Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(4): 301-305

Received: 20-05-2020
Accepted: 22-06-2020

Devajani Deka
Assistant Professor, Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences & A.H., Central Agricultural University, Selesih, Mizoram, India

M Das
MVSc Scholar, Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences & A.H., Central Agricultural University, Selesih, Mizoram, India

H Bayan
Assistant Professor, Department of Veterinary Surgery and Radiology, College of Veterinary Sciences & A.H., Central Agricultural University, Selesih, Mizoram, India

Corresponding Author:
Devajani Deka
Assistant Professor, Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences & A.H., Central Agricultural University, Selesih, Mizoram, India

Molecular detection of *Salmonella* Typhimurium and its antibiogram from marketed raw fish of inland fisheries sold in Aizawl, Mizoram, India

Devajani Deka, M Das and H Bayan

Abstract
The study aimed to detect *Salmonella* spp in 100 numbers of raw fish samples from inland fisheries sold in local markets of Aizawl. *Salmonella* was isolated and presumptively identified by using conventional bacteriological method and the *Salmonella* serovars were further confirmed by detection of species specific *16S-rRNA* gene and serotyping. The PCR positive *Salmonella* serovars were screened for the presence of virulence associated genes (*inv A* and *stn*) and antimicrobial susceptibility. From the 10 phenotypically positive *Salmonella* strains, 4 strains were confirmed as *Salmonella* by PCR (*16Sr-RNA* gene) and serotyped as *Salmonella* Typhimurium. All the 4 (100%) strains were subsequently positive for *invA* and *stn* genes. The antimicrobial sensitivity profile revealed that the *Salmonella* Typhimurium strains were sensitive to Amikacin, Gentamicin, Ofloxacine, Ceftazidime, Chloramphenicol and Imipenem and resistant to Tetracycline and Ciprofloxacin.

Keywords: Antibiogram, Mizoram, raw fish, *Salmonella* typhimurium, virulence genes

Introduction
Fish is loaded with many essential nutrients mainly good quality protein, iron, vitamin D and calcium along with unsaturated fat called omega-3 fatty acid with huge availability in most of the countries worldwide. The role of fisheries is increasingly recognized by national and global development policy makers for alleviation of hunger and malnutrition. In 2015, fish contributed for about 17 per cent of animal protein consumed by the global population and fish provided about 3.2 billion people with almost 20 percent of their average per capita intake of animal protein [8]. Globally, India holds 3rd position in fisheries and second in aquaculture and the per capita annual consumption of fish in India is 9kg in 2015 as per National Fisheries Development Board. Besides the capture fisheries, recent developments in inland fisheries make different kinds of fishes more available and favourite in most parts of world. In many developing countries with water and fishery resources, fish serves as an important source of nutrition, livelihoods and income for the rural poor who suffer from malnutrition including micronutrient deficiencies [33].

Like the raw meat, egg and other food products of animal origin, fish may also transmit many bacterial, viral and other microorganisms to human if appropriate food safety measures are not adapted. The hazardous factors may enter fish production chain during handling of fishes like catching, transportation, and marketing, slaughtering and processing for consumption [12]. Therefore, fish and other aquatic life forms are vulnerable to different kinds of environmental hazards [25]. Fish and fishery products are frequently contaminated with bacterial pathogens and have been recognized as an important carrier of food-borne pathogens [34]. Fishes are known to transmit *Salmonella* spp., *Staphylococcus* spp. and *Aeromonas* spp. which are the causal agents of human food borne infection and intoxication [11]. Common pathogens that are found in Indian seafood are *Salmonella*, *Vibrio*, *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* [26]. *Salmonella* infection in human and other animals occur due to the ingestion of under cooked fish contaminated during production and processing 20. *Salmonella* in fresh water fishes has been related to the faecal contamination of water from where fishes were harvested. The microbial contamination of fishes and detection of *Salmonella* in pond fishes has been recorded in some studies [5, 21]. Thus, fish is very much prone to be contaminated with different microorganisms which might threat consumer's health.

