International Journal of Multidisciplinary Research and Development
www.allsubjectjournal.com
Online ISSN: 2349-4182, Print ISSN: 2349-5979
Received: 01-07-2022, Accepted: 15-07-2022, Published: 28-07-2022
Volume 9, Issue 7, 2022, Page No. 93-98

Isolation and antimicrobial suscetibilities of *Salmonella* isolated from wild caught and aquaculture catfish and *Tilapia* in Zaria

Tangsom Hyellamada Chama[1, 2], Jacob K P Kwaga[2], Paul H Mamman[3]
[1] Department of Animal Science, Taraba State University, Jalingo, Taraba State, Nigeria
[2] Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
[3] Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

Abstract

Fish and Fish products have been recognized as major carriers of food-borne pathogens. The aim of this study was to investigate the occurrence and antimicrobial susceptibilities of *Salmonella* isolated from wild and aquaculture catfish and *Tilapia* in Zaria. A purposive sampling technique was employed in selection of samples. A total of 150 fish, consisting of 75 catfish and 75 *Tilapia* were sampled from wild and aquaculture fish. Portions of the gill, skin, and intestine of each fish were pooled and subjected to the conventional microbiological method of isolation and identification of *Salmonella*, followed by biochemical tests. Microbat 24E was used to confirm isolates that yielded presumptive isolates for conventional biochemical tests. Antibiotic susceptibility testing was performed according to Kirby-Bauer disc diffusion techniques on Mueller Hinton agar using standard guidelines according to CLSI (2012). Polymerase chain reaction (PCR) was also employed to detect *inv*A gene in the positive *Salmonella* isolates. An overall occurrence of 2.7% was recorded out of the 150 fish sampled. Fish from the wild had more positive samples (3%) than from cultured fish (2%). Catfish and *Tilapia* had equal occurrence rates of 2.7% each. Statistical analyses showed no significant difference in fish sources and species. The isolates were tested to a panel of 14 antibiotics. All *Salmonella* isolates (100%) showed resistance to at least 9 antibiotics which were cephazolin, ampicillin, amoxicillin, trimethoprim-sulphamethoxazole, cefuroxime, cefoxitin, kanamycin, colistin sulphate and nalidixic acid, while they were susceptible to gentamicin, chloramphenicol, ciprofloxacin and imipenem. Thus all *Salmonella* isolates were multidrug resistant, which may be attributable to antibiotic residues present in animal and human faecal matter and industrial effluents that are discharged into open water bodies such as dams. Multiple antibiotics resistance indices (MAR) of the four *Salmonella* isolates were greater than 0.2. All the isolates confirmed as *Salmonella* by Microbat were positive for *inv*A gene. The result of this study confirms the claim that as more cases of multidrug resistant bacteria are being recorded, consumption of fish could pose health threat to consumers and hence, strong control and mitigation measures be put in place to properly combat the menace of antimicrobial resistance as a global public health threat.

Keywords: Fish, *Salmonella,* Multi-drug resistance (MDR), multiple antimicrobial resistance index (MARI), invA gene

Introduction

The genus *Salmonella* comprises facultative anaerobic, non-spore forming rod-shaped and gram-negative motile bacillus bacteria belonging to the family *Enterobacteriaceae* that causes salmonellosis. They are mesophilic and are distributed geographically all over the world with aquatic environments serving as major reservoirs (Chia *et al*., 2016). To date, more than 2600 serotypes of *Salmonella* have been reported and many serovars have been associated with disease potentials (Yang *et al.*, 2015) [30]. Outbreaks of salmonellosis due to fish consumption have been reported in several countries (Titik *et al.*, 2013).

Fish is known for its high nutritional quality, relatively low-fat content, saturated fat, cholesterol and high polyunsaturated fatty acids, protein, and minerals such as calcium, phosphorus, sodium, potassium, and magnesium (Ibrahim *et al.*, 2014) [14]. Fishes account for approximately 17% of the global animal protein intake (Bibi *et al*., 2015) [5]. Aquaculture represents one means that supplements wild fish due to the growing demand of fish protein by the population (Grema *et al.*, 2015) [12]. However, the production of fish is faced with challenges that include, but not restricted to pathogenic microorganisms, antimicrobial or drug residues and environmental contaminants (Chia., 2016).

