resist and Infection Cont.* 2020; **9**:67-68.
10. Thornber K, Verner-Jeffreys D, Hinchliffe S, Rahman MM, Bass D, Tyler CR. Evaluating Antimicrobial Resistance in the Global Shrimp Industry. *Rev Aquaculture*. 2020; **12**:966–986.
11. Watts JE, Schreier HJ, Lanska L and Hale MS. The Rising Tide of Antimicrobial Resistance in Aquaculture: Sources, Sinks and Solutions. *Marine Drugs Resist.*2017; **15**:15-80
12. Sivaraman GK, Rajan V, Vijayan A, Elangovan R, Prendiville A, Bachmann TT. Antibiotic Resistance Profiles and Molecular Characteristics of Extended-Spectrum Beta-Lactamase (ESBL)-Producing *Escherichia coli* and *Klebsiellapneumoniae* Isolated from shrimp aquaculture farms in Kerala, India. *Frontier Microbiol.* 2021; **12**:622-891.
13. Montero L, Irazabal J Cardenas P, Graham JP, TruebaG. Extended-Spectrum Beta-Lactamase Producing-*Escherichia coli* isolated from irrigation Waters and Produce in Ecuador. *Frontier Microbiol.* 2021; **12**:70-9418.
14. Maniati M, Ikonomidis A,Mantzana P, Maniatis A, Pournaras S. *Pseudomonas aeruginosa*with a novel blaVIM-4/blaP1b and a Second Class-1 Integron, Effiux Pumps Overexpression and Repressed porin*OprD*. *IntJ Antimicrobial Agents.* 2007; 29:106-109.
15. Holmes AJ, Holley MP, Mahon A, Nield B, Gillings M. Recombination Activity of a Distinctive Integron-Gene Cassette System Associated with *Pseudomonas stutzeri*Populations in Soil. *J Bacteriol*. 2003;**185**: 918-928.
16. Ndi OL, Barton MD. Resistance Determinants of *Pseudomonas* Species from Aquaculture in Australia. *J Aquatic Research Development.* 2012; 3:119-200.

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17. Aghamelu OP, Nnabo PN, Ezeh HN.Geotechnical and environmental problems related to shales in the Abakaliki area, Southeastern Nigeria. *AfriJ Environ Science Technol.*2011; **5**(2):80-88
18. Cheesbrough, M (2006). District Laboratory practice in tropical countries (Part II), Cambridge University, p.19-110.
19. Akpu PO, Nomeh OL,MonethEC, StellaAOand Ogba RC, Peter IU, Iroha IR. Phenotypic Detection of AmpC beta-lactamase Producing *Escherichia coli* among Patients in Hospital Wards. J AdvMicrobiol. 2023; 23 (1):26-34
20. Ugwu MC, Shariff M, Nnajide C, Beri MK, Okezie UM, Iroha IR, Esimone CO.Phenotypic and Molecular Characterization of *β*-Lactamases among EnterobacterialUropathogens in Southeastern Nigeria. *Can J Infect Dis Med Microbiol.*2020; 12:1975–1978.
21. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-eighth edition (M100). Wayne, PA: Clinical and Laboratory Standards Institute; 2019
22. Nomeh OL, Chukwu EB, Ogba RC, Akpu PO, Peter IU., Nwuzo AC,Iroha IR. *Prevalence and Antibiogram Profile of Carbapenem-resistant Escherichia coli and Klebsiellapneumoniae among Patients with Urinary Tract Infection in Abakaliki, Nigeria.* IntJ Pathogen Res. 2022; 11 (3):14-28.
23. Assefa A, Regassa F, Dinka A, Abunna KA. Prevalence and antibiotic susceptibility pattern of *Escherichia coli* O157:H7 isolated from harvested fish at Lake Hayq and Tekeze Dam, Northern Ethiopia. *Heliyon*. 2019; **5**:29-96.
24. Amuneke KE, Igbodiegwu GC, Okeke PA Adibe AC. Bacteriological Profile of Selected Fish Species and Water Sample from Otuocha River Anambra State. *J Agric Food Sci.* 2022; **18**(1): 11-26.
25. Reza RH, Shipa SA, Naser NM, Miah F. Surveillance of *Escherichia coli* In A Fish Farm Of Sylhet, Bangladesh. *Bangladesh J Zool.* 2022; **48**(1):335-346.
26. Lihan S, Lee SY, Toh SC, Leong SS. Plasmid-Mediated Antibiotic Resistant *Escherichia coli* in Sarawak Rivers and Aquaculture Farms, Northwest of Borneo. *Antibiotics*. 2021;**10**:7-76.
27. Dewi RR, Hassan L, Daud HM, Matori MF, Nordin F, Ahmad NI, Zakaria Z. Prevalence and Antimicrobial Resistance of *Escherichia coli*, *Salmonella* and *Vibrio* Derived from Farm-Raised Red Hybrid Tilapia (*Oreochromis* species.) and Asian Sea Bass (Latescalcarifer, Bloch 1970) on the West Coast of Peninsular Malaysia. *Antibiotics*. 2022; **22**:11-136.