Mizoram has witnessed a good growth in fish production in recent years. Aquaculture has been contributing 90% of total fish production of 7630 MT during 2016-17. The pond productivity of fish is 1280kg/ ha/ yr in 2016-17 which is still far below than the national average [13]. Detection of the bacterial pathogen can be done by isolation and identification using traditional cultural method in combination with molecular tool such as polymerase chain reaction (PCR) [10]. Therefore, the present piece of work was carried out to detect *Salmonella* in raw fish from inland fisheries sold in local markets of Aizawl, Mizoram, its virulence associated genes and antibiogram profile.

Materials and methods

A total of 100 numbers of raw fish sample of different species from inland fisheries sold in local markets Aizawl, Mizoram were collected during October, 2015 to March, 2016. About 100grams of fish flesh was aseptically collected from each fish and processed for isolation and identification of *Salmonella* as per the standard guidelines from ISO 6579:2002 with slight modification. Different stages for isolation and identification of *Salmonella* involved pre-enrichment, selective enrichment, selective plating, Gram's staining and a set of biochemical tests**.** Twenty-five gram of fish flesh was aseptically ground and added with 225 ml of buffered peptone water (BPW) and incubated at 37ºC for 18 hours for pre-enrichment. One ml of pre-enriched broth was transferred into tubes containing 10 ml selenite cysteine broth and incubated at 37ºC for 24 hours. A loop-full of enriched culture was streaked onto selective agar plates of Xylose lysine deoxycholate agar (XLD) and Brilliant green agar (BGA) and incubated at 37º C for 24 hours. Gram negative bacterial colonies with specific morphological characteristics were biochemically analysed for Indole, Methyl red, Voges-Proskauer, Citrate utilisation and TSI as per the method described by Quinn *et al.* (1994) [22].

All the phenotypically positive strains were further screened for *16S-rRNA* genus specific PCR based detection of *Salmonella*. The template DNA was prepared from the pure cultured bacterial strains by using boiling and snap chill method. The bacterial isolates were grown in five ml single strength Luria Bertani (LB) broth and incubated at 37°C for 24 hours under constant shaking. After incubation, one ml of the bacterial broth culture was taken in a sterile micro-centrifuge tube and centrifuged at 8000 rpm at 4°C for 8-10 minutes. The bacterial pellet thus obtained was washed thrice with sterile normal saline solution (NSS, 0.85% w/v) by centrifuging at 8000 rpm at 4°C for five minutes and finally pellet was re-suspended in 100µl of nuclease free sterile distilled water. The bacterial suspension was boiled for 15-20 minutes in a boiling water bath followed by immediate chilling for 15 minutes at -20˚C. The lysate was centrifuged again at 5000 rpm for five minutes to sediment the cell debris and the supernatant was used as template DNA for PCR assay as per standard method.

The *16S- rRNA* gene was amplified (Master Cycler Gradient, Bio Rad) by using published oligonucleotide primers (Eurofins Genomics India Pvt. Ltd., Bangalore, India) which flanked a 480bp segment in reserved species specific gene sequence. The *16S-rRNA* gene positive *Salmonella* strains were processed for detection of virulence associated genes namely *inv*A and *stn.* Oligonucleotide primers used for detection of the targeted genes are given in Table-1. All the PCR mixtures consisted a final volume of 25µl containing 12.5 µL 2X Dream taq PCR Master Mix $Mgcl_2$ (20 mM), 1 µl (10 pmol) each of forward and reverse primer, 5 µl of template DNA (culture lysate) and nuclease free water to make up the volume 25 µl. The cycling condition of *16S-rRNA* gene and virulence genes are presented in Table-2. The final amplified products were analyzed by horizontal submarine electrophoresis with one per cent(w/v) agarose gel in 1X TAE buffer (Tris acetate 0.04 M, EDTA 0.001 M and pH adjusted to 8.0) [28].