Catfish *(Clarias gariepinus*) is one of the most widely farmed and harvested fish species in tropical Africa. This is because of its high-quality muscle, high production quality and great market values (Adeshina *et al.*, 2016) [1]. *Tilapia* (*Oreochromis niloticus*) is the most commonly cultivated species in many countries around the world (Salem, 2015) [20].

The use of antibiotics in aquaculture affects a wide range of bacteria and has potential impact on other components of the aquatic system such as receiving water bodies as well as in fish pathogens. Antibiotics routinely used for the treatment of human infections are also used in animal rearing, either for therapeutic or prophylactic purposes or for growth promotion (Njoku *et al*., 2015) [17]. and may accumulate in the water and sediments of fish farms and receiving water bodies that may lead to residues in fish tissues and consequently affect consumers (Agoba *et al.*, 2017) [2].The close confinement of finfish in ponds and sanitary shortcomings of these environments have resulted in the enormous use of wide variety of antimicrobials (Gelband *et al*. 2015) [11]. The greatest potential risk to public health associated with antimicrobial use in aquaculture is thought to be the development of a reservoir of transferable resistance genes in aquatic bacteria, from which such genes can be disseminated by horizontal gene transfer to other bacteria and ultimately reach human pathogens (Njoku *et al*., 2015) [17].

Materials and Methods

The sampling locations include Sabon Gari Market, Zaria dam and ABU dam in Zaria. Zaria is a town in Kaduna State of Nigeria located between latitude 110^{o} 3'N and longitude 704^{o} 2' E. It has a tropical climate that is characterised by cool humid wet seasons and cold or hot dry seasons. Study design was cross-sectional and Purposive sampling technique was used. Fish samples were collected within the period of three weeks (Jan 2020), 25 samples each of *Tilapia* and Catfish were bought and identified to make a total sample size of 150. Aquaculture fish were bought from Sabon Gari Market, while wild caught fish were bought from ABU and Zaria dams respectively. All samples were collected using standard aseptic protocol and transported live to the Bacterial Zoonosis laboratory in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria in a container with water to prevent sample deterioration on transit. Sample preparation was done using the conventional microbiological method of isolation as described by Chia *et al.* (2016) and Titik *et al*. (2011). Approximately 10 g of the skin, gills and intestine were harvested from each fish with the use of a sterile scalpel blade and pooled together, organs were placed on a sterile aluminium foil and chopped thoroughly with forceps and scissors and placed in a stomacher bag containing 90 ml 0.1% Peptone water (PW) and homogenized using a stomacher for 3 min at 200 rpm and incubated at 37° degrees for 24hrs. One ml aliquot of enriched culture was transferred into 9 ml of Rappaport Vassiliadis broth (*Salmonella* Enrichment Broth) and incubated at 37 °C for 24hrs. Following enrichment, a loopful of the culture was streak-plated onto Xylose-Lysine-Tergitol 4 (XLT4 Merck) and incubated at 37°C for 24-48 hrs. Organisms that grew as characteristic black colonies on XLT4 were considered as presumptive *Salmonella* specie and were subculture to obtain pure isolated colonies by streaking onto nutrient agar plates (Merck KGaA, Darmstadt, Germany). Biochemical screening tests for *Salmonella* species was also carried out which include Triple Sugar Iron (TSI), Urease, Indole, Citrate, Methyl red, Voges Proskauer, Motility and hydrogen sulphide production (Kaniz *et al.*, 2014; WHO, 2014) [15]. Fermentation of mannose, glucose, maltose, sucrose, arginine, xylose and lactose were also carried out. Microbact 24E was used to confirm presumptive *Salmonella* isolates. DNA extraction was performed using the quagen extraction kit. Antimicrobial susceptibility testing was performed according to Kirby-Bauer disk diffusion techniques (Bauer *et al*., 1966) [4]. on Mueller Hinton agar using the following antibiotic discs and concentrations (Oxoid): gentamicin (CN) 10 µg, tetracycline (TE) 30 µg, chloramphenicol (C) 30µg, trimethoprim-sulphamethoxazole (SXT) 25 µg, cephazolin (KZ) 30 µg, cefoxitin (FOX) 30 µg, cefuroxime (CXM) 30 µg, ampicillin (AMP) 10 µg, amoxicillin (AML) 10 µg, ciprofloxacin (CIP) 5 µg, imipenem (IPM) 10 µg, kanamycin (K) 30 µg, colistin sulphate (CT) 10 µg, and nalidixic acid (NA) 30 µg. The zones of inhibition were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2012). Multidrug resistance was defined as resistance to ≥3 classes of antimicrobials (Oteo *et al.*, 2005). Chi square was used to determine the Occurrence of *Salmonella* in Catfish and *Tilapia* while Fisher's exact Test was used to determine Occurrence of *Salmonella* in wild caught and aquaculture fish. Polymerase chain reaction (PCR) was carried out using specific primers for *inv*A genes namely; Forward (S139) and Reverse primers (S141) with the nucleotide sequence 5' GTG AAA TTA TCG CCA CGT TCG GGC AA -3'and 5´ TCATCG CAC CGT CAAAGG AAC C -3´ respectively (Rahn *et al*., 1992) which is expected to yield an Amplicon size of 284bp for targeted gene fragment. An aliquot of 10 µL of the PCR product was analysed by electrophoresis in 1% agarose gel, stained with Ethidium Bromide (EtBr) and photographed on a gel documentation unit.