28. Albuquerque R. *Escherichia coli* in Seafood: A Brief Overview.*AdvBiosci. Biotechnol*. 2013;**4**:450–454.
29. Sapugahawatte DN, Li C, Zhu C, Dharmaratne P, Wong KT, Lo N, IpM. Prevalence and Characteristics of Extended-spectrum-β-lactamase-producing and Carbapenemase-producing Enterobacteriaceae from Freshwater Fish and Pork in Wet Markets of Hong Kong. *mSphere*. 2020; 5:107-20.
30. Tran HL, Hong MH, Tran TTH. Antibiotic resistance and molecular characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* isolated from fish pond. *Can ThoUniv J Sci.* 2018; *54*(8), 114-123.
31. Moremi N, Manda EV, Falgenhauer L, Ghosh H, Imirzalioglu C, Matee M, Chakraborty T andMshana SE. Predominance of CTX-M-15 among ESBL Producers from Environment and Fish Gut from the Shores of Lake Victoria in Mwanza, Tanzania. *Frontier Microbiol*. 2016; **7**:18-62
32. Gufe C, Hodobo TC, Mbonjani B, Majonga O, Marumure J, Musari S, Jongi G, Makaya PV, Machakwa J. Antimicrobial Profiling of Bacteria Isolated from Fish Sold at Informal Market in Mufakose, Zimbabwe. *IntJ Microbiol*. 2019;**13**:34-56.
33. Baniga Z, Hounmanou YMG, Kudirkiene E, Kusiluka LJM, Mdegela RH, DalsgaardA. Genome-Based Analysis of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* in the Aquatic Environment and Nile Perch (*Latesniloticus*) of Lake Victoria, Tanzania. *FrontierMicrobiol.* 2020; **11**:108-109.
34. Musa N, Wei LS, Hamdan RH, Leong LK, Wee W, Amal MN, Kutty BM, Abdullah, SZ. Streptococcosis in Red Hybrid Tilapia (*Oreochromisniloticus*) Commercial Farms in Malaysia. *Aquaculture Res.* 2009; **40**:630–632.
35. Storteboom H, Arabi M, Davis JG, Crimi B, Pruden A. Identification of Antibiotic-resistance Gene Molecular Signatures Suitable as Tracers of Pristine River, Urban, Agricultural Sources. *Environ SciTechnol*2010; 44:1947–53.
36. Lastauskiene E, Valskys V, Stankevic˘iute J, Kalciene V, Gegžna V, Kavoliunas J, Ružauskas M, Armalyte˙ J. The Impact of Intensive Fish Farming on Pond Sediment Microbiome and Antibiotic Resistance Gene Composition. *Frontier Vet Sci.* 2021;**8**:673-756

37. Shaban SS. Prevalence of Extended Spectrum beta-lactamase producing *Escherichia coli* in Integrated Agro-aquaculture in Morogoro, Tanzania. https://www.suaire.sua.ac.tz/handle/123456789/2306.2017.
38. Apenteng JA, Osei-Asare C, Oppong EE, Amihere I, Hafiz MY. Antibiotic sensitivity patterns of microbial isolates from fish ponds: A study in the Greater Accra Region of Ghana. *AfriJ Pharmacy Pharmacol.* 2017; **11**(28):314320.
39. Chibuike KU, Iroha IR, Moses IB, Chukwunwejim CR, Peter IU, Edemekong CI, Ndugo CM, Ngene O, Egbuna NR, Okonkwo-Uzor NJ. Phenotypic screening of multidrug-resistant *E. coli* from water and fish collected from different fish farms within Abakaliki metropolis, Nigeria. *Scientific Res Essay.* 2020; **16**(2):15-19.
40. Schar D, Zhao C, Wang Y, Joakim Larsson DG, Gilbert M, Van Boeckel TP. Twenty-year trends in antimicrobial resistance from aquaculture and fisheries in Asia. *Nature Communications.* 2021; **12**:53-84
41. Gatya Al-Mayahie SM, Al-Guranie DRT, Hussein AA, Bachai ZA. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic*Escherichia coli*phylogroup B2 isolates from outpatients in Wasit Province/ Iraq. *Public Library of Science One.* 2022; **17**(1): 262-984
42. OgbaRC, Akpu PO,Nwuzo AC, Peter IU, Nomeh OL, Iroha IR. *Antibiotic Susceptibility Profile of Clinical Isolate of Carbapenem-Resistant Pseudomonas aeruginosa.* South Asian J Res Microbiol. 2022; 14 (2):14-23.
43. Nomeh OL, Federica OI, Joseph OV, Moneth EC, OgbaR C, Nkechi O A, Peter I U, Akpu P O, Edemekong, CI, IrohaIR. *Detection of Carbapenemase-Producing Escherichia coli and Klebsiellapneumoniae Implicated in Urinary Tract Infection*. Asian Journal of Research in Infectious Diseases. 2023; 12 (1):15-23
44. Akpu PO, Uzoeto, HO, Peter I U, Nomeh OL, Nwuzo AC, Ogba R CIroha IR.First Report Occurrence of CIT and DHA AmpC β-lactamase Gene in *Escherichia coli* and *Klebsiellapnuemoniae* from Clinical Sample in South Eastern, Nigeria. Asian J Biochem Genetic Mol Biol. 2023; 13 (1):30-36.