Table 1: Oligonucleotide primers used in PCR for detection of different genes of *Salmonella.*

Target gene	Primer sequence (5'-3')	Base pair	Reference
16S-rRNA	F-TAT CTG GCT ATC GCT GGC AGT G R- TCC GCT AAT CTT TTG GCA ACC	480	Whyte *et al.* (2002)[35]
Stn	F: TTGTGTCGCTATCACTGGCAACC R: ATTCGTAACCCGCTCTCGTCC	617	Murugkar *et al.* (2003)[19]
*inv*A	F:TGAAATTATCGCCACGTTCGGGCAA R:TCATCGCACCGTCAAAGGAACC	284	Rahn *et al.* (1992)[24]

Table 2: Thermal cycling conditions used for detection of different genes of *Salmonella.*

Stages of PCR	Different genes of *Salmonella*		
	16S-rRNA	***Stn***	***invA***
Denaturation	94ºC for 45 sec	94ºC for 1 min	94ºC for 30 sec
Annealing	59ºC for 45 sec	59ºC for 1 min	64ºC for 30 sec
Extension	72ºC for 45 sec	72ºC for 1 min	72ºC for 45 sec
Final extension for 1 cycle	72ºC for 6 min	72ºC for 10 min	72ºC for 10 min
No of cycle	30	30	30

The *16S-rRNA* gene positive *Salmonella* strains were serotyped on the basis of their somatic antigen at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh, India.

All the PCR positive *Salmonella* strains were tested for phenotypic antimicrobial sensitivity pattern by *in vitro* antimicrobial sensitivity test through disc diffusion method [1]. A panel of 12 commonly used antimicrobial agents were tested namely; Ampicillin (AMP, 10), Gentamicin (GEN, 10), Amikacin (AK, 30), Ciprofloxacin (CIP, 5), Norfloxacin (NX,10), Ofloxacin (OF,5), Ceftriaxone (CTR,30), Ceftazidime (CAZ,30), Cephalexine (CTX,30), Chloramphenicol (C, 30), Tetracycline (TE,30) and Imipenem (IPM, 10) (Hi-Media, Mumbai, India). Briefly, isolates were grown in a shaking water bath at 37°C Overnight and then the

bacterial suspension was spread over the entire surface of Mueller-Hinton agar plates and antibiotic disks were applied on the surface of the medium and incubated at 37°C for 18-24 hours. *Salmonella* strains were evaluated as susceptible and resistant according to the diameter of the zone of inhibition.

Results and discussion

From the 100 numbers of raw fish samples collected from different unorganized fish markets of Aizawl, 10 *Salmonella* strains were detected presumptively by conventional bacteriological method. From the 10 phenotypically positive *Salmonella* strains, four strains were found to be positive for genus specific gene (*16S-rRNA*) of *Salmonella* (Figure 1). All the four strains were serotyped as *S.* Typhimurium indicating the 4 per cent prevalence rate of the organism in raw fish sold in local markets.

According to Center for Disease Control and Prevention, *Salmonella* is the leading cause of bacterial foodborne illness causing approximately 1.4 million nontyphoidal illnesses, 15,000 hospitalizations and 400 deaths in USA annually. Thus, *Salmonella* is a pathogen responsible for severe foodborne infections which can be introduced into the fish production chain through inadequate handling/ hygiene or contact with contaminated water although it is not a biological contaminant originally reported in fish [9]. Its waterborne transmission has been well documented [6]. *Salmonella* has been isolated from fish and fishery products from India, though it is not psychotrophic or indigenous to the aquatic environment [18]. The occurrence of *Salmonella* in fish from other studies was found to be much higher than the findings of present study. However, the incidence of *Salmonella* infection due to aquatic food consumption is still low compared with salmonellosis associated with other foods while detection of *Salmonella* spp. in aquatic food cannot be skipped as it is responsible for much food borne gastroenteritis. Beshiru *et al.* (2019) [2] indentified *Salmonella* Enteridis (24.40%) and S. Typhimurium (31.40%) from shrimps sold in open market of Delta and Edo state, Nigeria.