Results

The overall occurrence of *Salmonella* species isolated was 2.7% from a total of 150 fish samples analyzed in this study. A rate of 2.7% was obtained for both catfish and *Tilapia,* while the rate obtained for both wild caught and aquaculture fish was 3% and 2% respectively. Statistical analysis showed Chi-square=0. df=1. P-value=1, Hence no significance difference based on species of catfish and *Tilapia.* Fisher's exact test=1.027. df=1. P-value=0.620. Hence no significance difference based on fish sourced from the wild and aquaculture**.**

Biochemical test suspected 24 isolates to be presumptive for *Salmonella* after which Microbact 24E confirmed four of the isolates to be Positive for *Salmonella*. Of the 24 isolates, each isolate was resistant to at least 6 antibiotics; All isolates (100%) were resistant to cephazolin, ampicillin, cefoxitin, kanamycin, colistin and nalidixic acid, while 1 (4%) was resistant to gentamicin, 22(92%) were resistant to amoxicillin, 5(21%) were resistant to tetracyclines, 8(33%) were resistant to trimethoprim sulphamethoxazole and 2(8%) were resistant to imipenem and ciprofloxacin each. All were (100%) susceptible to chloramphenicol, 23(96%) of the isolates were susceptible to gentamicin, 16(67%) were susceptible to trimethoprim sulphamethoxazole, 19(79%) were susceptible to tetracycline, 1(4%) was susceptible to cefuroxime, 22(92%) were susceptible to imipenem as well as Ciprofloxacin.

Antimicrobial susceptibility testing of the four confirmed *Salmonella* isolates showed that all were susceptible to gentamicin, chloramphenicol, ciprofloxacin, and imipenem, while cephazolin, cefuroxime, ampicillin, amoxicillin, cefoxitin, Trimethoprim sulphamethoxazole, colistin, kanamycin and nalidixic acid were all Resistant (Table 1.0). PCR amplification of *inv*A gene confirmed the four isolates to be positive for *inv*A gene with appearance of four molecular markers (Bands) with the amplicon size of 284bp. (Fig 1.0)

Table 1: Antimicrobial susceptibility of Four (4) *Salmonella* isolates from Catfish and *Tilapia* species in Zaria to 14 antimicrobial agents.

0	S	S	R	S	R	R	R	R	R	S	R	R	R	S
SAC6	S	R	R	S	R	R	R	R	R	S	R	R	R	S
ABUC23	S	R	R	S	R	R	R	R	R	S	R	R	R	S
ABUT25	S	R	R	S	R	R	R	R	R	S	R	R	R	S

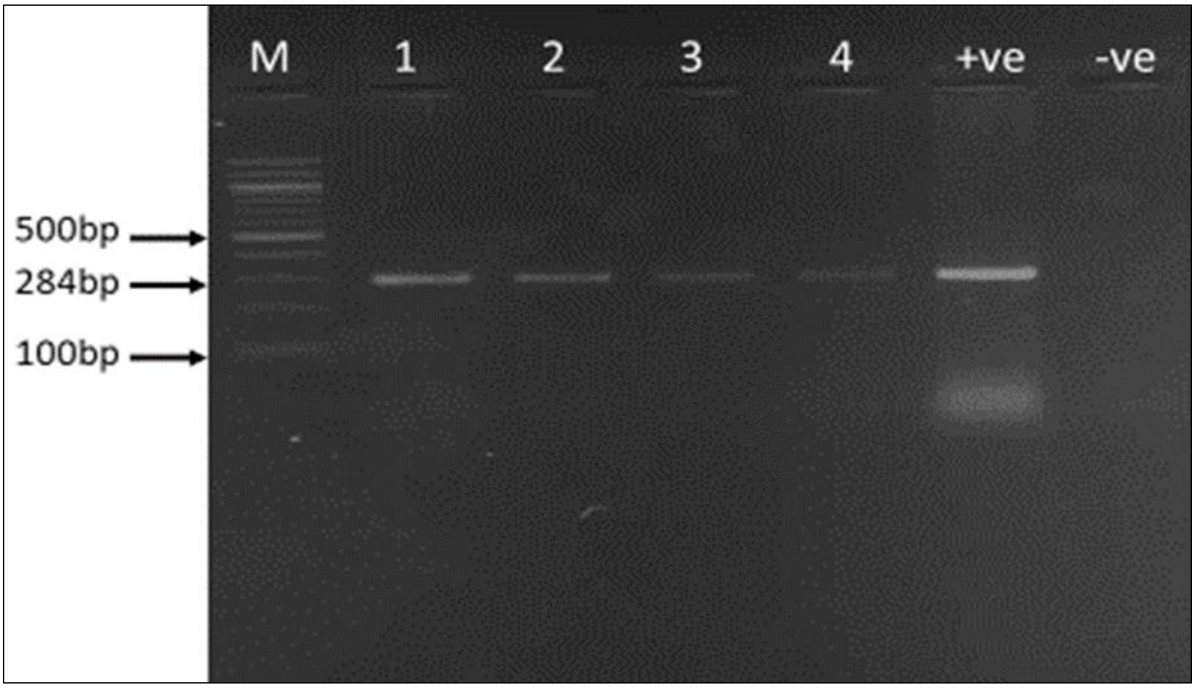

Plate 1: PCR amplification of *inv*A gene in four *Salmonella* isolates. Lanes M is 100bp molecular weight markers; lanes 1 to 4 are *Salmonella* isolates. Positive and negative controls are indicated.

Discussion

Salmonella is known be associated with fish as well as with other food and food products. Similar observation was made by Osungbemiro *et al.* (2014) who reported that *Salmonella* and *Shigella* species occurred on the skin, gills and intestines of *Clarias gariepinus*. This study is in line with the observation made by Shinkafi and Ukwaja (2020) [21] who reported 3.2% of *Salmonella* species isolated from the skin, gills and intestine of fish in Sokoto, and in contrast with the study by Saad *et al*. (2020) [19]. who failed to isolate *Salmonella* in fish samples in Egypt. This differences could be due to high ambient temperatures in the rivers where the fish was caught which is close to uptimum for many mesophilic organisms which also agrees with Grema *et al*. (2015) [12], that the microbial flora of freshly captured fish is usually a reflection of the environment in which it was harvested. The detection of the bacteria in fish in this study reflects the fact that water bodies such as dams, rivers, ponds and markets in Zaria are usually not free from *Salmonella*. This is in line with the study by Ellermeier and Slauch (2006) which revealed that cold-blooded animals such as catfish and *Tilapia* (FAO, 2010) are potential hosts for *Salmonella* species (Baker and Smitherman, 1983; Pal and Marshall, 2009). Generally, ponds and rivers that harbour fish may be the sources of the microorganisms due to contamination with pollutants from domestic, industrial and agricultural discharges, including indiscriminate dumping of human and animal excreta as well as other environmental wastes into natural water bodies or washing of excreta from land into water during the rainy season (Raufu *et al*., 2014; Grema *et al.*, 2015) [12]. The water bodies identified in this study may have been contaminated with wastes that are contaminated with *Salmonella*.