The occurrence of *Salmonella* serovars has also been reported in fresh water fish and sea foods from different parts of India. Kumar *et al.* (2008) [15] detected 23.20 per cent prevalence of *Salmonella* with 27 different serovars predominantly including *Salmonella* Typhimurium among all other serovars from seafood. *Salmonella* was also reported from the intestines of Silver Carp fish due to the microbial contamination of fishes grown in ponds in and around Calcutta [21].

In recent years, PCR has been used to study the distribution of genes present in *Salmonella* serovars associated with sea food in India [15, 4]. In the present study, all the *S.* Typhimurium strains were found to be positive (100%) for the virulence genes, *invA* and *stn* (Figures 2-3). These two genes are invariably present in all *Salmonella* serovars which can be used as PCR based detection tool for quick and less laborious identification of the organism. The *invA* and *stn* genes are responsible for invasiveness and enterotoxin production which make *Salmonella* an obligatory pathogen. Similar to the present findings, 100 per cent positivity to *invA* and *stn* gene in *Salmonella* isolates from fish was reported in an earlier study [32]. However, 100 per cent positivity of *invA* gene in *Salmonella* isolates from fishes was also reported [3, 29]. Kshirsagar *et al.* (2014) [14] reported that *Salmonella* strains originating from raw beef and offal were positive for *inv*A and *stn* genes.

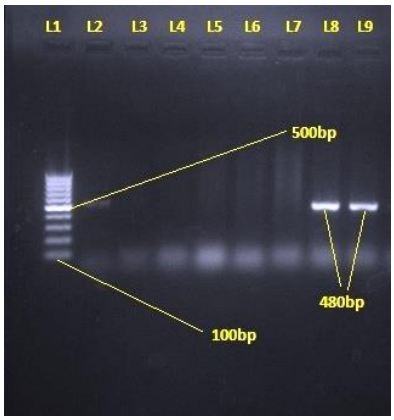

Fig 1: PCR amplicons of 16S- rRNA gene (480bp) obtained from Salmonella Typhimurium (L1: 100bp ladder; L8-9: Representative samples)

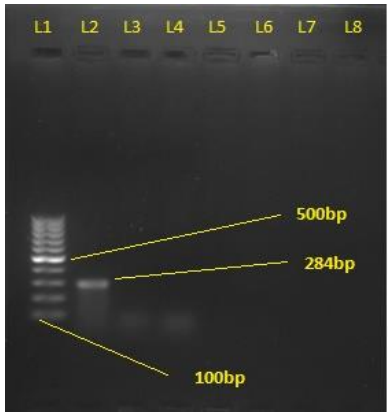

Fig 2: PCR amplicon of stn gene (284bp) obtained from Salmonella Typhimurium (L1: 100bp ladder; L2: Representative sample)

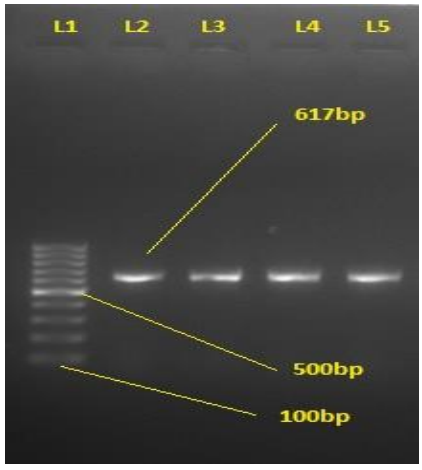

Fig 3: PCR amplicons of *invA* gene (617bp) obtained from *Salmonella* Typhimurium (L1: 100bp ladder; L2-5: Representative samples)

The antimicrobial resistance pattern among *Salmonella* strains isolated from environmental sources and food shows a variable incidence rate of resistant strains obtained from developed and developing countries. These observational studies varied in methodology and spectrum of antibiotics used by different investigators which might contribute to the high degree of variation in resistance pattern. Resistance to antimicrobials by *Salmonella* spp. can be transferred mainly due to consumption of food contaminated with antibiotics or eating food contaminated with faeces of animal or human carriers, who continue to suffer from the disease after various

incomplete or failed treatments [27].