The fish sampled from the wild were found to harbor *Salmonella* at the rate two fold higher than the ones caught from aquaculture pond, which is in agreement with the study by Mohammed and Lakshmanaperumalsamy (1997), who reported a higher prevalence of 30% of *Salmonella* in fish sampled from the wild compared to 5% in fish from aquaculture. This may be a result of the level of control put in place in aquaculture. There is little or no control over discharges that enter the natural waters (dams), making them to be susceptible to pollution from domestic, industrial and agricultural discharges that enhance the growth of *Salmonella* (Raufu *et al.*, 2014).

All four isolates (100%) identified as *Salmonella* in this study were multi-drug resistant. Of the 14 antibiotics used in this study, each Isolates identified as *Salmonella* was resistant (100%) to nine (9) which include cephazolin, cefoxitin, cefuroxime, ampicillin, amoxicillin, trimethoprim- sulphamethoxazole, colistin, kanamycin and nalidixic acid. This resistance is in line with the study by (Foley and Lynne) 2008; who reported *Salmonella* to be resistant to one or more antimicrobials. *Salmonella* isolates obtained from fish samples in this study were found to have multiple antibiotic resistance index of 0.64 for one isolate and 0.71 for three isolates respectively. The emergence of *Salmonella* serovars with high MAR index suggests that these serovars have originated from environments where antimicrobials are often used as therapeutic or as growth promoters in animal feeds (Krumperman, 1983; Singh *et al*., 2010). The occurrence of *Salmonella* with resistance to commonly used antibiotics in this study may be indicative of their use in farmed fish and humans. Also unhygienic way of farming or rearing, handling and processing of fish could aggravate the presence of *Salmonella.* Antimicrobial resistance is currently a special case of public health concern that need proper measure to curb the long-term effect. Misuse of drugs by addition of antibiotics to feed could be associated with drug resistance, hence multidrug resistant isolates in this study substantiates the claims that cases of resistance by bacteria are on the increase, which calls for proper public health measures to be put in place. More importantly, the findings that all isolates in this study were multi-drug resistant is cause for alarm. By implication this means that in near future there would be rarely any antibiotic that will be effective against bacterial infections like salmonellosis which agrees with Chia *et al.* (2016) [6] who reported that multidrug resistant *Salmonella* are increasingly being isolated.

All Four isolates identified as *Salmonella* in this study were susceptible to 4 antimicrobials which include gentamicin, ciprofloxacin, chloramphenicol and imipenem this is in line with the study by Odu *et al.*, (2021) [16]. who reported 100% potency of ciprofloxacin against all *Salmonella* species isolated from *Tilapia* in Port Harcourt but in contrast with the same study by Odu *et al. (*2021) [16]. who reported 100% resistance to gentamicin, these differences could be due to exposure of organism to antibiotics used in aquaculture either for therapeutic or prophylactics purposes or for growth promotions in animal feeds Njoku *et al*. (2017), could also be due to chemicals and harsh environmental conditions where these fishes live thus acquiring plasmids which enables these organisms transform to strains able to resist antibiotics they are normally susceptible to Odu *et al.* (2021) [16.]

In this study, *inv*A gene was detected in all four isolates by PCR, confirming them to be *Salmonella*. This aggress with the findings of Titik *et al*. (2013) who reported 100% detection of *inv*A gene in S. Typhymurium isolated from fishes and in contrast with the study by Yanestria *et al.* (2019*)* [29].who detected only 12.5% of *inv*A gene in *Salmonella* isolates confirmed by Microbact, the absence of target bands in some of the isolates could be because the strains of *Salmonella* obtained were not invasive or might also use other invasive mechanisms. The greatest potential risk to public health associated with antimicrobial use in aquaculture is thought to be the development of a reservoir of transferable resistance genes in aquatic bacteria, from which such genes can be disseminated by horizontal gene transfer to other bacteria and ultimately reach human pathogens (Njoku *et al*., 2015) [17].

Acknowledgment

I would like to acknowledge and thank my Supervisors, Dickson Anoibi Mathew and Staff of the Bacteria Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine Ahmadu Bello University Zaria Nigeria.

Competing Interests

Authors have declared that no competing interests exist.

Conclusion

It is evident from this study that *Salmonella* is present in fish which might cause health hazards to consumers of fish as well as to fish handlers. Multi-drug resistance observed in this study suggest indiscriminate use of antibiotics by fish farmers and environmental contamination, hence posing a threat to public health. Multiple antibiotics resistance (MAR) indices shows the ratio of ≥ 0.64 for all isolates, which indicates a problem of antibiotic resistance and complicates the choice of treatment in human disease with serious public health consequences. All four *Salmonella* isolates were found to harbour the *inv*A gene which indicates the virulence and pathogenicity of *Salmonella* to humans as well as animals posing a serious public health concern. The detection of *Salmonella* in fish can pose not only risk of disease to the fishes but public health hazard to consumers and humans in general. It is therefore recommended that Continuous monitoring and surveillance of antimicrobial resistant bacteria in fish and fish handlers and value chain be encouraged, Proper regulations on the use of antibiotics by fish farmers should be enforced by educating fish farmers as well as consumers on the dangers posed by fishes in the food chain; Open defecation and indiscriminate discharge of wastes and pollutants into water bodies should be discouraged and Finally Fish should be properly cooked before consumption

References

1. *Medicine* Adeshina I, Jenyo-Oni A, Ajani Ek, Adewale YA. Natural occurrence of *Diplostomum spp.* In farm raised African catfish (*Clarias gariepinus*) from Oyo state, Nigeria. International journal of Veterinary Science and Medicine,2016:4(2):41-45.
2. Agoba EE, Adu F, Agyare C, Boamah VE, Boakye YD. Antibiotic resistance patterns of bacterial isolates from hatcheries and selected fish farms in the Ashanti region of Ghana. Journal of Microbiology and Antimicrobial,2017:9(4):35-46.
3. Bashir KMI, Kim JS, An JH, Sohn JH, Choi JS. Natural food additives and preservatives for fish-paste products: A review of the past, present, and future states of research. Journal of Food Quality, 2017, 31.
4. Bauer AW, Kirby WM, Sheris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of clinical pathology,1966:45:149-158.
5. Bibi F, Qaisrani SN, Ahmad AN, Akhtar M, Khan BN, Ali Z. Occurrence of *Salmonella* in Fresh water Fishes: A Review. Journal of Animal and Plant Sciences,2015:25(3):303-310.
6. Chia MA, Abdulwahab R, Ameh I, Balogun JK, Auta J. Farmed *Tilapia* as an exposure route to microcystins in Zaria-Nigeria: A seasonal investigation. Environmental Pollution,2021:27(1):116-366.
7. Cheesbrough. District Laboratory Practice in Tropical Countries Part 2 Second Edition. Cambridge university press, 2006.
8. Danba E, Bichi A, Ishaku S, Ahmad M, Buba U, Bingari M, *et al*. Occurrence of pathogenic bacteria associated with *Clarias gariepinus* in selected fish farms of Kumbotso local government area of Kano state, Nigeria. Bayero Journal of Pure and Applied Sciences,2014:7(2):145-149.
9. Deekshit VK, Kumar BK, Rai P, Karunasagar I. Antibiotic resistance and molecular characterisation of sea food isolates of non thyphoidal *Salmonella* by PFGE. Procedia Food Science,2016:6:334-338.
10. Elhadi N. Prevalence and Antimicrobial Resistance of *Salmonella* spp. in Raw Retail Frozen Imported Freshwater Fish to Eastern Province of Saudi Arabia. Asian Pacific Journal of Tropical Biomedicine,2014:4(3):234-238.
11. Gelband H, Miller-Petrie M, Pant S, Gandra S, Levinson J, Barter D. The state of the world’s antibiotics. Wound Healing Southern Africa,2015:8:30-34.
12. Grema HA, Geidam YA, Suleiman A, Gulani IA, Birma RB. Multi-drug resistant bacteria isolated from fish and fish handlers in Maiduguri, Nigeria. International Journal of Animal and Veterinary Advances,2015:7(3):49-54.