The antibiogram study on *Salmonella* Typhimurium strains showed 100 per cent sensitivity to Gentamicin, Amikacin, Ofloxacin, Ceftazidime, Chloramphenicol and Imipenem where as all the strains were found to be completely resistant against Ciprofloxacin and Tetracycline. The *Salmonella* Typhimurium strains were 75 per cent resistant to Cephalexin, 50 per cent to Ampicillin and 25 per cent to Norfloxacin and Ceftriaxone (Figure 4).

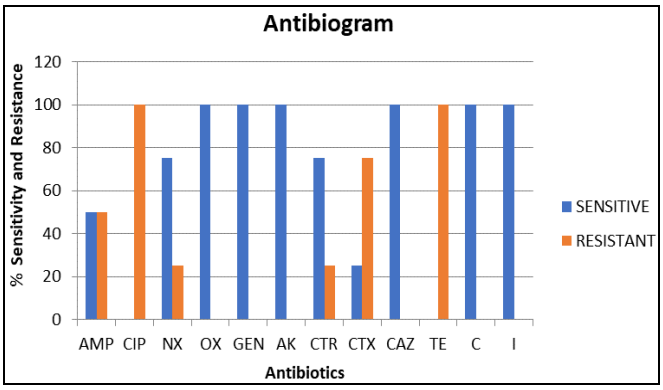

Fig 4: Antibiogram of *Salmonella* Typhimurium strains (n=4) isolated from fish

Martinez-Urtaza *et al.* (2004) [16], Kumar *et al.* (2008) [15] and Setti *et al.* (2009) [31] reported that 9, 82 and 49.10 per cent *Salmonella* strains isolated from marine environment showed antimicrobial resistance from Spain, India and Morocco, respectively. Seel *et al.* (2016) [30] reported that 86.95 per cent strains of *S.* Typhimurium were found to be resistant to Azithromycin and 91.30 per cent to Erythromycin while strains were 100 per cent sensitive to Ciprofloxacin and Gentamicin, 82.62 per cent to Norfloxacin and 86.95 per cent to Streptomycin. Elhadi (2014) [7] reported that *Salmonella* were highest resistant against Tetracycline (90%) followed by Ampicillin (70%) and Amoxycillin-clavulanic acid (45%). Beshiru *et al.* (2019) [2] reported that all *Salmonella* species recovered were resistant to Penicillin and Erythromycin with 100 per cent sensitivity to Cefotaxime, Cephalothin, Colistin and Polymyxin B. In a study by Rahimi *et al.* (2011) [23], *Salmonella* showed the highest resistance against Nalidixic acid (47.40%) followed by Tetracycline (36.80%), Streptomycin (15.80%), Trimethoprim (15.80%) and Ciprofloxacin (5.30%).

The occurrence of antimicrobial resistance in *Salmonella* was probably an indication of their frequent usage both in livestock, fish and human. Studies conducted in different parts of India and other countries have also indicated that the increase in the proportion of drug-resistant *Salmonella* isolates could be due to the irrational use of antimicrobials and inappropriateness of the prescription and dispensing methods in both the veterinary and public health setups [32, 17]. The contamination of fishes with antimicrobial resistant *Salmonella* in the present study might have resulted from the runoff water contaminating the ponds, use of animal and poultry offal as feed, indiscriminate use of antibiotics, improper handling of fishes during catching, storage, transportation and retailing.

Conclusion

The detection of antimicrobial resistant *S.* Typhimurium in fresh water fishes from Mizoram indicated the probable public health hazard contributed by irrational use of antibiotics in animal, fish and human, improper fish production management and handling.