13. Hohmann EL, Non typhoidal salmonellosis. *Clinical Infectious Diseases*,2001:32(2):263-269.
14. Ibrahim WA, Abd El-Ghany WA, Nasef SA, Hatem ME. A comparative study on the use of real time polymerase chain reaction (RT-PCR) and standard isolation techniques for the detection of Salmonellae in broiler chicks. International Journal of Veterinary Science and Medicine,2014:2(1):67-71.
15. Kaniz F, Rahman M, Datta S, Magnet MH. Comparative analysis of multi-drug resistance pattern of *Salmonella* sp. isolated from chicken feces and poultry meat in Dhaka city of Bangladesh. IOSR Journal of Pharmacy and Biological Science,2014:9(2):147-154.
16. Odu NN, Ogbonna DN, Daminabo V. Antibiogram of Listeria and *Salmonella* Species Isolated from *Tilapia* Fish (Oreochromis nitolicus) and Snail (Archachatina marginata) Sold in Port Harcourt, Nigeria. Journal of Advances in Microbiology,2021:21(3):46-58.
17. Njoku OE, Agwa OK, Ibene AA. Antibiotic Susceptibility Profile of Bacteria Isolates from Some Fishponds in Niger Delta Region of Nigeria. *British Microbiology Research Journal*,2015:7(4):167-173.
18. Rahn K, De Grandis SA, Clarke RC, Curtiss R, Gyles CL. Amplification of an *inv*A gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of Salmonella. Molecular and Cellular Probes,1992:6(4):271-279.
19. Saad SM, Hassanien FS, Mousa M. Monitoring of some Pathogenic bacteria in Nile fish. Benha Veterinary Medical Journal,2020:38(2):65-70.
20. Salem MAA. Studies on Some Predominant Bacterial Diseases in Some Fresh Water Fishes in Dakahlian Farms. M. V. Sc. Thesis, Faculty of Veterinary Medicine, Mansoura University, Egypt, 2015.
21. Shinkafi SA, Ukwaja VC. Bacteria Associated with Fresh *Tilapia* Fish (Oreochromis niloticus) sold at Sokoto Central Market in Sokoto, Nigeria. Nigerian Journal of Basic and Applied Sciences, 2010, 18(2).
22. Singleton P. Bacteria in Biology, Biotechnology and Medicine.4th edition. John Wiley and Sons; Ltd,1997:36(3):345-346.
23. Smith PV, Alday-Sanz J, Matysczak G, Moulin CR, Lavilla-Pitogo, Prater D. Monitoring and surveillance of antimicrobial resistance in microorganisms associated with aquatic animals. Scientific and Technical Review: International Office of Epizootics,2013:32(2):583-593.
24. Threlfall EJ, Antimicrobial drug resistance in *Salmonell*a: problems and perspectives in food-and water-borne infections. FEMS Microbiology Reviews,2002:26(2):141-148.
25. Thrustfield M. *Veterinary Epidemiology*, 3rd Ed. Blackwell Science Ltd, a Nackwell Publishing Company, UK, 2008, 228-242.
26. Tikit B, Rusul G, Wan-Abdullah WN, Arip YM, Ahmad R, Thong KL. Prevalence, antibiotic resistance and plasmid profiling of Salmonella in catfish (Clarias gariepinus) and Tilapia (Tilapia mossambica) obtained from wet markets and ponds in Malaysia. Aquaculture,2013:372:127-132.

27. Upadhyay BP, Utrarachkij F, Thongshoob J, Mahakunkijcharoen Y, Wongchinda N, Suthienkul O, *et al*. Detection of *Salmonella inv*A gene in Shrimp Enrichment Culture by Polymerase Chain Reaction. Southeast Asian Journal of Tropical Medicine and Public Health,2010:41(2):426-435.
28. WHO. A global *Salmonella* surveillance and laboratory support project of the World Health Organization. Laboratory protocols. Level 1 training course. Identification of Salmonella, 2003.
29. Yanestria SM, Rahmaniar RP, Wibisono FJ, Effendi MH. Detection of *invA* gene of *Salmonella* from milkfish (*Chanos chanos*) at Sidoarjo wet fish market, Indonesia, using polymerase chain reaction technique. Veterinary world,2019:12(1):170-175.
30. Yang X, Wu Q, Zhang J, Huang J, Chen L, Liu S, et al. Prevalence, enumeration, and characterization of *Salmonella* isolated from aquatic food products from retail markets in China,2015:57:308-313.