Acknowledgement

Authors are highly grateful to the Dean, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India for providing the necessary facilities and support for successfully carrying out the work.

References

1. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standard single disk method. American Journal of Clinical Pathology. 1966; 45(4):493-496.
2. Beshiru A, Igbinosa IH, Igbinosa IO. Prevalence of antimicrobial resistance and virulence gene elements of *Salmonella* Serovars From Ready-to-Eat (RTE) Shrimps. Frontier Microbiology, 2019. https://doi.org/10.3389/fmicb.2019.01613
3. Bhatta KS, Pattnaik AK, Behera BP. Further contribution to the fish fauna of Chilika lagoon-A Coastal wetland of Orissa. GIOBIOS. 2001; 28(2-3):97-100.
4. Bhowmick PP, Srikumar S, Devegowda D, Shekar MHA, Ruwandeepika D, Karunasagar I. Serotyping and molecular characterization for study of genetic diversity among seafood associated non typhoidal *Salmonella* serovars. Indian Journal of Medical Research. 2012; 135(3):371-381.
5. Bibi F, Ahmed AN, Quaisrani NS, Akhtar M. Occurrence of *Salmonella* in freshwater fishes: 4. A review. Journal of Animal and Plant Sciences. 2015; 25(3):303-310.
6. Cabral JP. Water microbiology: bacterial pathogens and water. International Journal of Environtal Research and Public Health. 2010; 7(10):3657-703.
7. 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.
8. FAO. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. 2018. Rome. Licence: CC BY-NC-SA 3.0 IGO.
9. Fernandes DVG, Castro VS, Neto AC, Figueiredo S. *Salmonella* spp. in the fish production chain: a review. Ciencia Rural. 2018; 48(8). https://doi.org/10.1590/0103-8478cr20180141
10. Gaertner J, Wheeler PE, Obafemi S, Valdez J, Forstner MRJ, Bonner TH *et al*. Detection of Salmonellae in fish in a natural river system. Journal of Aquatic Animal Health. 2008; 20:150-157.
11. Gold WL, Salit IE. *Aeromonas hydrophila* infections of the skin and soft-tissue: Report of 11 cases and review. Clinical Infectious Diseases. 1993; 1:69-74.
12. Hastein T, Hejltnes B, Lillehug A, Skare JU. Food safety hazards that occur during the production stage: Challenges for fish farming and the fishing industry. Revue scientifique et technique (International Office of Epizootics). 2006; 25(2):607-25.
13. Hussan A, Chakrabarti PP, Sundaray JK, Das A, Mohapatra BC, Ananth PN. Status and future of aquaculture development in Mizoram, India. International Journal of Fisheries and Aquatic Studies. 2018; 6(4):42-48.
14. Kshirsagar DP, Singh S, Brahmbhatt MN, Nayak JB.

Isolation and molecular characterization of virulence associated genes of *Salmonella* from buffalo meat samples in Western Region of India. Israel Journal of Veterinary Medicine. 2014; 69(4):228-233.
15. Kumar R, Surendran PK, Thampuran N. Evaluation of culture, ELISA and PCR assays for the detection of *Salmonella* in sea food. Letters in Applied Microbiology. 2008; 46:221-226.
16. Martinez UJ, Liebana E, Garcia ML, Perez PP, Saco M. Characterization of *Salmonella enterica* serovar Typhimurium from marine environments in coastal waters of Galicia (Spain). Applied Environmental Microbiology. 2004; 70:4030-4034.
17. Mebrat E, Legesse G, Zabishwork A, Walelgn W. Prevalence and antimicrobial resistance of *Salmonella* isolated from animal origin food items in Gondar, Ethiopia. Biomedical Research International, 2016. https://doi.org/10.1155/2016/4290506
18. Mol S, Cosansu S, Alakavuk DU, Ozturan S. Survival of *Salmonella enteritidis* during salting and drying of horse mackerel (*Trachurus trachurus*) fillets. International Journal of Food Microbiology. 2010; 139:36-40.
19. Murugkar HV, Rahman H, Dutta PK. Distribution of virulence genes in *Salmonella* serovars isolated from man and animals. Indian Journal of Medical Research. 2003; 117:66-70.
20. Novotony L, Dovorska L, Lorencova L, Beran V, Puvlik L. Fish: a potential source of bacterial pathogens for human beings. Veterinary Medicine Czech Republic. 2004; 49(9):343-358.
21. Pal D, Gupta C. Microbial pollution in water and its effect on fish. Journal of Aquatic Animal Health. 1992; 4(1):32-39.
22. Quinn PJ, Carter ME, Markey BK, Carter GR. Clinical Veterinary Microbiology. Wolf Publishing, London, 1994, 648.
23. Rahimi E, Shakerian A, Falavarjani AG. Prevalence and antimicrobial resistance of *Salmonella* isolated from fish, shrimp, lobster and crab in Iran. Comparative Clinical Pathology. 2013; 22:59-62.
24. Rahn K, De Grandis SA, Clarke RC, McEwen SA, Galan JE, Ginocchio C *et al*. Amplification of an *inv*A gene sequence of *Salmonella* Typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*. Molecular Cell Probes. 1992; 6(4):271-27.
25. Raufu AI, Hauwa SB, Lawan FA, Musa AS. Occurrence and antimicrobial susceptibility profiles of *Salmonella* serovars from fish in Maiduguri, Sub Saharah, Nigeria. Egyptian Journal of Aquatic Rsearch. 2014; 40:59-63.
26. Raymond A, Ramachandran A. Bacterial pathogens in sea food- Indian Scenario. Fishery Technology. 2019; 56:1-22.
27. Rivera UJ, Sánchez PM, Ferro-García MÁ, Prados-Joya G, Ocampo-Pérez R. Pharmaceuticals as emerging contaminants and their removal from water. A review. Chemosphere. 2013; 93:1268-1287.
28. Sambrook J, Russel DW. Plasmid and their usefulness in molecular cloning. Molecular cloning: A Laboratory Manual. Edn 3, Cold Spring Harbour Laboratory press, New York, 2001, 1-32.
29. Saroj SD, Shashidhar R, Bandekar JR. Genetic Diversity of food isolates of *Salmonella enterica* serovar Typhimurium in India, International Journal of Food Science Technology. 2008; 14:151-156.
30. Seel SK, Kabir SML, Islam MAB. Characterization of *Salmonella* spp. isolated from fresh fishes sold in selected Upazila markets of Bangladesh Journal of Veterinary Medicine. 2016; 14(2):283-287.
31. Setti I, Rodriguez-Castro A, Pata MP, Cadarso-Suarez C, Yacoubi B, Bensmael L *et al*. Characteristics and dynamics of *Salmonella* contamination along the coast of Agadir, Morocco. Applied Environmental Microbiology. 2009; 75:7700-7709.
32. Tekale AA, Savalia CV, Kshirsagar DP, Brahmbhatt MN, Chatur YA. Detection and virulence gene characterization of *Salmonella* isolates from fish by conventional and molecular methods. Journal of Veterinary Public Health. 2015; 13(1):43-46.
33. Thompson B, Subasinghe R. Aquaculture's role in improving food and nutrition security. Thompson and L Amoroso eds., FAO, Rome, 2011, 150-162.
34. Upadhyay BP, Utrarachkij F, Thongshoob J, Mahakunkijcharoen Y, Niracha WN, Suthienkul O *et al*. Detection of *Salmonella invA* gene in Shrimp enrichment culture by polymerase chain reaction. Southeast Asian Journal of Tropical Medicine and Public Health. 2010; 41(2):426-431.
35. Whyte P, Gill K, Collin JD, Gormley E. The prevalence and PCR detection of *Salmonella* contamination in raw poultry. Veterinary Microbiology. 2002; 89(1):53-60.